Yokosuka Science Festa 2009

8th Pan-Pacific Connective Tissue Societies Symposium (PPCTSS)

41st Annual Meeting of the Japanese Society of Connective Tissue Research (JSCTR)

56th Annual Meeting of the Japan Matrix Club (JMC)

Concurrent Meeting:

Yokosuka International Conference on Cancer Microenvironments (YICCM)

Program & Proceedings

June 4 (Thu) – June 7 (Sun), 2009
Shonan Village Center

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Shonan Village Center <URL: http://www.shonan-village.co.jp/svc/>
Conference Web Site <URL: http://www.kokuhoken.or.jp/ysf2009/index.html>

Welcome to Yokosuka Science Festa

Dear Colleagues and Friends,

We are delighted to welcome you to "Yokosuka Science Festa," which will be held from June 4 (Thurs) to June 7 (Sun), 2009, in Yokosuka, located on the west side of Tokyo Bay. The Yokosuka Science Festa is composed of a complex of meetings. It includes the 8th Pan Pacific Connective Tissue Societies Symposium in association with the ISMB, Kanagawa Dental College, and it incorporates the 41st Annual Meeting of the Japanese Society for Connective Tissue Research, the 56th Annual Meeting of the Japan Matrix Club, and The Yokosuka International Conference on Cancer Microenvironments.

Connective tissues are characterized by an abundance of extracellular matrix (ECM) components in addition to various kinds of cells, and the ECM is important for connecting tissues and cells in many circumstances, including both normal as well as pathological states of the human body.

On behalf of the Organizing Committee of the Yokosuka Science Festa, we invite all basic scientists and clinicians interested in the structure, biosynthesis, and function of the ECM and in the biology and pathology of connective tissues to join us for this meeting. We are also much interested in new functions of the ECM in the development of normal and cancerous tissues.

We look forward to discussing many new aspects of the ECM and connective tissues at the Yokosuka Science Festa, and we hope that the Festa will be a catalyst that will connect many people working in the diverse sub-specialties of this field.

Best Wishes,

Ryu-Ichiro Hata, Ph.D.
Chairperson of the Organizing Committee
Yokosuka Science Festa
Professor and Chair
Dept. Biochem. & Mol. Biol.
Director, High-Tech Research Center
Kanagawa Dental College

Yokosuka Science Festa 2009
Joint Conference of 8th PPCTSS, 41st JSCTR, and 56th JMC
Concurrent Meeting: YICCM

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Yokosuka Science Festa 2009

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(Kanagawa Dent. Coll., Yokosuka, Japan)

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Keiichi Tsukinoki
(Kanagawa Dental College, Yokosuka, Japan)

How to get there?

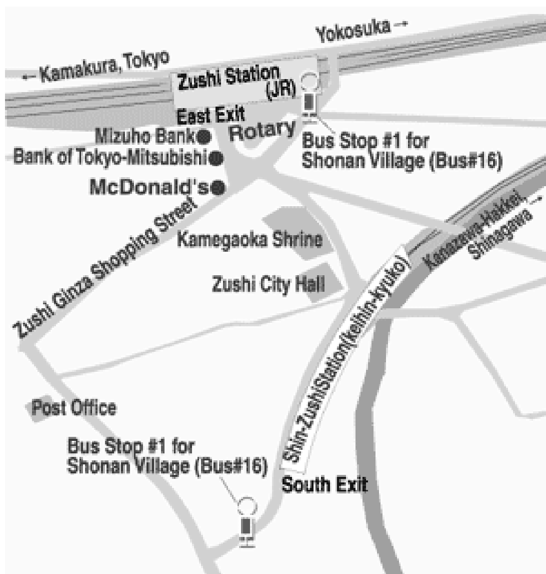
Shonan Village Center, in the city of Kanagawa, which is situated approximately 50 km south- west of Tokyo. The center is located on a hill commanding a view of Mt. Fuji and overlooking Sagami Bay.

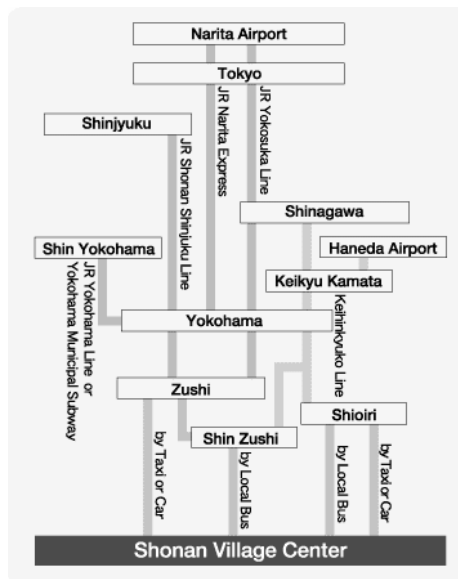
Tokyo-Narita airport is the nearest international airport to Kanagawa.

From Tokyo-Narita airport

- by JR Sobu-Yokosuka Line Rapid Train ('Airport Narita'). Get off at Zushi station (journey time is approximately 2 hours and a half). From JR Zushi Station, take Keikyu bus no. 16. Get off at Shonan Kokusaimura Center Mae. (approximately 30 min.).
- by JR Narita Express. Change at Yokohama station to JR Yokosuka Line. Get off at Zushi station (approximately 2 hours). From JR Zushi Station, take Keikyu bus no. 16. Get off at Shonan Kokusaimura Center Mae. (approximately 30 min.).
- Take a bus from Narita airport to Yokohama City Air Terminal (YCAT) .
- Change at Yokohama station to JR Yokosuka Line no.9 (Train for Zushi or Kurihama). Get off at Zushi station(approximately 2 hours). From JR Zushi Station, take Keikyu bus no. 16. Get off at Shonan Kokusaimura Center Mae. (approximately 30 min.).
- YCAT: Timetable

  <http://www.ycat.co.jp/bus/narita%20e.htm>





From Tokyo

- by JR Yokosuka Line. Get off at Zushi Station (about 60 min. from Tokyo station). From JR Zushi, take Keikyu bus no. 16. Get off at Shonan Kokusaimura Center Mae. (approx. 30 min.).
- by JR Shonan-Shinjuku Line. Get off at Zushi Station (60 min. from Shinjuku station). From JR Zushi, take Keikyu bus no. 16. Get off at Shonan Kokusaimura Center Mae. (approx. 30 min.).
- by Keihin Kyuko (Keikyu) Line. Get off at Shin-Zushi or Shioiri station. (50 min. approx. from Shinagawa station). From Keikyu Shin-Zushi or Shioiri stations, take Keikyu bus no. 16. Get off at Shonan Kokusaimura Center Mae. (approx. 30 min.).

From Haneda airport

- by Keikyu Haneda Airport Line. Change at Keikyu Haneda station to a train heading for Yokohama. Get off at Shin-Zushi or Shioiri station (60 min. approx. from Haneda airport). From Keikyu Shin-Zushi or Shioiri station, take Keikyu bus no. 16 for Shonan Village. Get off at Shonan Kokusaimura Center Mae. (approx. 30 min.).

From Shin-Yokohama

- by JR Yokohama Line. Change at Yokohama station to JR Yokosuka Line. Get off at Zushi station. (50 min. approx. from Shin-Yokohama station). From Zushi station take Keikyu bus no. 16 for Shonan Village. Get off at Shonan Kokusaimura Center Mae. (approx. 30 min.).
- Japan Rail : http://www.japanrail.com/
- East Japan Railway Company :

  http://www.jreast.co.jp/ehttp://www.shonan-village.co.jp/

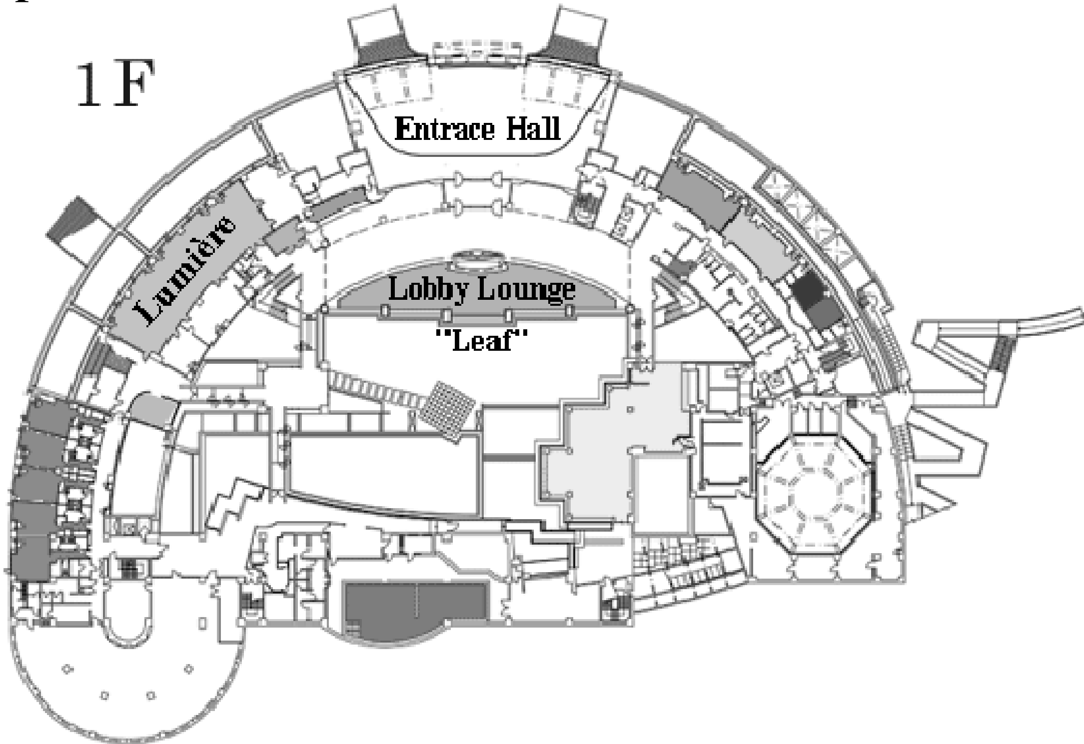
from either JR Zushi or Keikyu Shin-Zushi station.

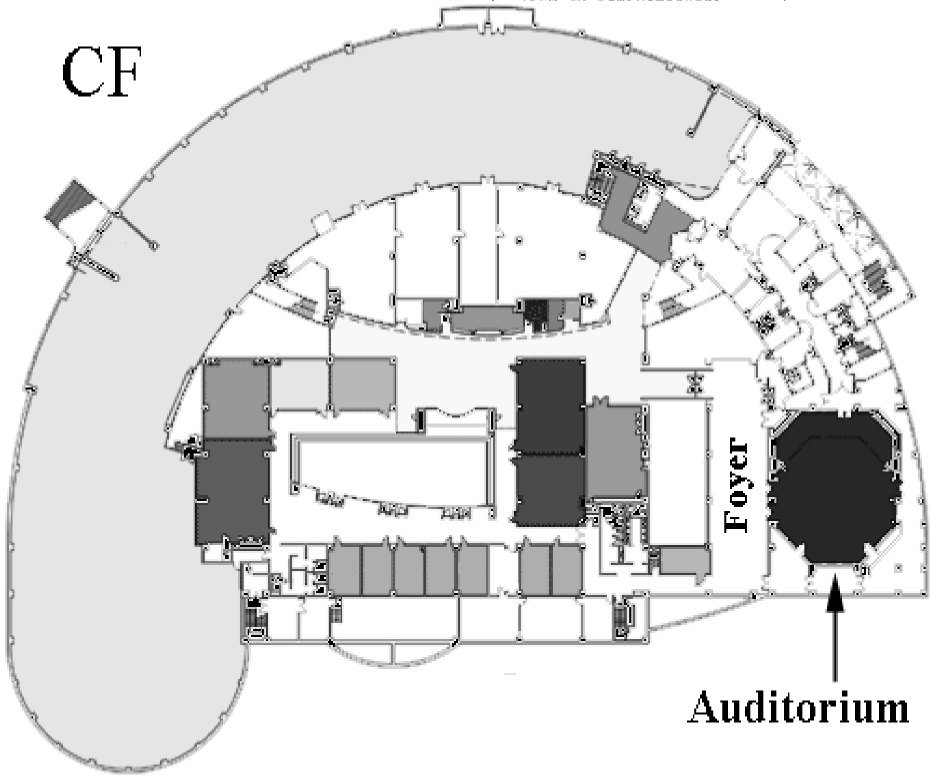
- Take bus no. 16 for Shonan Village, which leaves from bus stop number 1 at both stations (approx. 30 min.). A one way bus ticket costs JPY 340.

from Keikyu Shioiri station.

- Take bus no. 16 for Shonan Village, which leaves from bus stop number 2 (approx. 35 min.). A one way bus ticket costs JPY 380.

Floor Map





General Informations

Registration (Foyer)

June 4 (Thursday): 11:00-17:00
June 5 (Friday): 08:00-17:00
June 6 (Saturday): 08:00-17:00
June 7 (Sunday): 08:00-13:00

Reception and Meal

Registration-fee Includes:
-Access to all sessions of YSF 2009 (Except Accompanying Person).
-Satchel complete with program and abstract book (Except Accompanying Person).
-Morning teas, luncheon seminars and afternoon teas.
-Welcome Party (June 4) and Conference Dinner (June 6).
-Registration fee of "Yokosuka International Conference on Cancer Microenvironments".
Registration-fee Does not Include: Dinner on June 5 (Friday), 2009.

Oral Session

Lecturs & Symposiums: Bring not only own PC but also a power-point file saved on USB flash memory. Be sure to bring an AC adaptor and also an appropriate convertor if your PC does not have the "Mini D-sub 15 pins" connection with you. Earlier check-in is recommended because congestion is anticipated at the Presenter Registration desk just before the Session.
Workshops: Please bring your Power Point File (97-2003 format (.ppt)) on USB flash memory (we do not accept CD-R, CD-RW, DVD-R, and MO, Zip) and load your file onto the Conference PC at the Presenter Registration desk during 08:00-16:15 on June 5.
(Presentation files loaded on to the PC provided will be completely deleted by the Secretariat after your session).

Poster Session

Poster size is 120 cm wide X 180cm long. Pins are available at the congress site.

	Poster Session I	**Poster Session II**
Mounting	14:00-16:00, June 4 (Thursday)	16:30-20:00, June 5 (Friday)
Discussion	12:00-14:00, June 5 (Friday)	14:30-15:15, June 6 (Saturday)
Removal	16:00-16:30, June 5 (Friday)	18:45-19:15, June 6 (Saturday)

No Smorking Regulations

Smoking shall be prohibited in all Public Places and Places of Employment within the Shonan Village Center, unless specifically exempted. Please use smoking room.

Cell Phones and Beepers

As courtesy to your colleagues, please turn off your cell phone or beeper, or set it on "vibrate" before you enter any room in which a lecture session is taking place.

Sponsors:

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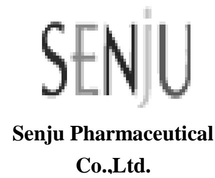

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High-Tech Research Center KDC

Time Schedule of the Conference

Thursday, June 4

(10:00-11:00) (JMC Boad Member Meeting)

(11:00-12:00) (JSCTR Boad Member Meeting)

(12:00-12:45) (Joint Boad Member Meeting of JSCTR & JMC)

Joint Conference of 8th PPCTSS(*1), 41st JSCTR(*2) & 56th JMC(*3)

11:00-17:00 **Registration** (Foyer)

13:00-13:10 Opening Address (Auditorium)

13:10-14:00 **Opening Lecture** (Auditorium)
Kazuhiro Nagata (*Kyoto University, Kyoto, Japan*)

14:00-15:30 Symposium I (Auditorium)
"*Developmental Biology and the Basement Membrane*"

15:30-16:00 Coffee Break (Foyer)

16:00-17:30 Symposium II (Auditorium)
"*Genetics and Connective Tissue Disorders*"

17:30-18:30 Welcome Concert (Auditorium)

18:30-20:00 Welcome Party (Foyer)

Friday, June 5

09:00-09:50 **Keynote Lecture I** (Auditorium)
Toshio Suda (*Keio University, School of Medicine, Japan*)

09:50-10:00 Coffee Break (Foyer)

10:00-12:00 Symposium III (Auditorium)
"*ADAMS*"

12:00-14:00 **Lunch & Poster Discussion I** (Foyer)
(*incuding 41st JSCTR Award Competition*)

(13:30-14:00 Editorial Boad meeting of Connective Tissue Research "JSCTR page")

14:00-16:00 Symposium IV (Auditorium)
"*Biosynthesis and Assembly of ECM*"

16:00-16:30 Coffee Break (Foyer)

16:30-18:42 Workshop I (A&B) (Auditorium) Workshop II (A&B) (Lumière)
(*incuding 8th PPCTSS Award Competition*)

Saturday, June 6

09:00-09:50 **Keynote Lecture II** (Auditorium)
Francesco Ramirez (*Mount Sinai School of Medicine, New York, NY, USA*)

09:50-10:00 Coffee Break (Foyer)

10:00-11:45 Symposium V (Auditorium)
"*Molecular Pathology and Molecular Therapy of Muscular Dystrophy*"

11:45-12:45 Luncheon Seminner: Sponsored by Nippi, Inc.
Ryoji Nagai (*Japan Women's University, Tokyo, Japan*)

12:45-14:30 Symposium VI (Auditorium)
"*Molecular and Cellular Mechanisms of Tissue Remodeling and Fibrosis-Their Therapeutic Implications*"

14:30-15:15 Poster Discussion II & Coffee Break
(*incuding 56th YIA Competition*)

15:15-16:15 Business Meeting (PPCTSS, JSCTR, and JMC) (Auditorium)

16:15-16:45 Otaka Prize Lecture (in Japanese) (Auditorium)

Concurrent Meeting:
Yokosuka International Conference on Cancer Microenvironments

16:45-18:45 Symposium VII (Auditorium)
"*Cytokine, Chemokine and Cancer*"

19:00-21:00 **Dinner Party** (Lobby Lounge "Leaf")

Sunday, June 7

09:00-09:50 **Keynote Lecture III** (Auditorium)
Mina J. Bissell (*Life Sciences Division, Lawrence Berkeley National Laboratory, USA*)

09:50-10:00 Coffee Break (Foyer)

10:00-11:30 Symposium VIII (Auditorium)
"*Microenvironments Regulate Cancer Progression*"

11:30-12:30 Luncheon Seminner: Sponsored by Open Research Center, KDC
Sadao Sato (*Kanagawa Dental College, Yokosuka, Japan*)

12:30-14:30 Symposium IX (Auditorium)
"*Bone Metastasis & Acidic Microenvironments*"

14:30-14:40 Closing Remarks (Auditorium)

(*1)**8th PPCTSS:** 8th Pan-Pacific Connective Tissue Societies Symposium
(*2)**41st JSCTR:** 41st Annual Meeting of the Japanese Society of Connective Tissue Research
(*3)**56th JMC:** 56th Annual Meeting of the Japan Matrix Club

Program

Thursday, June 4

11:00-17:00 Registration (Foyer)

13:00-13:10 Opening Address (Auditorium)
Eiro Kubota (*President of YSF2009, Dean of Kanagawa Dental College, Yokosuka, Japan*)

13:10-14:00 Opening Lecture (Auditorium)
Chairperson: **Tomoatsu Kimura** (*Toyama University, Toyama, Japan*)
Revisiting of collagen-specific molecular chaperone Hsp47: Fate of procollagen with or without Hsp47
Kazuhiro Nagata (*Kyoto University, Kyoto, Japan*)

14:00-15:30 Symposium I (Auditorium)
"*Developmental Biology and the Basement Membrane*"
Chairpersons: **Yoshifumi Ninomiya** (*Okayama University, Okayama, Japan*)
Kiyotoshi Sekiguchi (*Osaka University, Suita, Japan*)

S1-1 **Customization of the basement membrane during embryonic development**
Sekiguchi, K. (*Osaka University, Suita, Japan*)

S1-2 **Laminin-511 plays a key role in dermal stem cell development and hair formation**
Marinkovich, P. (*Program in Epithelial Biology, Stanford University, Stanford, USA*)

S1-3 **Developmental studies of *Drole*, *Drosophila* type XV/XVIII collagen**
Momota, R., Naito, I., Ninomiya, Y., Ohtsuka, A. (*Okayama University, Okayama, Japan*)

S1-4* **Peptide-chitosan membranes can mimic the biological activities of laminin α1 LG4 module**
Hozumi, K., Yamagata, N., Fujimori, C., Katagiri, F., Kikkawa, Y., Kadoya, Y. Nomizu, M. (*University of Pharmacy and Life Sciences, Hachioji, Tokyo, Japan; Kitasato University, Sagamihara, Kanagawa, Japan*)
***Short talk chosen from the abstracts**

15:30-16:00 Coffee Break (Foyer)

16:00-17:30 Symposium II (Auditorium)
"*Genetics and Connective Tissue Disorders*"
Chairpersons: **Kathryn Cheah** (*University of Hong Kong, Hong Kong*)
Yukihide Iwamoto (*Kyushu University, Fukuoka, Japan*)

S2-1 **Integrated approach toward bone and joint diseases using human and mouse genetics**
Ikegawa, S. (*Center for Genomic Medicine, RIKEN, Japan*)

S2-2 **The new paradigm for OI genetics and the functional effects of recessive CRTAP and P3H1/LEPRE1 Mutations**
Marini, J.C. (*Eunice Kennedy Shriver National Institute of Child Health and Human Development, NIH, Bethesda, MD, USA*)

S2-3* **A new categorized *COL3A1* mutation detected by genome scanning with vascular Ehlers-Danlos syndrome (vEDS)**
Watanabe, A., Tang, B.N., Shimada, T. (*Nippon Medical School, Japan; Nippon Medical School Hospital, Japan*)
***Short talk chosen from abstracts.**

17:30-18:30 Welcome Concert by Ayano Ninomiya (violin) & Shinich Iino (piano) (Auditorium)

18:30-20:00 Welcome Party (Foyer)

Friday, June 5

09:00-09:50 Keynote Lecture I (Auditorium)
Chairperson: **Yuji Hiraki** (*Kyoto University, Kyoto, Japan*)
Cancer stem cells and their niche
Toshio Suda (*Keio University, School of Medicine, Japan*)

09:50-10:00 Coffee Break (Foyer)

10:00-12:00 Symposium III (Auditorium)
"*ADAMS*"
Chairpersons: **Yasunori Okada** (*Keio University, Tokyo, Japan*)
Amanda Fosang (*University of Melbourne & MCRI, Melbourne, Australia*)

S3-1 Insights into aggrecan and collagen degradation using knockin mice
Fosang, A.J., Gauci, S.J., Kurth, L.M., Little, C.B., Lee, E.R., and Sims, N.A., Tatarczuch, L., Mackie, E.J. (*University of Melbourne, Parkville, Australia; University of Sydney at the Royal North Shore Hospital, St. Leonards, Australia; McGill University, Montreal, Canada*)

S3-2 Induction of aggrecanases in cartilage by fibronectin fragments is mediated by α5β1 integrin and TLR4.
Nagase, H. (*Imperial College London, London, UK*)

S3-3 Studies from TACE Mutant Mice
Horiuchi, K. (*Keio University, Japan*)

S3-4 The role of ADAM28 in cancer cell proliferation and progression
Mochizuki, S., Shimoda, M., Soejima, K., Tanaka, R., Okano, H.J., Tsuji, O., and Okada, Y. (*Keio University; Chemo-Sero-Therapeutic Research Institute, Japan*)

S3-5* Gene transfer of ADAMTS1 induced apoptosis in endothelial cells and inhibited tumor growth
Hirohata, S., Obika, M., Takahashi, K., Miyoshi, T., Ogawa, H. Cilek, M.Z., Hatipoglu, O.F., Yamamoto, K., Kusachi, S., Ninomiya, Y. (*Okayama University, Japan*)
***Short talk chosen from abstracts**

12:00-14:00 Lunch and Poster Discussion I (*incuding 41st JSCTR Award Competition*) **(Foyer)**

14:00-16:00 Symposium IV (Auditorium)
"*Biosynthesis and Assembly of ECM*"
Chairpersons: **Hans-Peter Bächinger** (*Shriners Hospital for Children, Portland, OR, USA*)
Koji Kimata (*Aichi Medical University, Aichi, Japan*)

S4-1 Biochemical characterization of the P3H1/CRTAP/CypB complex as a prolyl 3-hydroxylase, a PPIase and a molecular chaperone
Bächinger, H.P., Ishikawa, Y., Vranka, J., Wirz, J., Pokidysheva, E. and Nagata, K. (*Shriners Hospital for Children, Portland, OR, USA; Oregon Health & Science University, Portland, OR and, Kyoto University, Kyoto, Japan*)

S4-2 Generation and characterization of chondroitin sulfate E-deficient mice
Ohtake-Niimi, S., Kondo, S., Ito, T., Ohta, T., Habuchi, H., Kimata, K., Habuchi, O. (*Aichi University of Education, Aichi, Japan; Aichi Medical University, Aichi, Japan*)

S4-3 Role of the sulfation pattern of chondroitin sulfate in its neuritogenic activities
Kitagawa, H. (*Kobe Pharmaceutical University, Kobe, Japan*)

S4-4 Hyaluronan as a key adhesion molecule in the liver
Kubes, P. (*University of Calgary, Calgary, Canada*)

S4-5* Brevican determines specialization of the hyaluronan-binding nodal matrix assemblies at the large diameter nodes of Ranvier in the CNS
Bekku, Y., Rauch, U., Ninomiya, Y., Oohashi, T. (*Okayama University, Okayama, Japan; Lund University, Lund, Sweden*)
***Short talk chosen from abstracts**

16:00-16:30 Coffee Break (Foyer)

16:30-17:42 Workshop I-A (Auditorium) (*incuding 8th PPCTSS Award Competition[c)]*)

Chairpersons: **Masahiro Saito** (*Tokyo University of Science, Noda, Japan*)
Shunji Hattori (*Nippi Research Institute of Biomatrix, Toride, Japan*)

16:30 1W-01[c)] ADAMTS1 is induced by hypoxia in endothelial cells and HIF-1 binds to the ADAMTS1 promoter
Omer Faruk Hatipoglu, Satoshi Hirohata, Mehmet Zeynel Cilek, Toru Miyoshi, Yoshifumi Ninomiya (*Okayama University, Okayama, Japan*)

16:42 1W-02[c)] Model organism approaches to understand the role of WISP3, the gene that is mutated in progressive pseudorheumatoid dysplasia
Nakamura, Y., Kato, H., Warman, M.L. (*Shinshu University, Nagano, Japan; Howard Hughes Medical Institute, Children's Hospital and Harvard Medical School, Boston MA, USA*)

16:54 1W-03[c)] Suppression of Akt activation on collagen gels (sAag); in the case of cancer cell lines
Fujisaki, H., Sasaki, J., Hattori, S. (*Nippi Research Institute of Biomatrix, Japan Institute of Leather Research, Ibaraki, Japan*)

17:06 1W-04[c)] A quantitative estimation system for fibrosis in non-alcoholic steatohepatitis by using transgenic collagen promoter/luciferase reporter mouse
Moro, T. Nakao, S. Higashiyama, R., Mikami, K., Fukumitsu, H., Ueda, Y., and Inagaki, Y. (*Tokai University, Isehara, Japan; Minophagen Pharmaceutical Co., Ltd., Zama, Japan*)

17:18 1W-05[c)] Versican expression is transient during wound healing but continues at high levels in keloid: Role of versican in keloid formation in a new mouse model
Araki, E., Naitoh, M., Aota, S., Matsui, S., Suzuki, S., Miyachi, Y., Utani, A. (*Kyoto University, Kyoto, Japan; RIKEN, Japan*)

17:30 1W-06 ADAMTSL4 improves microfibril of Marfan syndrome derived cells
Saito, M., Tsutsui, K., Suda, N., Ganjargal, G., Sekiguchi, K., Tsuji, T., and Yoneda, T. (*Tokyo University of Science; Osaka University, Osaka, Japan; Tokyo Medical and Dental University, Tokyo, Japan*)

17:42-18:42 Workshop I-B (Auditorium)

Chairpersons: **Yoshinori Kuboki** (*Hokkaido University, Sapporo, Japan*)
Hideto Watanabe (*Aichi Medical University, Aichi, Japan*)

17:42 1W-07 Optimal spaces for bone regeneration created by artificial ECM of titanium web
Kuboki, Y., Takita, H., Yoshimoto, R., Kaku, T., Ohguro, T., Ametani, A., Yoshino, K. Shima, T., Seki, Y. (*Hokkaido University, Sapporo, Japan; Medical Science University of Hokkaido, Tohbetu Hokkaido; [3]Yoshida Dental MFG Co., Tokyo; [4]Hi-Lex Corporation, Takarazuka, Japan*)

17:54 1W-08 Versican/PG-M assembles hyaluronan into extracellular matrix and inhibits CD44-mediated signaling toward premature senescence in embryonic fibroblasts
Watanabe, H., Suwan, K., Choocheep, K., Hatano, S., Kongtawelert, P., Kimata, K. (*Aichi Medical University, Aichi, Japan; Chiang Mai University, Thailand*)

18:06 1W-09 Ovalbumin-induced airway hyperresponsiveness is increased in SHAP-hyaluronan complex deficient mice
Zhuo, L., Zhu, L., Kimata, K., Yamaguchi, E., and Baba, K. (*Aichi Medical University, Aichi, Japan*)

18:18 1W-10 Essential role of β3GnT7 for efficient KS-GAG production in cultured cells
Akama, T. and Nakamura, T. (*Kansai Medical University, Osaka, Japan; Burnham institute for Medical Research, La Jolla, CA, USA*)

18:30 1W-11 Fractones: specialized extracellular matrix structures governing the stem cell niches
Douet, V., Saint Georges Chaumet, M., Kerever, A., Arikawa-Hirasawa, E., Mercier, F. (*University of Hawaii, Honolulu, USA; Juntendo School of Medicine, Tokyo, Japan*)

16:30-17:30 Workshop II-A (Lumière)

Chairpersons: **Takaki Koide** (*Waseda University, Tokyo, Japan*)
Yasuyuki Sasano (*Tohoku University, Sendai, Japan*)

16:30 2W-01 Bone formation and ECM remodeling cease within a limited period regardless of completion of bone healing in the rat calvarial defect
Sasano, Y. (*Tohoku University, Sendai, Japan*)

16:42 2W-02 Effect of collagen tripeptide of type I collagen on proliferation, migration and collagen synthesis in human aortic smooth muscle cells
Lihua, T., Sakai, Y., Katsuda, S. (*Kanazawa Medical University, Ishikawa, Japan; Jellice Co., Ltd., Miyagi, Japan*)

16:54 2W-03 Integrin-dependent cell adhesion to the peptide-based artificial collagen
Yamazaki, C.M., Kadoya, Y., Koide, T. (*Waseda University, Tokyo, Japan; Kitasato University, Kanagawa, Japan*)

17:06 2W-04 Proteomic characterization of cartilage matrix synthesis and breakdown
Wilson, R., Zivkovic, S., Rowley, L., Diseberg, A., Gorman, J., Bateman, J. (*Murdoch Childrens Research Institute, Royal Children's Hospital, Melbourne, Vic and [2]Queensland Institute of Medical Research, PO Royal Brisbane Hospital, Qld*)

17:18 2W-05 Collagen in frozen mammoths
Senoo, H., Imai, K., Miura, M., Tikhonov, A., Kiriyama, T., Yoshikawa, K., Mezaki, Y., Hattori, S., Yamaguchi, N., Fujiwara, M. (*Akita University, Akita, Japan; Russian Academy of Sciences, St. Petersburg, Russia; Nippi Research Institute of Biomatrix, Tokyo, Japan, Japanese Red Cross Medical Center, Tokyo, Japan*)

17:30-18:30 Workshop II-B (Lumier)

Chairpersons: **Tomoyuki Nakamura** (*Kansai Medical University, Moriguchi, Japan*)
Yoshiaki Hirako (*Nagoya University, Nagoya, Japan*)

17:30 2W-06 The role of fibulins in elastic fiber assembly of mouse aorta
Horiguchi, M., Inoue, T., Noda, K., Nakamura, T. (*Kyoto University, Kyoto, Japan; Kansai Medical University, Moriguchi, Japan*)

17:42 2W-07 The carboxyl-terminal region of laminin beta chains modulates the integrin-binding affinities of laminins
Taniguchi, Y., Ido, H., Sanzen, N., Hayashi, M., Sato-Nishiuchi, R., Futaki, S., and Sekiguchi, K. (*Osaka University, Osaka, Japan*)

17:54 2W-08 Calcium influences hemidesmosome formation and processing of laminin332
Hirako, Y., Yonemoto, Y., Katsura, T., Owaribe, K. (*Nagoya University, Nagoya, Japan*)

18:06 2W-09 TGFbeta-dependent localization of MT1-MMP regulates epithelial tuburogenesis in 3D collagen
Weaver, S., Wolters, B., Ito, N., and Itoh, Y. (*Imperial College London, London, UK*)

18:18 2W-10 A prolonged decrease in phospholipase D activity modulates ECM turnover by increasing EGF receptor signal transduction in cultured human fibroblasts
Yoshida, H., Sugiyama, Y., Inoue, S. (*Kanabo Cosmetics INC. Basic Reserch Laboratory*)

Saturday, June 6

09:00-09: 50 Keynote Lecture II (Auditorium)
Chairperson: **Hidekatsu Yoshioka** (*Oita University, Oita, Japan*)
Fibrillin-rich microfibrils; an instructive view from outside the cell
Francesco Ramirez (*Mount Sinai School of Medicine, New York, NY, USA*)

09:50-10:00 Coffee Break (Foyer)

10:00-11:45 Symposium V (Auditorium)
"***Molecular Pathology and Molecular Therapy of Muscular Dystrophy***"
Chairpersons: **Shireen Lamande** (Murdoch Childrens Res. Inst., Melbourne, Australia)
Eri Arikawa-Hirasawa (Juntendo Univ. School of Medicine, Tokyo, Japan)

S5-1 **Significance of the dystrophin-glycoprotein complex that connects the cytoskeleton to the basal lamina**
Takeda, S. (*National Center of Neurology and Psychiatry, 4-1-1 Ogawa-higashi, Tokyo, Japan*)

S5-2 **Zebrafish integrin-linked kinase is required in skeletal muscles for strengthening the Integrin-ECM adhesion complex.**
Postel, R., Vakeel, P., Topczewski, J., Knöll, R., and Bakkers, J. (*Hubrecht Laboratory and Interuniversity Cardiology Institute of the Netherlands; University Hospital Göttingen, Germany; Northwestern University, Chicago, USA*)

S5-3 **Role of perlecan, a heparan sulfate proteoglycan, in skeletal muscle maintenance**
Arikawa-Hirasawa, E., Zhuo, X., Ichikawa, N., Kosaki, K., and Yamada, Y. (*Juntendo University School of Medicine, Tokyo, Japan; NIDCR, NIH, Bethesda, Maryland, USA*)

S5-4 **Pathogenic mechanisms in the collagen VI muscular dystrophies**
Lamandé, S.R. (*Murdoch Childrens Research Institute, Royal Children's Hospital, Parkville, Vic, Australia*)

S5-5* **Myostatin functions in the rat masseter muscle hypertrophied by cenbuterol, a β_2 adrenergic agonist**
Yamane, A., Fukui, T., Iida, R., Suga, T., Morito, M. (*Tsurumi University, Yokohama, Japan*)
***Short talk chosen from abstracts.**

11:45-12:45 Luncheon Seminar:
Ryoji Nagai (*Japan Women's University, Tokyo, Japan*)
Sponsored by Nippi, Inc.

12:45-14:30 Symposium VI (Auditorium)
"***Molecular and Cellular Mechanisms of Tissue Remodeling and Fibrosis-Their Therapeutic Implications***"
Chairpersons: **Yutaka Inagaki** (*Tokai University, Isehara , Japan*)
Haruki Senoo (*Akita University, Akita, Japan*)

S6-1 **House dust mite allergen Der f 1 can activate latent TGF-β, leading to the expression of profibrogenic genes**
Nakao, A. (*University of Yamanashi, Yamanashi, Japan*)

S6-2 **Role of endothelial progenitor cells for organ regeneration**
Asahara, T. (*Kobe Institute of Biomedical Research and Innovation/ RIKEN Center of Developmental Biology, Kobe, Japan; Tokai University, Isehara, Japan*)

S6-3 **Role of bone marrow in pathophysiology of hepatic fibrosis and regeneration**
Higashiyama, R. (*Tokai University, Kanagawa, Japan*)

S6-4 **Resolution of tissue fibrosis by siRNA HSP47 encapsulated in vitamin A bound liposome.**
Niitsu, Y. (*Sapporo Medical University, Sapporo, Japan*)

S6-5 **Hepatic stellate cells in liver fibrosis**
Yamaguchi, N., Abe, K., Yoshikawa, K., Mezaki, Y., Imai, K., Miura, M., Kasai, S., Senoo, H. (*Akita University, Akita, Japan, Eisai Co. Tokyo, Japan*)

14:30-15:15 Poster Discussion II & Coffee Break (Foyer)

15:15-16:15 Business Meeting (PPCTSS, JSCTR, JMC)

16:15-16:45 JSCTR Otaka Prize Lecture (in Japanese) (Auditorium)
(16:15-16:30) Kozo Hosono (*Nagoya University, Nagoya, Japan***)**
(16:30-16:45) Hiroshi Wachi (*Hoshi University School of Pharmacy and Pharmaceutical Sciences, Japan***)**

Concurrent Meeting:
Yokosuka International Conference on Cancer Microenvironments

16:45-18:45 Symposium VII (Auditorium)
"*Cytokine, Chemokine and Cancer*"
Chairpersons: **Masayuki Miyasaka** (*Osaka University, Osaka, Japan*)
Ryu-Ichiro Hata (*Kanagawa Dental College, Yokosuka, Japan*)

S7-1 The role of CD44-ECM interactions in tumor invasion
Miyasaka, M. and Sugahara, K. (*Osaka University, Osaka, Japan*)

S7-2 Maturation of blood vessels in the tumor environment
Takakura, N. (*Osaka University, Osaka, Japan*)

S7-3 "Mouse models for colon cancer invasion and metastasis"
Taketo, M.M. (*Kyoto University, Kyoto, Japan*)

S7-4 The BRAK box is opening
Hata, R-I. (*Kanagawa Dental College, Yokosuka, Japan*)

19:00-21:00 Dinner Party (Lobby Lounge "Leaf")

Sunday, June 7

09:00-09:50 Keynote Lecture III (Auditorium)

Chairperson: **Toshihiko Hayashi** (*Teikyo Heisei University, Ichihara, Japan*)

Modeling normal mammary gland to understand breast cancer: The Yin and Yang of the ECM and ECM-degrading enzymes
Mina J. Bissell (*Life Sciences Division, Lawrence Berkeley National Laboratory, USA*)

09:50-10:00 Coffee Break (Foyer)

10:00-11:30 Symposium VIII (Auditorium)

"*Microenvironments Regulate Cancer Progression*"

Chairpersons: **Hiroyasu Esumi** (*National Cancer Center Hospital East, Kashiwa, Japan*)
Motoharu Seiki (*The University of Tokyo, Tokyo, Japan*)

S8-1 Metabolic characteristics of cancer microenvironment and its implication in malignant progression of cancer
Esumi, H., Hirayama, A., Kami, K., Fujii, S., Soga, T., Ochiai, A. (*National Cancer Center Hospital East; Keio University, Japan*)

S8-2 MT1-MMP as a potent modulator of tumor microenvironment
Seiki, M. (*The University of Tokyo, Tokyo, Japan*)

S8-3 Macrophages, microenvironment and metastasis
Pollard, J.W. (*Louis Goldstein Swan Chair in Women's Cancer Research, Albert Einstein College of Medicine, NY, NY, USA*)

11:30-12:30 Luncheon Seminar:

Sadao Sato (*Kanagawa Dental College, Yokosuka, Japan*)
Sponsored by Open Research Center, KDC

12:30-14:30 Symposium IX (Auditorium)

"*Bone Metastasis & Acidic Microenvironments*"

Chairpersons: **Eric W. Thompson** (*St. Vincent's Inst. and Univ. of Melbourne, Australia*)
Yasumasa Kato (*Kanagawa Dental College, Yokosuka, Japan*)

S9-1 NHE1 (Na^+/H^+ exchanger 1) promotes invadopodia ECM degradation and invasion through the spatially restricted acidification of the peri-invadopodial space
Busco, G., Cardone, R.A., Bellizzi, A., Greco, M.R., Antelmi, E., Casavola, V., Paradiso, A., Reshkin, S.J. (*University of Bari, Bari, Italy; National Cancer Institute Giovanni Paolo II, Bari, Italy*)

S9-2 Acidic pH signaling in metastasis
Kato, Y. (*Kanagawa Dental College, Yokosuka, Japan*)

S9-3 Role of acid microenvironment in cancer-induced bone pain
Yoneda, T. (*Osaka University, Osaka, Japan*)

S9-4 Targeting MMP13 in human breast cancer metastasis to bone
Shah, M., Blick, T., Huang, D., Pinto, C., Trinh, J., Reiter, L.A., Hardink, J.R., Waltham, M., Thompson, E.W. (*St. Vincent's Institute & University of Melbourne, St. Vincent's Hospital, Melbourne, Australia; Pfizer Global Research and Development, Groton Laboratories, Groton, CT, USA*)

S9-5 Quantitative proteomics of breast cancer identifies new substrates and roles for MMPs
Overall, C.M. (*University of British Columbia, Vancouver, BC, Canada*)

14:30-14:40 Closing Remarks (Auditorium)

Poster Discussion I (12:00-14:00, June 5, Foyer) (*41st JSCTR Award Competition* [a])

1P-01[a] **Novel chondro-protective mechanisms of hyaluronic acid: down-regulation of ADAMTS-7 and ADAMTS-12, and reduced COMP release from articular cartilage**
Minoru Takasaki, Jun-ichi Fukushi, Yukihide Iwamoto (*Kyushu University, Japan*)

1P-02[a] **Ectopic bone formation after implantation of thermoreversible gelation polymer as a carrier of bone morphogenetic protein-2**
Emiko Saito, Akira Saito, Shigeru Takahashi, Tsuneyuki Yamamoto, Yoshiyuki Honma, Masamitsu Kawanami (*Hokkaido University, Sapporo, Japan.*)

1P-03[a] **Critical role of the TGF-β type I receptor ALK5 in skeletal development**
Tomoya Matsunobu, Yukihide Iwamoto, Yoshihiko Yamada (*National Institute of Dental and Craniofacial Research, NIH, USA; Kyushu University, Fukuoka, Japan*)

1P-04[a] **BMP-2 regulates expression of Gas6 during osteoblast differentiation**
Takashi Matsumoto, Atsushi Yamada, Dai Suzuki, Masamichi Takami, Tetsuo Suzawa, Yoichi Miyamoto, Kazuyoshi Baba, Ryutaro Kamijo (*Showa University, Tokyo, Japan*)

1P-05[a] **Role of carbonic anhydrase IX in chondrocyte differentiation**
Toshifumi Maruyama, Yoichi Miyamoto, Kentaro Yoshimura, Atsushi Yamada, Tetsuo Suzawa, Masamichi Takami, Kazuyoshi Baba, Ryutaro Kamijo (*Showa University, Tokyo, Japan*)

1P-06[a] **Reactive oxygen species reduce the eexpression of BRAK/CXCL14 in human head and neck squamous cell carcinoma cells**
Yojiro Maehata, Shigeyuki Ozawa, Chihiro Miyamoto, Kyo Kobayashi, Yasumasa Kato, Fumihiko Yoshino, Masaichi-Chang-il Lee, Ryu-Ichiro Hata (*Kanagawa Dental College, Yokosuka, Japan*)

1P-07[a] **Optical imaging of mouse articular cartilage using the glycosaminoglycans binding property of fluorescent-labeled octaarginine**
Toshitaka Oohashi, Kiichi Inagawa, Keiichiro Nishida, Yoshifumi Ninomiya (*Okayama University, Okayama, Japan*)

1P-08[a] **The application of elastin haploinsufficiency mice on lung disease with aging**
Yuichi Shimizu, Ayako Koga, Yoshitaka Ai, Risa Nonaka, Hiroshi Wachi, Yoshiyuki Seyama (*Hoshi University School of Pharmacy and Pharmaceutical Sciences, Japan*)

1P-09[a] **Phenotype of vascular smooth muscle cells and aortic calcification in elastin haploinsufficiency mice**
Risa Nonaka, Yoshitaka AI, Yuichi Shimizu, Takuya Azechi, Ayako Saito, Hiroshi Wachi (*Hoshi University School of Pharmacy & Pharmaceutical Sciences, Shinagawa, Tokyo, Japan*)

1P-10[a] **Involvement of lipid raft-associated signaling in EMMPRIN gene expression and secretion**
Takashi Sato, Miwa Ishii, Keisuke Imada, Akira Ito (*Tokyo University of Pharmacy and Life Sciences, Hachioji, Tokyo, Japan*)

1P-11[a] **Suppression of EMMPRIN-mediated tumor cell migration by syndecan-1**
Kei Hashimoto, Takashi Sato, Keisuke Imada, Motoyoshi Nomizu, Akira Ito (*Tokyo University of Pharmacy and Life Sciences, Hachioji, Tokyo, Japan*)

1P-12[a] **Release of emmprin as glycocalyceal bodies**
Mikiko Aoki, Kazuki Nabeshima, Kaori Koga, Hiroshi Iwasaki (*Fukuoka University, Fukuoka, Japan*)

1P-13[a] **Primary culture of hepatocytes on A13 peptide derived from laminin alpha1 chain**
Yamato Kikkawa, Naoya Takahashi, Yuji Matsuda, Takahiro Miwa, Taneyasu Akizuki, Akira Kataoka, Fumihiko Katagiri, Kentaro Hozumi, Motoyoshi Nomizu (*Tokyo University of Pharmacy and Life Sciences, Hachioji, Tokyo, Japan*)

1P-14[a] **Evaluation of dermal degeneration in photoaged skin using polarization-sensitive optical coherence tomography**
Shingo Sakai, Masahiro Yamanari, Arata Miyazawa, Masayuki Matsumoto, Noriaki Nakagawa, Tomoko Sugawara, Keigo Kawabata, Toyohiko Yatagai, and Yoshiaki Yasuno (*Kanebo COSMETICS INC.; University of Tsukuba, Japan*)

1P-15 ADAMTS-4 and ADAMTS-5 in degradation of inner and outer zones of the meniscus
Fuller ES, Little CB and Melrose J (*Kolling Institute of Medical Research, Institute of Bone and Joint Research, University of Sydney, Royal North Shore Hospital, St. Leonards, NSW, Australia*)

1P-16 MIG-17/ADAMTS interact with nidogen and UNC-6/netrin to guide the reader cell of organ
Yukihiko Kubota, Kayo Nagata, Kiyoji Nishiwaki (*Kwansei-Gakuin University, Hyogo, Japan; RIKEN CDB, Hyogo, Japan*)

1P-17 Analysis of the role of caspase-14 in ameloblast differentiation
Agasa Miyazono, Tetsuo Suzawa, Matsuo Yamamoto, Ryutaro Kamijo (*Showa University, Tokyo, Japan*)

1P-18 *In Vitro* calcification by dentin phosphoprotein and effects of cationic peptides
Ryuichi Fujisawa, Morimichi Mizuno, Masato Tamura (*Hokkaido University, Sapporo Japan*)

1P-19 Differential expression of basement membrane type IV collagen α chains as a prognostic factor in extrahepatic bile duct carcinoma
Kotaro Hirashima, Ken-ichi Iyama, Yoshifumi Baba, Yoshikazu Sado, Yoshifumi Ninomiya, Hiroshi Takamori, Hideo Baba (*Kumamoto University; Sigei Medical Research Institute; Okayama University, Japan*)

1P-20 Crucial effect of ultraviolet radiation on mammalian skin under the Antarctic ozone hole
Takayuki Ogura, Tomomi Kiriyama, Keisuke Tanaka, Tetsuya Takahashi, Shinkichi Irie, Shunji Hattori (*Nippi Research Institute of Biomatrix; Shimane University; Japan Institute of Leather Research, Japan*)

1P-21 Clinical and genetic features of Japanese patients with the vascular-type of Ehlers-Danlos syndrome
A. Hatamochi, M. Funakoshi, Y. Shimaoka, H. Namikawa, Y. Kitamura, S. Hayashi, Y. Hamasaki, M. Kaneda, Y. Mitsuhashi, T. Tanaka, T. Yanagisawa, K. Yamazaki, K. Hashimoto, Y. Aoki, A. Ohtake, S. Yamazaki (*Dokkyo Medical University, Tochigi, Japan; Osaka University, Osaka, Japan; Tokyo Medical University, Tokyo, Japan; Shiga Medical University, Ohtsu, Japan; Saitama Cardiovascular Respiratory Center, Saitama, Japan; Ehime University, Ehime, Japan; Tokoku University, Sendai, Japan; Saitama Medical University*)

1P-22 Expression and localization of lysyl oxidase in the presumptive dermis of chick limb bud
Yosuke Yamazaki, Yoshikazu Mikami, Maki Yuguchi, Yuichi Namba, Keitaro Isokawa (*Nihon University, Tokyo, Japan*)

1P-23 Culture conditions affecting cellular clump formation accompanying intercellular accumulation of type V collagen fibrils
Kenji Uchida, Takanori Kihara, Yongchol Shin, Toshihiko Hayashi, Yasutada Imamura (*Kogakuin University; The University of Tokyo; Teikyo Heisei University, Chiba, Japan*)

1P-24 Intercellular accumulation of type V collagen fibrils in accordance with cell aggregation
Takanori Kihara, Yasutada Imamura, Yukitoshi Takemura, Kazunori Mizuno, Eijiro Adachi, Toshihiko Hayashi (*The University of Tokyo; Kogakuin University; Shriners Hospital for Children, Portland Research Center, OR, USA; Kitasato University, Kanagawa, Japan; Teikyo Heisei University, Chiba, Japan*)

1P-25 Substrate recognition of collagen-binding domains derived from bacterial collagenases
Osamu Matsushita, Nozomu Nishi, Takaki Koide, ST Leena Philominathan, Joshua Sakon, Robert C Gensure, Hironobu Iwashiro, Eijiro Adachi (*Kitasato University; Kagawa University, Waseda University, University of Arkansas, [5]Ochsner Clinic Foundation, Japan*)

1P-26 Physical and biological functions of soluble elastin from Pisces
Eri Shiratsuchi, Kana Nishiyama, Misako Nakaba, Iori Maeda, Hiroyuki Ito, Kouji Okamoto (*Kyushu Institute of Technology; Hayashikane Sangyo Co., Ltd; Kinki University, Japan*)

1P-27 Protective effect of the fibronectin-derived peptide PHSRN in cultured human corneal epithelial cells
Tai-ichiro Chikama, Ryoji Yanai, Wu-Yong Quan, Ji-Ae Ko, Teruo Nishida (*Yamaguchi University, Yamaguchi, Japan*)

1P-28 The production and purification of recombinant human laminin-332 in *Leishmania tarentolae* expression system
Marisa Sugino, Hoang-Phuong Phan, Tomoaki Niimi (*Nagoya University, Nagoya, Japan*)

1P-29 Expression of laminin α3B chain in vascular and epithelial basement membranes and its possible functions
Taizo Mori, Yoshinobu Kariya, Chie Yasuda, Takashi Ogawa and Kaoru Miyazaki (*Yokohama City Univercity, Japan*)

1P-30 G1 domain of versican in transitional granulation tissue in pressure ulcer
Yusuke Murasawa, Chika Orii, Ken Watanabe, Zenzo Isogai (*National Center for Geriatrics and Gerontology, Aichi, Japan*)

1P-31 Regulation of fibrillin-1 fiber formation and tropoelastin deposition in Rho-ROCK signaling pathway
Hiroshi Wachi, Tatsuya Ogawa, Risa Nonaka, Yoshiyuki Seyama (*Hoshi University School of Pharmacy and Pharmaceutical Sciences*)

1P-32 Cyclosporin A suppresses up-regulated matrix metalloproteinase (MMP)-9 expression together with caspase-3/7 activity from keratinocyte in high calcium condition
Takashi Kobayashi (*National Defense Medical college, Tokorozawa, Japan*)

1P-33 IL-1 beta stimulates activin βA mRNA expression in human skin fibroblasts through MAP kinase pathways, NF-κB pathway and prostaglandin E2
KY Arai, M Ono, C Kudo, Y Nomura. T Nishiyama (*Tokyo University of Agriculture and Technology, Tokyo, Japan.*)

1P-34 Gadolinium promotes osteogenic differentiation in MC3T3-E1 cells and human adipose tissue-derived mesenchymal stem cells: a possible role of gadolinium on ectopic calcification of nephrogenic systemic fibrosis
Masayoshi Yamanaka, Etsuko Okada, Osamu Ishikawa (*Gunma University, Maebashi, Japan*)

1P-35 Decorin regulates osteoblastic differentiation of mesenchymal stem cell
Yoshinao Hosaka, Takeshi Tsuka, Tomohiro Imagawa, Masato Uehara (*Tottori University, Tottori, Japan*)

Poster Discussion II (14:30-15:15, June 6, Foyer) (*56th JMC YIA Competition[b)]*)

2P-01 Production of BRAK knockout mice
Nobuyuki Yajima, Ryu-Ichiro Hata (*Kanagawa Dental College, Yokosuka, Japan*)

2P-02 Suppression of growth of Lewis lung carcinoma cell xenografts in BRAK transgenic mouse: Production of cancer resistant mouse
Kazuhito Izukuri, Kenji Suzuki, Shigeyuki Ozawa, Eiro Kubota, Ryu-Ichiro Hata (*Kanagawa Dental College, Yokosuka, Japan*)

2P-03 Chemokine BRAK stimulates apoptosis elicited by gefitinib in oral squamous cell carcinoma
Shin Ito, Shigeyuki Ozawa, Naoto Shiiki, Keiichi Tsukinoki, Eiro Kubota, Yasumasa Kato, Takahide Taguchi, Yukari Imagawa-Ishiguro, Mamoru Tsukuda, and Ryu-Ichiro Hata (*Kanagawa Dental College; Yokohama City University, Japan*)

2P-04 Basic study on prescription of effective conservative combined therapy for malignant tumor using quantitative imaging analysis for vascular structure
Takashi Sakurai, Ryota Kawamata, Isamu Kashima (*Kanagawa Dental College, Yokosuka, Japan*)

2P-05 Expression of BRAK/CXCL14 is associated with antitumor efficacy of gefitinib in head and neck squamous cell carcinoma
Shigeyuki Ozawa, Yasumasa Kato, Shin Ito, Reika Komori, Kenji Suzuki, Keiichi Tsukinoki, Yojiro Maehata, Takahide Taguchi, Yukari Imagawa-Ishiguro, Mamoru Tsukuda, Eiro Kubota, and Ryu-Ichiro Hata (*Kanagawa Dental College, Yokosuka, Japan; Yokohama City University, Yokohama, Japan*)

2P-06[b)] Functional analysis of promoter region of human BRAK/CXCL14, a tumor progression suppressor
Reika Komori, Shigeyuki Ozawa, Yasumasa Kato, Hisaaki Shinji, Shigenari Kimoto, and Ryu-Ichiro Hata (*Kanagawa Dental College, Yokosuka, Japan*)

2P-07[b)] ADAMTS1 as a hypoxia sensing biomarker
Mehmet Zeynel Cilek, Satoshi Hirohata O.Faruk Hatipoglu, Toru Miyoshi, Yoshifumi Ninomiya (*Okayama University, Okayama, Japan*)

2P-08[b)] Matrix array as a novel research tool for analysis of cell-ECM interactions
Jun Sasaki, Keisuke Tanaka, Testuya Ebihara, Shinkichi Irie, Shunji Hattori (*Nippi Research Institute of Biomatrix, Nippi Collagen Industries Ltd., Japan*)

2P-09[b)] Osteogenesis-mimicking Matrices as models of remodeling extracellular matrix in osteogenesis
Takashi Hoshiba, Naoki Kawazoe, Tetsuya Tateishi, Guoping Chen (*National Institute for Materials Science, Tsukuba, Japan*)

2P-10[b)] Sp1 and CBF/NF-Y transcription factors up-regulate the proximal promoter of mouse α3(V) collagen gene in osteoblasts
Yunfeng Wu, Noritaka Matsuo, Hideaki Sumiyoshi, Hidekatsu Yoshioka (*Oita University, Oita, Japan*)

2P-11[b)] Distinct mechanisms in Maintaining calvaria and long bone mass in adult mouse
Takami Furuhama, Kouji Naruse, Yuko Mikuni-Takagaki (*Kanagawa Dental College, Yokosuka, Japan*; *Kitasato University, Japan*)

2P-12[b)] Sequential remodeling and loss of epithelial basement membrane type IV collagen α chains in the intraepithelial neoplasia (CIN) and squamous cell carcinoma of the uterin cervix
Naoko Imamura, Yasuji Ishimaru, Tsuguharu Asato, Sonoko Ishihara, Yumi Honda, Yoshikazu Sado, Yoshifumi Ninomiya, Ken-ichi Iyama (*Kumamoto University; Shigei Medical Institute; Okayama University, Japan*)

2P-13[b)] Etracellular matrix in frozen mammoths-protein profile and amino acid sequencing using LC/MS
Tomomi Kiriyama' Masashi Kusubata, Yuki Taga, Katsuyuki Imai, Noriko Yamaguchi, Haruki Senoo, Alexei Tikhonov, Testuya Ebihara, Nobue Kubo, Shunji Hattori (*Nippi Research Institute of Biomatrix, Ibaraki, Japan; Akita University, Akita, Japan; Russian Academy of Sciences, St. Petersburg, Russia; Nippi collagen industries Ltd., Fujinomiya, Japan*)

2P-14[b)] Cell-cell contacts differently regulate alpha-smooth muscle actin expression and collagen production in hepatic stellate cells
Yoshitaka Ueda, Tadashi Moro, Reiichi Higashiyama, Sachie Nakao, Kenichiro Mikami, Hiroshi Fukumitsu, and Yutaka Inagaki (*Tokai University, Isehara, Japan*)

2P-15[b)] Little contribution of epithelial-to-mesenchymal transition of biliary epithelial cells to the progression of experimental biliary fibrosis
Sachie Nakao, Tadashi Moro, Reiichi Higashiyama, Kenichiro Mikami, Hiroshi Fukumitsu, Yoshitaka Ueda, Kazuo Ikeda*, and Yutaka Inagaki (*Tokai University, Isehara, Japan; Nagoya City University, Nagoya, Japan*)

2P-16[b)] Autophagy eliminates misfolded procollagen aggregates in the endoplasmic reticulum for cell survival
Yoshihito Ishida, Akitsugu Yamamoto, Akira Kitamura, Shireen R. Lamandé, Tamotsu Yoshimori, John F. Bateman, Hiroshi Kubota, and Kazuhiro Nagata (*Kyoto University; Nagahama Institute of Bio-Science and Technology; University of Melbourne; Osaka University; Hokkaido University; Akita University, Japan*)

2P-17 Inflammatory alveolar bone resorption in mouse model of Marfan syndrome
Ganburged Ganjargal, Naoto Suda, Yusuke Takahashi, Nobushiro Hamada, Keiji Moriyama (*Tokyo Medical & Dental University, Tokyo, Japan; Kanagawa Dental College, Yokosuka, Japan; Global Center of Excellence (GCOE) Program, International Research Center for Molecular Science in Tooth and Bone Diseases, Tokyo, Japan*)

2P-18 Interaction of hemidesmosome protein and focal contact protein in healing wound
Toshiyuki Ozawa, Daisuke Tsuruta, Masamitsu Ishii, Kazuo Ikeda, Teruichi Harada, Jonathan C. R. Jones, and Hiromi Kobayashi (*Osaka City University, Osaka, Japan; Nagoya City University, Aichi, Japan; Northwestern University the Feinberg School of Medicine*)

2P-19 Eosinophil cationic protein (ECP) protects hearts against myocardial infarction
Takashi Ohtsuki, Satoshi Hirohata, Shigeshi Kamikawa, Shogo Watanabe, Shozo Kusachi, Masaharu Seno, Yoshifumi Ninimiya (*Okayama University, Okayama, Japan*)

2P-20 Recombinant α1 chain of human type I collagen in the silkworms *Bombyx mori*: production of human gelatin as a novel biomaterial
Takahiro Adachi, Masanobu Obara, Xiaobiao Wang, Hidenori Akutsu, Masakazu Machida, Akihiro Umezawa, Masahiro Tomita (*Hiroshima Pref. Inst. Ind. Sci. Tech., Hiroshima; NeoSilk Co., Ltd., Hiroshima; Hiroshima Univ., Hiroshima, Nat. Res. Inst. Chi. Heal. Devel., Tokyo, Japan*)

2P-21 A 384-well format screening of the compounds that inhibit collagen-protein interactions
Hitomi Kosugi, Shinichi Asada, Osamu Matsushita, Kouki Kitagawa, and Takaki Koide (*Niigata University of Pharmacy and Applied Life Sciences; Kitasato University; Engineering, Waseda University, Japan*)

2P-22 APC-induced MMP activation in human diseased chondrocytes requires EPCR and thrombomodulin
Miriam T Jackson, Margaret M Smith, Christopher Jackson, and Christopher B Little (*Raymond Purves Bone and Joint Research Laboratories, Sutton Arthritis Research Laboratories;Kolling Institute of Medical Research, Institute of Bone and Joint Research, University of Sydney, Royal North Shore Hospital, St. Leonards, NSW, Australia*)

2P-23 Development of ELISA measurement for urinary 3-hydroxyproline containing peptides and its preliminary application to community healthy persons and cancer patients
Yasutada Imamura, Junichi Saito, Joji Itoh, Shigeo Matsuyama, Akie Maruta, Toshihiko Hayashi, Chu Sato, Norihito Wada, Kazuo Kashiwazaki, Yutaka Inagaki, Tetsu Watanabe, Yuko Kitagawa and Isao Okazaki (*Kogakuin University; Keio University; Inagi Municipal Hospital, Inagi; Itoh Co. Ltd.; National Defense Medical College; Teikyo Heisei University; Sato Clinic, Ebina City Medical Association; KKR Tachikawa Hospital; Tokai University; International University of Health and Welfare, Japan*)

2P-24 Insulin–like growth factor binding protein-related protein 1 (IGFBP-rP1/TAF) synergistically modulates tumor cell adhesion with laminin-332 (laminin-5)
Eriko Komiya, Marii Ise, Yuichiro Sato, Shouich Higashi, Kaoru Miyazaki (*Yokohama City University, Japan*)

2P-25 Effects of nicotine and lipopolysaccharide on the expression of MMPs, PAs, and their inhibitors in human osteoblasts
Takayuki Kawato, Tomoko Katono, Hideki Tanaka, Masafumi Motohashi, Masao Maeno (*Nihon University, Tokyo, Japan*)

2P-26 Immobility-induced cartilage degeneration differed at three specific areas
Akira Ando, Yoshihiro Hagiwara, Eiichi Chimoto, Yoshito Onoda, Hideaki Suda, Eiji Itoi (*Tohoku University, Sendai, Japan*)

2P-27 The role of type I collagen in full-thickness articular cartilage repair
Mitsuhiko Kubo, Tomohiro Mimura, Kazuya Nishizawa, Susumu Araki, Shinji Imai, Yoshitaka Matsusue (*Shiga University of Medical Science, Shiga, Japan*)

2P-28 Effects of cyclic compression on human synovium-derived cells.–analysis of histology and time related changes of gene expression–
Yuutetsu Akamine, Ken Nakata, Takashi Kanamto, Yasuhiro Take, Hideyuki Kouda, Kazunori Shimomura, Kenji Kakudo, Hideki Yoshikawa (*Osaka University, Osaka, Japan; Osaka Dental University, Osaka, Japan*)

2P-29 Molecular profiles of basement membranes during early stages of mouse embryogenesis
Sugiko Futaki, Itsuko Nakano, Ri-ichiroh Manabe, Ko Tsutsui, Noriko Sanzen, Yoshikazu Sado, Kiyotoshi Sekiguchi (*Osaka University, Osaka; RIKEN, Yokohama, Shigei Medical Research Institute, Okayama, Japan*)

2P-30 *Xenopus* dicalcin, a novel mediator of sperm-egg interaction in the extracellular egg-coating membrane in *Xenopus laevis* eggs
N. Miwa, M. Ogawa, Y. Hiraoka, K. Takamatsu, and S. Kawamura (*Toho University; Kitasato University; Keio University; Osaka University, Japan*)

2P-31 Sustained activation of β1-integrins induces proliferative arrest or apoptosis in fibroblasts
Masaki Matsumura, Mayu Eguchi, Toshiyuki Owaki, Fumio Fukai (*Tokyo University of Science, Chiba, Japan*)

2P-32 Promotion of PDGF-dependent cell proliferation through β1-integrin activation
Tatsuya Takai, Toshiyuki Owaki and Fumio Fukai (*Tokyo University of Science, Chiba, Japan*)

2P-33 Chemosensitization of malignant tumor cells to anticancer drugs through β1-integrin activation
Mai Kobayashi, Miyoko Komatsu, Toshiyuki Owaki, Fumio Fukai (*Tokyo University of Science, Chiba, Japan*)

2P-34 The expression and the distribution of epiplakin on wound healing
Kazushi Ishikawa, Hideaki Sumiyoshi, Mizuki Goto, Hirokazu Kitamura, Hidekatsu Yoshioka, Sakuhei Fujiwara (*Oita University, Oita, Japan*)

2P-35 The study of fibrogenesis using a wound healing model
Hideaki Sumiyoshi, Noritaka Matsuo and Hidekatsu Yoshioka (*Oita University, Oita, Japan*)

2P-36 Monitoring of pressure ulcer detecting ECM fragments from wound surface
Chika Orii, Yusuke Murasawa, Naoko Matsumoto, Masahiko Yoneda, Zenzo Isogai (*National Center for Geriatrics and Gerontology, Obu, Aichi, Japan, [2]Aichi Prefectural College of Nursing and Health, Nagoya, Aichi, Japan*)

Memo

Opening Lecture

Revisiting of Collagen-specific Molecular Chaperone Hsp47: Fate of Procollagen with or without Hsp47

Kazuhiro Nagata*, Yusaku Masago and Yoshihito Ishida
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Hsp47 is a collagen-specific molecular chaperone in the ER, and has a pivotal role in the folding and/or assembly of procollagens. In Hsp47-disrupted mice, was observed the impairment of triple helix formation, secretion, propeptide processing and fibril formation of type I and/or type IV collagens, which resulted in embryonic lethality of mice at 10.5 dpc. Recently, we have succeeded in making conditional knockout mice of Hsp47 in the cartilage, and observed the disorder in cartilage and bone formation, causing the death of mice just after birth, which suggests that Hsp47 is also essential for proper folding of type II collagen.

In the absence of Hsp47, type I procollagen accumulated in the ER as detergent-insoluble aggregates. We found that such misfolded procollagen is degraded by autophagy, not by ER-associated degradation (ERAD), which was shown by lysosomal inhibitors and by knockdown of Atg5 in Hsp47-null cells as a model of collagen misfolding. This was also true in collagen misfolding diseases in general. In mov13 cells where only α2 chain of type I collagen is produced, collagen triple helix was correctly formed and secreted by transfection of wild type α1 chains. However, transfection of α1 chains that make trimers with α2 chains without making correct triple helices caused the degradation of misfolded collagen by autophagy, while transfected α1 chains that do not make trimers are degraded by ERAD. Thus, procollagens accumulated in the ER as aggregates and those without making trimers are degraded by different pathways.

Dr. Nagata's Biosketch

Dr Kazuhiro Nagata has had a lifelong affiliation with Kyoto University. After earning a bachelor's degree in physics there in 1971, he worked as a research fellow at Morinaga's Central Research Institute for 6 years. He returned to Kyoto University in 1976 for his graduate studies, and he has continued there ever since. After completing his PhD in biophysics in 1979, he joined the Chest Disease Research Institute, first as lecturer and later as Professor and Chairman of the Department of Cell Biology of that institute. From 1984 to 1986, while still a lecturer, Dr Nagata held an additional post as Visiting Associate in the Laboratory of Molecular Biology at NIH's National Cancer Center.

In 1998 Dr Nagata was named Professor and Chairman of the Department of Molecular and Cellular Biology at the Institute for Frontier Medical Sciences of Kyoto University, a position that he continues in today. In the last few years he has augmented his teaching duties with Visiting Professorships at the University of the Air and at Akita University.

Dr Nagata has a long record of service to the professional scientific community. He served as president of the Japan Society for Cell Biology and the Cell Stress Society International, and he has served in various editorial capacities for a number of scientific journals.

Keynote Lecture I

Cancer Stem Cells and their Niche

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The unique characteristics of stem cells, specifically pluripotency and self-renewal, are critical for sustaining the lifelong functionality of organs. Stem cells reside in a special microenvironment called the niche. Stem cells interact with the niche via adhesion molecules and exchange molecular signals that maintain the specific features of stem cells. A better understanding of the nature of stem cells and their niches is expected to provide an alternative approach to the treatment of cancer in clinical practice. It has been suggested that tumor tissue contains a type of stem cell referred to as a cancer stem cell. Interestingly, there are a number of molecules that are commonly expressed in normal and cancer stem cells that lead to different phenomena depending on the local conditions. In this presentation, the hematopoietic system is used as an example to show how stem cells interact with different niches. The regulatory mechanisms of two kinds of bone marrow niche, osteoblastic and vascular, are covered. Furthermore, the involvement of the niche in cancer stem cell regulation, tumor invasion and metastasis, and its response to oxidative stress will be presented, and novel therapeutic approaches involving the interactions between cancer stem cells and their niches will be proposed

Dr. Suda's Biosketch

Dr. Toshio Suda was graduated from the Yokohama City University School of Medicine with an MD degree in 1974. After serving residencies at Jichi Medical School and Kanagawa Children's Medical Center from 1974-1977, he took a position in 1978 as a Research Associate in the Division of Hematopoiesis, Institute of Hematology, at Jichi Medical School. In 1982 he carried his research work to the United States as a Research Associate in the laboratory of Dr Makio Ogawa at the Medical University of South Carolina.

In 1984 Dr Suda returned to Japan and to Jichi Medical School to take a position as Assistant Professor in the Division of Hematopoiesis, Institute of Hematology. He was promoted to the rank of Associate Professor at the same institution in 1991. In 1992, he accepted an appointment as Professor of the Department of Cell Differentiation, Institute of Molecular Embryology and Genetics, at Kumamoto University School of Medicine. He remained in Kumamoto for 10 years, until 2002, at which time he moved to Tokyo to accept the position he currently holds as Professor of Developmental Biology at the Sakaguchi Laboratory of the Department of Medicine at Keio University.

Dr Suda is a distinguished scholar who has won the Baelz Prize twice, first in 1991 and then again in 2003. He has advanced the profession of hematology not only with a number of outstanding publications, but also by his active participation on a three editorial boards. We are honored to have Dr Suda with us at the Yokosuka Science Festa.

Keynote Lecture II

Fibrillin-rich Microfibrils; an Instructive View from Outside the Cell

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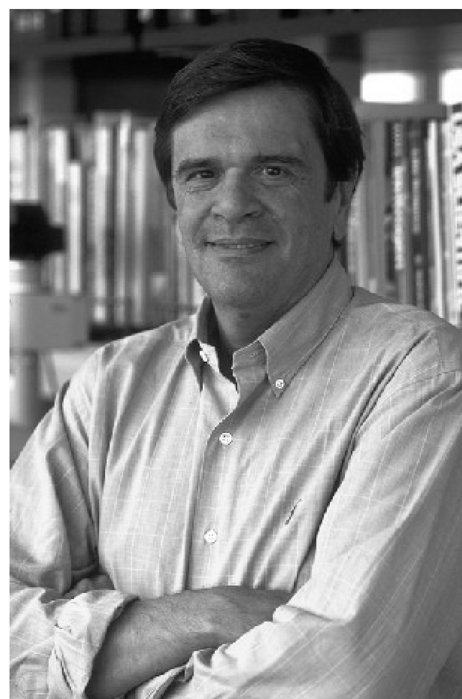
Fibrillins 1 and 2 are large cysteine-rich glycoproteins that serve two key physiological functions; the function of a structural support that imparts tissue integrity, and the function of a regulator of signaling events that instruct cell performance[1]. The structural role of fibrillins is exerted through the temporal and hierarchical assembly of microfibrils and elastic fibers, whereas the instructive role reflects the ability of fibrillins to sequester TGFβ and BMP complexes in the extracellular matrix. Characterization of fibrillin mutations in human patients and genetically engineered mice has demonstrated that perturbation of either function manifests in disease[2]. Early findings correlated promiscuous TGFβ signaling with impaired lung development, mitral valve prolapse, muscle hypoplasia, and aortic aneurysm in *Fbn1* mutant mice that replicate the progressively severe form of Marfan syndrome (MFS). Loss of fibrillin-1 production in a mouse model on neonatal lethal MFS has more recently implicated improper MAPK signaling in TGFβ-driven disease progression, perhaps as a result of perturbed cell-matrix interactions. Additional insights into the instructive role of microfibrils have been gathered from the study of skeletal manifestations in *Fbn* mutant mice. Previous characterization of syndactyly in *Fbn2*-null mice indicated that fibrillin-2 microfibrils are positive regulators of BMP7 signaling in the developing autopods and that, despite robust expression, fibrillin-1 cannot compensate for loss of fibrillin-2 during this particular developmental process. Ongoing investigations on bone remodeling have shown that *Fbn2*-null osteoblast cultures fail to mineralize due to heightened TGFβ signaling, whereas *Fbn1*-null osteoblasts mature properly even though TGFβ signaling is enhanced because of increased availability of otherwise matrix-bound BMPs. Collectively, these studies suggest that the relative composition of fibrillin-rich microfibrils imparts contextual specificity to TGFβ and BMP signaling by either concentrating the ligands locally so as to regulate cell differentiation within a spatial context during organ formation (positive regulation) or by restricting their bioavailability so as to modulate cell performance in a timely fashion during tissue remodeling/repair (negative regulation).

[1] Ramirez F., Sakai L.Y., Dietz H.C. and Rifkin D.B. (2004) *Physiol. Genomics*19, 151-154.
[2] Ramirez F. and Dietz H. C. (2007) *Curr. Opin. Genet. Dev.* 17, 252-258.

Dr. Ramirez's Biosketch



After earning an MA in Liberal Arts in 1965 at the Liceo Classico Garibaldi in Palermo, Italy, Dr Francesco Ramirez then studied genetics and embryology at the University of Biological Sciences, also in Palermo. He was graduated with a PhD from that institution in 1969, and he remained there for the next three years as a research associate in the Department of Genetics and Comparative Anatomy until 1972.

An appointment as a research associate drew him to the United States. He continued his research work in the Department of Human Genetics and Development, Columbia University, until 1979, at which time he became an assistant professor in the departments of Obstetrics/Gynocology and Biochemistry at Rutgers Medical School. He was promoted to the rank of Associate Professor in 1981, and he continued his research and teaching at Rutgers Medical School until 1986.

After holding a professorship in the Department of Microbiology and Immunology at the SUNY Health Science Center at Brooklyn for three years, Dr Ramirez continued his career at the Mr Sinai School of Medicine in New York. At various times during his tenure there from1989-2002, he held several distinguished professorships, he served as interim chair of the Departments of Biochemistry and

Molecular Biology, and he served as the Dean of Research at that institution. In 2002 he added two new paths to his career by accepting the St. Giles Chair of Genetics at Chief Scientific Officer Hospital for Special Surgery and by accepting professorships in several of the departments of the Weill Medical College of Cornell University.

In 2005 career opportunities arose for Dr Ramirez in New Jersey, and he accepted an endowed professorship and directorate of the Child Health Institute of New Jersey as well as a professorship at the UMDNJ-Robert W. Johnson Medical School in New Brunswick, New Jersey. After continuing there for three years, Dr Ramirez returned to the Mount Sinai School of Medicine, where he currently is the Amy and James Elster Professor, an appointment that encompasses both the Department of Pharmacology and Systems Therapeutics and the Department of Medicine.

The outstanding contributions of Dr Ramirez earned him the Antoine Marfan Award in 1993 and numerous MERIT awards for Scientific Excellence from the NIH. He has served on a wide variety of editorial boards and advisory committees. His research legacy is long and distinguished, and over his career he has published over 260 peer-reviewed papers spanning topics in medicine, physiology, biochemistry, and molecular biology.

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Memo

Keynote Lecture III

Modeling Normal Mammary Gland to Understand Breast Cancer: The Yin and Yang of the ECM and ECM-Degrading Enzymes

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The ability of epithelial cells to organize into polarized three dimensional (3D) structures correlates closely with their normal or malignant status. In a versatile model of morphogenesis, we have shown in past studies that inhibiting a number of key signaling pathways in human breast cancer cells grown in laminin-rich ECM gels leads to 'reversion' of the malignant phenotype. The resulting growth-arrested polarized structures resemble normal 'acini' and have helped us to understand how polarity of the normal gland structures may be disrupted as breast cancer progresses. We now have used two additional models to study signaling integration in single mammary cells and also mammary organoids, where we have modeled mammary invasion into the stromal collagen during branching morphogenesis to learn how tumor cells usurp these pathways to invade. We provide additional proof for our contention that all signaling pathways must directly or indirectly communicate to maintain homeostasis. We show how biochemical and mechanical signaling from the ECM, the ECM receptors and MMPs interconnect with cell and tissue architecture in reciprocal and reiterating loops in tissue specificity. It is precisely this integration and reciprocity that must get disrupted for the tumor to succeed.

Dr. Bissell 's Biosketch

MINA J. BISSELL, Ph.D.
Distinguished Scientist, Life Sciences Division
Faculty, Comparative Biochemistry, UC Berkeley
Ernest Orlando Lawrence Berkeley National Laboratory
Berkeley, California

Dr. Bissell is a pioneer in the area of the role of extracellular matrix (ECM) and microenvironment in regulation of tissue-specific function with special emphasis in breast cancer, where she has changed some established paradigms. She earned an A.B. with honors in chemistry from Harvard/Radcliffe College and a Ph.D. in bacterial genetics from Harvard University. She joined the Lawrence Berkeley National Laboratory in 1972, became Director of Cell & Molecular Biology in 1988, and was appointed Director of all of Life Sciences in 1992. Upon stepping down as the Life Sciences Division Director, she was named Distinguished Scientist. She is also the OBER/DOE Distinguished Scientist Fellow in Life Sciences.

Dr. Bissell has authored more than 300 publications, is member of 5 international scientific boards, and is on the editorial board of a dozen scientific journals, including Science magazine. She has given more than 90 'named and distinguished' lectures. Her awards include the Lawrence Award and medal, the Mellon Award from the University of Pittsburgh, the Eli Lilly/Clowes Award from AACR, the first "Innovator Award" of the US DOD for breast cancer research, the Brinker Award from Komen Foundation, the Discovery Health Channel Medical Honor and medal, the H. Lee Moffitt Cancer Center Ted Couch Lectureship and Award, the Pezcoller Foundation–AACR International Award for Cancer Research, the 2008 Excellence in Science Award from FASEB. She has been awarded the 2008 Mina J. Bissell Award by the University of Porto and the 2008 American Cancer Society's Medal of Honor for Basic Research Award.

Dr. Bissell was elected as a Fellow of AAAS, the Institute of Medicine of the National Academies, the American Academy of Arts and Sciences, and the American Philosophical Society. She served as President of the American Society of Cell Biology and the International Society of Differentiation. She has received honorary doctorates from Pierre & Marie Curie University in Paris and the University of Copenhagen.

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Symposium I:

S1-1

Customization of the Basement Membrane during Embryonic Development

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A hallmark of the extracellular matrix (ECM) is its diversity of molecular composition. Individual cell types have their own customized extracellular environment with a distinct molecular composition. Basement membrane is a thin sheet of ECM that underlies epithelial cells and surrounds muscle cells, blood vessels, and peripheral nerves. Basement membrane serves as a physical as well as functional interface of epithelial-mesenchymal interactions, thereby transducing signals in both directions (from the epithelium to the mesenchyme and vice versa) to orchestrate a complex series of organogenetic processes. To better understand the molecular entities of the customized extracellular environment, we set out to comprehensively localize >40 basement membrane proteins in mouse embryos at different embryonic stages [1]. We converted the immunohistochemical data to digital images and compiled them into a database in which individual images can be browsed on the web at desired magnification (http://www.matrixome.com/bm/). Our results are consistent with the concept that ECM composition is regulated developmentally and such customization of ECM composition plays an important role in organogenesis and cell fate determination.

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S1-2

Laminin-511 plays a key role in dermal stem cell development and hair formation

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Hair formation requires communication and cooperation between the epithelial and dermal layers of the skin but how this occurs is not fully understood. Here we show that the basement membrane protein laminin-511, located at the interface of epithelial and dermal layers, orchestrates this communication. Initially we found that laminin-511 deficient mice completely lacked hair formation. However, introduction of purified laminin-511 into the skin of these mice dramatically triggered the restoration of fully formed hair. In studying the mechanism of this process, we found laminin-511 induced dramatic changes in the development of the collection of dermal stem cells known as the dermal papilla (DP). DP from laminin-511 null skin showed multiple defects during development, most notably a lack of expression of the key morphogen noggin. This led to a lack of sonic hedgehog (Shh) expression in laminin-511 deficient mouse skin. DP cells from laminin-511 null skin also showed defective formation of primary cilia, which are small microtubule based organelles involved in Shh signaling. We found that addition of exogenous purified laminin-511 restored primacy cilia in laminin-511 null DP. We are currently trying to identify key hair morphogenic domains on the large laminin-511 molecule. While deletion of the heparan binding G45 domain on the α3 chain appears to have no effect on laminin-511's hair promoting activity, the integrin binding G1-3 domain of laminin-511 was absolutely essential. Consistent with this, antibody inhibition or genetic deletion of α3β1 integrin (laminin-511's receptor) both reduced DP primary cilia and inhibited hair formation. In summary, we have shown that laminin-511 is an early epithelial hair induction signal which binds to α3β1 integrin on the dermal papilla, stimulates primary cilia development, and sets off a reciprocal noggin-Shh signaling loop between the follicular epithelium and the DP which ultimately leads to hair follicle elongation. Further studies are needed to pinpoint the minimal required sequences for laminin-511's hair promoting activities and to determine whether any forms of alopecia might respond to laminin-511.

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S1-3
Developmental Studies of *Drole*, *Drosophila* Type XV/XVIII Collagen

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Type XV/XVIII collagens, components of basement membranes (BMs), form a distinct subgroup called Multiplexin among the collagen family, characterized by multiple glycosaminoglycan attachment sites and by the central triple helical region with multiple interruptions flanked by N-terminal thrombospondin type I repeat and C-terminal endostatin domain [1]. They have been conserved widely among the metazoans and are suggested to be important in skeletal muscle stability [2], cell migration, axon guidance [3], but the underlying mechanism is unknown yet. To further explore its biological function, we examined *Drosophila* type XV/XVIII collagen homologue, which we named "*Drole*" (DROsophila coLlagen with Endostatin). We identified two major forms of transcripts, generated from distantly located promoters. *In situ* hybridization using specific probes on whole embryos exhibited an accumulation in the central nervous system. Immunostaining with anti-*Drole* exhibited a unique segmental expression pattern in a subset of cells in the central nervous system, as well as in the peripheral nervous system in the developing embryos. Loss of function mutants displayed multiple defects such as low surviving ratio, neural defect and an altered BM ultrastructure, which may mimic the deposits observed in the retina of *Col18a1-/-* mice [4]. Overall, our results indicate an important role for *Drole* during early embryogenesis.

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S1-4
Peptide-Chitosan Membranes Can Mimic the Biological Activities of Laminin α1 LG4 Module

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Keywords: Cell adhesion, Chitin/chitosan, Laminin, Integrin, Syndecan, Peptide

Objective: Laminin α1 chain LG4 module is multifunctional and binds to syndecans and integrin α2β1 via AG73 (RKRLQVQLSIRT) and EF-I (DYATLQLQEGRLHFMFDLG) sites, respectively. We previously reported that AG73 site is necessary for cell attachment and that the EF-1 site is for cell spreading activity. Here, we conjugated the AG73 and EF1zz (ATLQLQEGRLHFXFDLGKGR, X: Nle) peptides on a chitosan membrane in various ratios to mimic the multifunction of recombinant laminin α1 chain LG4 module
Results: The AG73-chitosan membrane promoted strong cell attachment through syndecan with membrane ruffling and the EF1zz-chitosan membrane promoted integrin-mediated cell adhesion with well-organized actin stress fibers to the human dermal fibroblasts. When both AG73 and EF1zz were conjugated on a chitosan membrane with 1:9 molar ratio, the mixed-peptide chitosan membrane promoted the strong cell attachment and spreading similar to that fibroblasts on the recombinant LG4 protein. Well organized actin stress fibers and vinculin accumulated focal contacts were observed in the cells attached on the AG73:EF1zz (molar ratio = 1:9)-chitosan membrane. Further, AG73:EF1zz (molar ratio = 1:9)-chitosan membrane promoted neurite outgrowth to PC12 cells as LG4 protein.
Conclusion: The mixed peptide-chitosan membrane interacts with both syndecans and integrin α2β1, and can mimic the biological activities of the multifunctional LG4 protein. The molar ratio of AG73:EF1zz = 1:9 on the chitosan membrane is a critical point to get the synergistic effect of the integrin- and syndecan-mediated cell attachment. The mixed peptide-chitosan approach is useful to develop a multifunctional biomaterial for cell and tissue engineering.

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Symposium II:

S2-1

Integrated Approach toward Bone and Joint Diseases using Human and Mouse Genetics

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One of the challenges in the "post-genome sequence" era is to utilize the genome information to the research of diseases, in particular, common polygenic diseases. There are many "common" diseases, life-style associated diseases in bone and joint, including osteoarthritis (OA), rheumatoid arthritis, lumbar disc disease and osteoporosis. These diseases are serious concern for the world health and economy, as exemplified by the WHO campaign of "Bone and Joint Decade" (2001-2010); however, most of their etiology are unknown and their pathogenesis are unclear, resulting in lack of effective and fundamental treatment.

Recent advance in molecular genetics and genome medicine has revealed that genetic factors play a critical role in etiology and pathogenesis of these common bon and joint diseases. Identification of the genetic factors (i.e., susceptibility genes) is the first, mandatory step toward the innovative treatment and "order-made" medicine. To identify susceptibility genes, we have been performing systemic large-scale association studies followed by linkage–disequilibrium mapping in various diseases. Though these projects, we have found genes for OA, *ASPN* [1], *GDF5* [2] and *DVWA* [3], which are supported by functional evidence and replication in different ethnic populations, as well as genes for lumbar disc disease, *CILP* [4], *COL11A1* [5], *TBSP2* [6] and *MMP9* [6]. Identification of these genes gave us many insights into the molecular mechanism of the diseases, which would lead to the logical invention of innovative treatment.

In this talk, I explain the detail of our approach for the common diseases, using OA study as an example.

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S2-2

The New Paradigm for OI Genetics and the Functional Effects of Recessive CRTAP and P3H1/LEPRE1 Mutations

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Osteogenesis Imperfecta is a well-known autosomal dominant bone dysplasia caused by mutations in either of the genes that encode type I collagen, COL1A1 or COL1A2. Most clinically significant cases of dominant OI are caused by point mutations that result in substitutions for glycine residues in the collagen helical region. More recently, the genes responsible for recessive OI have been identified; these cases comprise 5-7% of OI. Recessive OI is caused by deficiency of either of two proteins, CRTAP and P3H1, involved in the prolyl 3-hydroxylation of types I, II and V and components of the ER 3-hydroxylation complex along with cyclophillin B. The phenotype of recessive OI types VII and VIII ranges from severe to lethal, similar to types II/III OI, except with white sclerae. A founder mutation from West Africa was identified in the *LEPRE1* gene, with a prevalence of >1% in contemporary West-Africans and 1:200-300 in African-Americans. Recessive OI is characterized by the loss of CRTAP or P3H1 message and protein and lack of 3-hydroxylation of type I collagen. In contrast, collagen from these probands is overmodified and their collagen secretion is increased. P3H1 and CRTAP protein is absent or minimally detectable in *CRTAP*-null or *LEPRE1*-null fibroblasts, despite normal levels of *LEPRE1* or *CRTAP* transcripts in these cell lines, respectively. This suggests that CRTAP and P3H1 are mutually protected in the ER prolyl-3-hydroxylation complex. Transfection of full length *CRTAP* expression constructs into *CRTAP*-null cells can rescue P3H1 protein and reduce overmodification of type I collagen. Protein degradation pathways were also investigated using proteinase inhibitors. Examination of ER stress in proband fibroblasts showed increased expression of *IRE1*, *BiP* and *EDEM1*, while HSP47 protein levels were increased to help relieve the ER stress burden. Loss of the 3-hydroxylation complex and ER stress adaptation may contribute to the recessive OI phenotype.

S2-3

A new categorized *COL3A1* mutation detected by genome scanning with vascular Ehlers-Danlos syndrome (vEDS)

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Keywords: vascular Ehlers-Danlos syndrome, *COL3A1*

Objective: Vascular type of Ehlers-Danlos syndrome (vEDS), also known as EDS type IV (NIM#130050) is a life-threatening autosomal dominant inherited disorder of connective tissue, caused by mutations of the *COL3A1* gene. Vascular EDS causes severe fragility of connective tissues with arterial and intestinal ruptures and complications associated with both surgical and radiological treatment. The genetic testing of *COL3A1* is important to diagnose vEDS. After making a positive diagnosis of *COL3A1*, the establishment of a network among medical specialists to perform a long-term follow-up for vEDS may help to improve the management of vascular and visceral complications.

Case: We describe a 20-year-old Japanese male with both pneumothorax and cervical artery dissections. His brother suffered sudden death at of 25 years of age due to an aortic rupture.

Results: The sequencing of cDNA containing the triple-helical domain of *COL3A1* from cultured skin fibroblasts obtained from the patient showed no nucleotide abnormalities. However, a DNA analysis of the *COL3A1* gene revealed a nonsense mutation (c.2491C>T; Gln831Stop). A possible reason for this discrepancy may be due to nonsense-mediated mRNA decay and needs to be discussed.

Conclusion: This is a first report with a nonsense *COL3A1* mutation in individuals who exhibited symptoms of vEDS. We would therefore like to stress that a genomic DNA analysis of *COL3A1* should be performed in all patients when there is a strong suspicion of vEDS despite negative findings in a cDNA analysis of *COL3A1*.

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Symposium III

S3-1

Insights into Aggrecan and Collagen Degradation using Knockin Mice

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Accelerated catabolism of aggrecan and type II collagen is a feature of cartilage destruction in arthritis. ADAMTS-5 is the major aggrecanase in mouse cartilage and MMP-13 is the major cartilage collagenases in several species including humans. One approach to studying the activity of these enzymes is to mutate the aggrecan and collagen II substrates, rendering them resistant to aggrecanases or collagenases, respectively. We have generated the Bailey mouse which is resistant to collagenase cleavage in the triple helical region of type II collagen, and the Jaffa mouse which is resistant to ADAMTS cleavage in the aggrecan interglobular domain.

Degradation of fibrillar collagens is initiated by collagenase cleavage at a highly conserved site in the triple helix. We mutated the mouse *col2a1* gene to change amino acids PQG$^{775}\downarrow^{776}$LAG to PPG$^{775}\downarrow^{776}$MPG in collagen II. Bailey collagen II is resistant to *all* collagenases. The Jaffa mouse whose aggrecan is resistant to ADAMTS cleavage was made by mutating the *agc1* gene to change amino acids EGE$^{373}\downarrow^{374}$ALG to EGE$^{373}\downarrow^{374}$NVY. Aggrecanases do not recognise this sequence as a cleavage site.

The enzyme-resistant Jaffa and Bailey mice offer distinct advantages over the ADAMTS-5 and MMP-13 null mice for studying aggrecanolysis and collagenolysis, because the consequences of targeted mutations in the aggrecan or collagen substrates are not confounded by the effects of null mutations in enzymes, on other substrates, or compensation by other enzymes. We have compared the extent of aggrecan loss and cartilage erosion in inflammatory arthritis, between Jaffa and Bailey mice. This study will identify the contributions of aggrecanases and collagenases to key phases of arthritic disease by determining whether ablation of one or both activities can modulate disease initiation and/or disease progression. These results will identify whether single or combination therapies are required for the management of arthritic disease.

S3-2

Induction of aggrecanases in cartilage by fibronectin fragments is mediated by α5β1 integrin and TLR4.

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Abundant extracellular matrix (ECM) components in cartilage are important in maintaining the joint function and the degradation of the ECM is the main cause of osteoarthritis (OA). This process progresses upon ageing accompanied by an increased production of matrix-degrading metalloproteinases. While tissue injury, mechanical loading, inflammatory cytokines and growth factors are considered to stimulate cartilage catabolism, endogenous factors that sustain the prolonged production of destructive proteinases in adult cartilage have not been clearly defined. A series of recent studies demonstrating that ECM components, when degraded by proteinases, reveal cryptic biological functions including induction of matrix metalloproteinases (MMPs) led us to consider the role of fibronectin fragments (FNfs) in cartilage degradation, because the synthesis of FN is elevated and its fragments are found in OA cartilage. We identified two key regions in FN located in type III repeats, III (8-10) and III (14), which induced cartilage aggrecan degradation. The degradation of aggrecan was primarily due to the elevated activity of aggrecanases. The fragment III (8-10) contains two integrin binding sites and the III (14) has an integrin binding site and a heparin binding site. The action of III (8-10) region is mediated through α5β1 integrins and that of III (14) through TLR4. Fragments III (8-10) and III (14) acted synergistically with each other and with IL-1 or TNFα. These studies suggest the complexity of inductive stimuli that cause cartilage matrix catabolism.

S3-3

Studies from TACE Mutant Mice

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The TNFα converting enzyme (TACE/ADAM17) is involved in the proteolytic release of the ectodomain of diverse cell surface proteins with critical roles in development, immunity and hematopoiesis. As the perinatal lethality of TACE-deficient mice has prevented an analysis of the roles of TACE in adult animals, I took advantage of the Cre-LoxP system and generated conditional *Tace*-deficient mice. Using this mutant line, I previously showed that TACE- inactivation in myeloid cells or temporal inactivation offers strong protection from endotoxin shock lethality in mice by preventing increased TNFα serum levels [1]. To gain further insight on the roles of TACE in vivo, I next generated a mutant line in which a Cre recombinase gene is expressed under the control of a *Sox9* promoter [2]. SOX9 is an essential transcription factor for skeletal development and is expressed in all osteo-chondroprogenitor cells as well as in many other organs, including the pancreas, heart, lung, brain and skin, but not in hematopoietic cells. These mutant mice survived up to 9-10 months, but exhibited severe growth retardation as well as skin defects and infertility. The analysis of the skeletal system revealed shorter long bones and prominent bone loss, characterized by an increase in osteoclast and osteoblast activity. In addition, these mice exhibited hypercellularity in the bone marrow and extramedullary hematopoiesis in the spleen and liver. Flow cytometric analysis of the bone marrow cells showed a sharp increase in granulopoiesis and in the population of c-Kit-1$^+$ Sca-1$^+$ lineage$^-$ cells, and a decrease in lymphopoiesis. Taken together, these observations reveal unexpected involvements of TACE in normal growth, skin development, bone metabolism and hematopoiesis, and therefore further underscore the importance of ectodomain shedding *in vivo*.

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S3-4
The role of ADAM28 in cancer cell proliferation and progression

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ADAMs (a disintegrin and metalloproteinases) are new gene family of proteins with sequence similarity to the reprolysin family of snake venomases and share the metalloproteinase domain with matrix metalloproteinases (MMPs). Recent studies suggest that ADAMs are related to both cancer development and progression [1]. We have previously demonstrated that ADAM28 is selectively overexpressed by carcinoma cells in human invasive breast carcinomas and involved in breast carcinoma cell proliferation through cleavage of insulin-like growth factor binding protein-3 (IGFBP-3) [2]. More recently, we have established an experimental mouse model to monitor cancer cell metastasis by *in vivo* bioluminescence imaging. In this method, transplanted cells expressing luciferase and green fluorescent protein (GFP) can be readily detected within the tissues of live animals after administration of luciferin. We found that injection of ADAM28-expressing carcinoma cells, which express luciferase and GFP, via tail vein show metastasis in the lungs and minute metastasis foci are readily detected by immunostaining of GFP. In mice receiving the ADAM28 siRNA/ atellocollagen complex, this metastasis was inhibited by 80-90%, suggesting that ADAM28 plays a key role in cancer cell metastasis. To explore functions of ADAM28 in cancer cell invasion and metastasis, we screened interacting proteins for ADAM28 by yeast two-hybrid system, and identified von Willebrand factor (vWF) and connective tissue growth factor (CTGF) as candidate proteins. In this symposium, we will also discuss ADAM28-induced cancer cell invasion and metastasis by cleavage of vWF and/or CTGF.

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S3-5
Gene transfer of ADAMTS1 induced apoptosis in endothelial cells and inhibited tumor growth

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It has been argued whether ADAMTS1 (a disintegrin and metalloproteinase with thrombospondin motifs1) has anti- or pro-angiogenic property. Here we examined the effect of gene transduction of ADAMTS1 with two constructs, full-length ADAMTS1 (full ADAMTS1) and catalytic domain-deleted ADAMTS1 (delta ADAMTS1) on endothelial cells. Conditioned medium derived from both full ADAMTS1- and delta ADAMTS1-transfectred cells increased the number of Annexin V-positive endothelial cells. Both conditioned medium induced caspase-3 activity. These conditioned medium inhibited endothelial cell survival and migration, and these effects were observed in endothelial cells but not in smooth muscle cells, skin fibroblasts and CHO-K1 cells. Both constructs also inhibited endothelial tube formation. Gene transduction of both full ADAMTS1 and delta ADAMTS1 significantly inhibited subcutaneous tumor growth while decreasing the number of tumor-induced blood vessels. Collectively, these results demonstrated the novel mechanism of anti-angiogenic property of ADAMTS1 and indicated for the first time the potential therapeutic use of ADAMTS1 for cancer dormancy.

Symposium IV:

S4-1

Generation and Characterization of Chondroitin Sulfate E-deficient Mice

Biochemical Characterization of the P3H1/CRTAP/CypB Complex as a Prolyl 3-Hydroxylase, a PPIase and a Molecular Chaperone

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The rough endoplasmic reticulum resident protein complex consisting of prolyl 3-hydroxylase 1, CRTAP and cyclophilin B can be isolated from chick embryos on a gelatin Sepharose column, indicating some involvement in the biosynthesis of procollagens. Prolyl 3-hydroxylase 1 modifies a single proline residue in the α-chains of type I, II and III collagens to 3(*S*)-hydroxyproline. The peptidyl-prolyl *cis-trans* isomerase activity of cyclophilin B was previously shown to catalyze the rate of triple helix formation. The cyclophilin B in the complex shows peptidyl-prolyl cis-trans isomerase activity and the P3H1/CRTAP/CypB complex has another important function: It acts as a chaperone molecule when tested with two classical chaperone assays: The P3H1/CRTAP/CypB complex inhibits the thermal aggregation of citrate synthase and is active in the denatured rhodanese refolding and aggregation assay. The chaperone activity of the complex is higher than that of protein disulfide isomerase (PDI), a well characterized chaperone. The P3H1/CRTAP/CypB complex also delays the in vitro fibril formation of type I collagen, indicating that this complex is also able to interact with triple helical collagen, and acts as a collagen chaperone. Human mutations in P3H1 and in CRTAP lead to a recessive form of Osteogenesis Imperfecta (OI) and a mutation in CypB in the American Quarter Horse leads to Hyperelastosis cutis (HC). The P3H1 null mouse shows low bone density, fragile skin and disorganized tendon fibrils. The disorganized tendon fibrils are also observed in the HC horse.

S4-2

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N-acetylgalactosamine 4-sulfate 6-*O*-sulfotransferase (GalNAc4S-6ST) transfers sulfate to position 6 of GalNAc($4SO_4$) residue of chondroitin sulfate to yield chondroitin sulfate E (CS-E). We identified cDNA of human GalNAc4S-6ST on the basis of amino acid sequences of the purified squid GalNAc4S-6ST. GalNAc4S-6ST is expressed in various adult mouse tissues including cerebellum, cerebrum, heart, kidney, liver, spleen, mesentery, and large intestine. Relatively higher proportion of CS-E was observed in liver, spleen, kidney, and small intestine. To investigate functions of GalNAc4S-6ST and CS-E, we generated GalNAc4S-6ST-null mice by homologous recombination. The GalNAc4S-6ST-null mice produced normal-sized litters, showed no abnormality in gross appearance. Alcian Blue positive cells in the small intestine were more sparse in GalNAc4S-6ST-null mice than in wild mice. CS-E disappeared systemically in the GalNAc4S-6ST-null mice, indicating that GalNAc4S-6ST is a sole sulfotransferase involved in the final sulfation step of CS-E. Because CS-E is found in bone marrow derived mast cells (BMMCs) as the major glycosaminoglycan attached to serglycin proteoglycan, we compared BMMCs derived from wild mice (wild-BMMCs) and those derived from the GalNAc4S-6ST-null mice (KO-BMMCs). Chondroitin sulfate (CS) synthesized by KO-BMMCs contained no CS-E unit and the chain length of the CS was slightly larger than CS synthesized by wild-BMMCs. Although the expression level of mRNA of mMCP-6, a major mast cell tryptase, in KO-BMMCs was nearly the same as the level in wild-BMMCs, mMCP-6 protein as well as tryptase activity of KO-BMMCs were markedly lowered than those of wild-BMMCs. Morphological observation of BMMCs by May Grünwald/Giemsa staining showed a tendency that KO-BMMCs had more empty vacuoles than wild-BMMCs. Observations of BMMCs by transmission electron microscopy supported such morphological difference. These observations suggest that CS-E may function in packaging active mMCP-6 in the granules and contribute to normal granule formation in BMMCs.

S4-3
Role of the Sulfation Pattern of Chondroitin Sulfate in its Neuritogenic Activities.

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Chondroitin sulfate (CS) is a representative sulfated glycosaminoglycan, which is covalently attached to a panel of core proteins to form proteoglycans (CSPGs), and is ubiquitously located in extracellular matrices and on cell surfaces in various tissues. CSPGs regulate diverse physiological phenomena such as cytokinesis, morphogenesis, and infections with viruses and bacteria. In particular, the pathologic functions of CS moieties of CSPGs as major axon growth-inhibitory molecules in the injured adult central nervous system (CNS) have attracted widespread attention, and prompted research aimed at overcoming their barrier effects on neuronal regeneration processes. Although axonal regeneration is indeed improved by the removal of CS moieties around lesion sites, CS does not always impede neurite outgrowth. For example, several CS preparations serve as stimulatory substrata for neurite outgrowth of cultured primary neurons.

The apparently contradictory actions of CS in the CNS are thought to be attributable to its structural diversity. CS is a linear polysaccharide that contains repeating disaccharide units consisting of glucuronic acid (GlcUA) and *N*-acetyl-D-galactosamine (GalNAc). The building blocks can be substituted with sulfate groups at various positions, thereby producing characteristic "sulfation codes". CS polysaccharides are divided into subclasses based on their disaccharide composition. The major CS subclasses found in mammalian tissues contain monosulfated disaccharide units, A [GlcUA-GalNAc(4-*O*-sulfate)] and C [GlcUA-GalNAc(6-*O*-sulfate)]. CS polysaccharides rich in A and C units are poorly permissive for neurite extension, probably reflecting the inhibitory nature of typical mammalian CS. In contrast, squid cartilage-derived CS-E polysaccharide possesses strong neuritogenic activity toward primary hippocampal neurons. CS-E is characterized by the predominant disulfated disaccharide E unit, [GlcUA-GalNAc (4,6-*O*-disulfate)]. We have recently demonstrated the involvement of a cell adhesion molecule, contactin-1, in CS-E-mediated neuritogenesis in a neuroblastoma cell line and primary hippocampal neurons. Our data provide the evidence for functional expression of CS through the CS receptor-mediated signaling pathway(s).

S4-4
Hyaluronan As A Key Adhesion Molecule In The Liver

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White cells must attach to the vessel wall before they can emigrate into tissues. A series of molecules including selectins and integrins are needed to allow for recruitment in most tissues. One major exception is the liver which does not use these molecules. Neutrophils will adhere in both sinusoids and post-sinusoidal venules but blocking integrins and selectins only blocks neutrophil adhesion in the post-sinusoidal vessels. Using a adhesion molecule screen, we discovered that hyaluronan is expressed most in liver and mainly in the sinusoids. Removal of hyaluronan or inhibition of its receptor CD44 prevented this recruitment. CD44 and hyaluronan were not sufficient alone to induce adhesion in sinusoids. We ultimately indentified a hyaluronan structure modifying protein as key to allowing for cell adhesion in sinusoids. Hyaluronan functions as a key molecule in neutrophil recruitment.

S4-5
Brevican determines specialization of the hyaluronan-binding nodal matrix assemblies at the large diameter nodes of Ranvier in the CNS

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Keywords: Brevican, Node of Ranvier, Hyaluronan, Tenascin-R, Phosphacan

Objective: Brevican is known to be an abundant extracellular matrix (ECM) component in the adult brain and a structural constituent of perineuronal nets (PNN). It also acknowledged as an extracellular component at the node of Ranvier in the CNS. To explore the role of brevican in the formation of the nodal matrix, immuno-histochemical staining was conducted in the facial nerve tract of wild-type and brevican-deficient mice.
Results: We herein show that brevican, tenascin-R (TN-R) and phosphacan are present at the nodes of Ranvier on myelinated axons with a particularly large diameter in the CNS. A brevican deficiency resulted in a reorganization of the nodal matrices, which was characterized by the shift of TN-R, and concomitantly phosphacan, from an axonal diameter-dependent association with nodes to an axonal diameter independent association. Supported by the co-immunoprecipitation results, these observations indicate that the presence of TN-R and phosphacan at nodes is normally brevican-dependent, while in the absence of brevican these molecules can also be recruited by versican V2. The versican V2 and Bral1 distribution was not affected, thus indicating a brevican-independent role of these two molecules for establishing hyaluronan-binding matrices at the nodes.

Conclusions: Our results revealed that brevican plays a crucial role in determining the specialization of the hyaluronan-binding nodal matrix assemblies in large diameter nodes.

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Symposium V:
S5-1
Significance of the dystrophin-glycoprotein complex that connects the cytoskeleton to the basal lamina.

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Duchenne muscular dystrophy (DMD) is a lethal muscle disorder caused by mutations of the *DMD* gene, which codes a 427 kDa spectrin-like cytoskeletal protein, dystrophin. Dystrophin is localized just beneath the sarcolemma, and binds F-actin *via* its N-terminal domain and beta-dystroglycan *via* its cysteine-rich domain, forming a vital link between the actin cytoskeleton and extracellular matrix in skeletal and cardiac muscle. The lack of dystrophin, accompanied by loss of dystrophin-binding proteins, weakens the muscle membrane, leading to degeneration of myofibers. There is no effective treatment for the disease at present, but exon skipping by antisense oligonucleotides is a novel method to restore the reading frame of the mutated *DMD* gene, and rescue dystrophin production.
We recently reported that systemic delivery of Morpholino antisense oligonucleotides targeting exon 6 and 8 of the canine *DMD* gene, efficiently recovered functional dystrophin proteins at the sarcolamma of dystrophic dogs, and improved performance of affected dogs without serious side effects (Yokota et al, Ann Neurol, in press). To optimize therapeutic antisense Morpholinos for more frequent mutations of the *DMD* gene, we designed 14 kinds of antisense Morpholinos targeting exon 51 of the mouse *DMD* gene, and injected them separately or in combination into the muscles of *mdx*52 mice, in which exon 52 has been deleted by a gene targeting technique. A combination of two Morpholinos showed an excellent restoration of sarcolemmal dystrophin in injected muscle. We, therefore, intravenously injected them into *mdx*52 mice at 7 times weekly. Two weeks after the final injection, dystrophin was expressed at the sarcolemma throughout the body, with an average of about 10-50% normal levels. This was accompanied by amelioration of dystrophic pathology, and improvement of contractile force of EDL, grip power test, and treadmill performance. This study provides a proof of concept for exon 51 skipping in the DMD animal model and that can be applicable to DMD patients.

S5-2

Zebrafish Integrin-linked Kinase is required in Skeletal Muscles for strengthening the Integrin-ECM Adhesion Complex.

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Deficiencies that influence the stability of skeletal muscle cells in humans often lead to various forms of muscular dystrophy (MD). MD is a group of autosomal recessively inherited muscular disorders characterised by hypotonia and weakness at birth or within the first few month of life. The disease is caused by deficiencies in components that facilitate and regulate the connection of the skeletal muscle plasma membrane with the basement membrane and the cytoskeleton. Identification of novel components involved in this connection increases our understanding on the cause of MD. By using the model system zebrafish we identified the focal adhesion protein integrin-linked kinase (Ilk) as a novel component involved in connecting the skeletal muscle plasma membrane with the actin cytoskeleton in vertebrates. Via laminins in the extracellular matrix (ECM) and integrin α7β1 in the skeletal muscle plasma membrane, Ilk connects via β-parvin the actin cytoskeleton. Loss of Ilk in zebrafish results in skeletal muscle instability and eventually detachment of the skeletal muscle cells from the myotendinous junction. This reveals Ilk as the link between the cytoskeleton and integrins in skeletal muscle cells. In addition, the laminin/integrinα7β1/Ilk/β-parvin complex acts in parallel with the dystrophin glycoprotein complex (DGC) in maintaining mechanical stability of skeletal muscles in zebrafish. Deficiencies in components of the DGC have been shown before to be involved in the cause of MD and therefore, Ilk is a potential new factor involved in MD. Interestingly, we identified an interaction between Ilk and the mechanical stretch sensor protein MLP (muscle LIM protein), suggesting a link between Ilk and the stretch sense response in skeletal muscles cells.

S5-3

Role of perlecan, a heparan sulfate proteoglycan, in skeletal muscle maintenance

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Mutations in extracellular matrix molecules such as collagen VI and laminin-2 cause myopathy phenotypes. Schwartz-Jampel syndrome (SJS), which is characterized by myotonia and mild chondrodysplasia, is caused by functional mutations in the perlecan gene. Perlecan is a large heparan sulfate proteoglycan expressed in all basement membranes. Perlecan binds extracellular matrix molecules, growth factors, and receptors and is implicated in many biological functions. We have created a mouse model for SJS by rescuing the perinatal lethality of perlecan-null mice by expressing recombinant perlecan specifically in cartilage under the control of a cartilage-specific promoter. The mutant mice survived and exhibited myotonic myopathy. The mutant mice also developed muscle degeneration and hypertrophy, and changes in the proportion of muscle fiber types. These results suggest that perlecan is required not only for adult muscular function, but also to maintain muscle homeostasis.

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S5-4
Pathogenic mechanisms in the Collagen VI Muscular Dystrophies

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Collagen VI is a widely expressed extracellular matrix protein and mutations in the collagen VI genes, *COL6A1*, *COL6A2* and *COL6A3*, cause muscular dystrophy, indicating an integral role for collagen VI in skeletal muscle. The collagen VI disorders, Bethlem myopathy and Ullrich congenital muscular dystrophy (UCMD) were thought to be separate disorders with distinct modes of inheritance; however, it is now clear that they form a spectrum of clinical severity from relatively mild muscle weakness to profound disability. Our studies, defining mutations and their effects on collagen VI assembly, are providing fundamental new information about the domains important for intracellular assembly of monomers, dimers and tetramers, and extracellular microfibrils. These studies now allow us to draw genotype-phenotype correlations and understand why apparently similar mutations produce very different clinical outcomes. The majority of the dominant structural mutations cluster towards the N-terminal end of the triple helix and have a clear dominant negative effect on intracellular assembly. We are examining the intracellular pathways responsible for eliminating mutant collagen VI and determining if a cellular stress response ensues. A smaller subset of mutations is found in the A-domains flanking the triple helix but little is known about how they affect assembly or why they cause muscular dystrophy. Our new studies are revealing abnormal folding in some mutant A-domains suggesting the mutations disturb protein-protein interactions in the matrix. Other A-domain mutations lead to rapid intracellular degradation of mutant chains while another allows normal intracellular assembly but prevents microfibril formation. Despite this progress in understanding how the mutations affect collagen VI assembly we still know very little about the downstream pathogenic processes in these disorders. We are conducting detailed gene expression studies in muscle from collagen VI knockout mice to determine if the downstream pathogenic events are similar to other muscular dystrophies or involve novel processes.

S5-5
Myostatin functions in the Rat Masseter Muscle hypertrophied by Cenbuterol, a β_2 adrenergic Agonist

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Keywords: Myostatin, Clenbuterol, Hypertrophy

The function of myostatin, a negative regulator for skeletal muscle development, was investigated in the hypertrophy of the rat masseter muscle induced by clenbuterol, a β_2 adrenergic agonist. Clenbuterol induced the hypertrophy of rat masseter muscle between 3 and 14 days of oral administration. However, at 21 days, the clenbuterol-induced hypertrophy terminated, the action of myostatin was up-regulated, and apoptosis was down-regulated. These results suggest that the up-regulation of myostatin and the down-regulation of apoptosis are involved in the termination of the clenbuterol-induced hypertrophy. At day 1, clenbuterol + ActRIIB/Fc, an antagonist of myostatin, stimulated the differentiation of C2C12 more than clenbuterol alone, whereas at day 2 it inhibited the differentiation. Clenbuterol + ActRIIB/Fc at days 1 and 2 induced an elevation in apoptosis in comparison with clenbuterol alone. These results suggest that, at day 1, the suppression of myostatin with ActRIIB/Fc stimulates the differentiation of C2C12 myogenic cells, but, at day 2, it inhibited the differentiation due to excess apoptosis.

Conclusions: Myostatin plays a role in the hypertrophy of rat masseter muscle induced by clenbuterol to terminate the hypertrophy and to protect myofibers from harmful effects such as excess apoptosis.

Symposium VI:

S6-1

House dust mite allergen Der f 1 can activate latent TGF-β, leading to the expression of profibrogenic genes

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Abstract
Rationale: It remains uncertain whether the protease activity of a major mite allergen Der f 1 affects airway remodeling in asthma. Transforming growth factor (TGF)-β, a key cytokine for airway remodeling, is secreted as a latent complex (latent TGF-β) in which TGF-β is non-covalently associated with the latency-associated peptide (LAP). LAP proteolysis is required for the release of TGF-β from the latent complex and its binding to the receptors.
Objective: This study investigated whether Der f 1 can cleave LAP via its proteolytic activity and activate latent TGF-β, thereby leading to expression of profibrogenic genes.
Methods: The effects of Der f 1 on the activation of latent TGF-β *in vitro* and *in vitro* were examined by the detection of TGF-β activity using TGF-β signaling reporter cells and mice, real-time PCR for TGF-β target gene expression, and histological examination.
Measurements and Main Results: Der f 1 cleaved LAP and induced the activation of latent TGF-β *in vitro*, which was inhibited by E-64, a cysteine protease inhibitor. The intratracheal or intranasal exposure of Der f 1 to mice induced TGF-β activity in the bronchoalveolar lavage (BAL) fluid, expression of TGF-β, Smad7, and type I and IV collagen mRNAs in the lung, and subepithelial fibrosis which was inhibited by E-64. The Smad promoter activity increased in the lung of Der f 1-challgenged TGF-β/Smad signaling-reporter mice.
Conclusions: Der f 1 can induce the activation of latent TGF-β via its protease activity, leading to expression of profibrogenic genes involved in airway remodeling in asthma.

S6-2

Role of Endothelial Progenitor Cells for Organ Regeneration

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Recently the regenerative potential of stem cells has been under intense investigation. In vitro, stem and progenitor cells possess the capability of self-renewal and differentiation into organ-specific cell types. In vivo, transplantation of these cells may reconstitute organ systems, as shown in animal models of diseases. In contrast, differentiated cells do not exhibit such characteristics. Human endothelial progenitor cells (EPCs) have been isolated from the peripheral blood of adult individuals, expanded in-vitro and committed into an endothelial lineage in culture. The transplantation of these human EPCs has been shown to facilitate successful salvage of limb vasculature and perfusion in athymic nude mice with severe hindlimb ischemia, while differentiated endothelial cells (human microvascular endothelial cells) failed to accomplish limb-saving neovascularization**.**
These experimental findings call into question certain fundamental concepts regarding blood vessel growth and development in adult organisms. Postnatal neovascularization has been previously considered synonymous with proliferation and migration of pre-existing, fully differentiated ECs resident within parent vessels, i.e. angiogenesis**.** The finding that circulating EPCs may home to sites of neovascularization and differentiate into ECs in situ is consistent with "vasculogenesis"**,** a critical paradigm for establishment of the primordial vascular network in the embryo. While the proportional contributions of angiogenesis and vasculogenesis to postnatal neovascularization remain to be clarified, our findings together with the recent reports from other investigators suggest that growth and development of new blood vessels in the adult is not restricted to angiogenesis but encompasses both embryonic mechanisms.
Furthermore, recent studies indicate optional role of EPCs for organ regeneration, including anti-inflammatory and anti-fibrotic effects for the preparation of organ regenerations. I will discuss this issue in the symposium.

Reference

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S6-3

Role of Bone Marrow in Pathophysiology of Hepatic Fibrosis and Regeneration

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Objective: It is recently reported that bone marrow (BM)-derived cells participate in either progression or regression of liver fibrosis by expressing collagen and matrix metalloproteinases (MMPs) [1], respectively. Here we examined the functional role of BM in hepatic fibrosis and regeneration.
Methods: BM of wild type mice was replaced by cells obtained from transgenic mice harboring a promoter of alpha2(I) collagen gene (COL1A2) linked to enhanced green fluorescent protein (EGFP) gene. Liver fibrosis was introduced into those mice or their BM recipients by repeated carbon tetrachloride (CCl_4) injections. To examine the effects of MMPs on migration and function of BM cells, MMP-13 knockout (KO) mice and recombinant adenoviruses overexpressing MMP-13 were used in the CCl_4-induced liver fibrosis model.
Results: A large number of EGFP-expressing cells were observed in fibrotic liver of transgenic COL1A2/EGFP mice. In contrast, there were few, if any, EGFP-expressing cells detected in the fibrotic liver of COL1A2/EGFP recipients. Experiments using MMP-13 KO mice indicated that BM cells-derived MMP-13 certainly contributes to the regression of liver fibrosis. Overexpression of MMP-13 remarkably enhanced the migration of BM-derived cells into the parenchyma of fibrotic liver, and some of which exhibited the phenotype of sinusoidal endothelial cells.
Conclusions: By using a specific experimental system which detects exclusively BM-derived collagen-producing cells, the role of BM-derived cells was very limited in collagen production during hepatic fibrosis. On the other hand, overexpression of MMP-13 enhanced the migration of BM-derived cells and their differentiation, which suggests the therapeutic implications in the repair and regeneration of fibrotic liver.

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S6-4

Resolution of Tissue Fibrosis by siRNA HSP47 encapsulated in Vitamin A bound Liposome.

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There are currently no approved antifibrotic therapies for liver cirrhosis. We used vitamin A–coupled liposomes to deliver small interfering RNA (siRNA) against gp46, the rat homolog of human heat shock protein 47, to hepatic stellate cells.

Our approach exploits the key roles of these cells in both fibrogenesis as well as uptake and storage of vitamin A. Five treatments with the siRNA-bearing vitamin A–coupled liposomes almost completely resolved liver fibrosis and prolonged survival in rats with otherwise lethal dimethylnitrosamine-induced liver cirrhosis in a dose- and duration-dependent manner.

Rescue was not related to off-target effects or associated with recruitment of innate immunity. Receptor-specific siRNA delivery was similarly effective in suppressing collagen secretion and treating fibrosis induced by CCl4 or bile duct ligation. The eficacy of the approach using both acute and chronic models of liverfibrosis suggests its therapeutic potential for reversing human liver cirrhosis.

Because recent investigations suggest wide distribution of stellate cells in other tissues, we then extended our exploration to examine if our approach is also valid for other organ fibrosis including lung fibrosis, chronic pancreatitis and myelofibrosis. Results so for obtained indicate promise of our approach in application to these fibrosis.

Mechanisms underlying such dramatic effect involved 1) inhibition of collagen secretion from stellate cells by siRNAHSP47, 2) resolution of predeposited collagen fiber by metalloproteinase in the fibrosis tissue and 3) apoptosis of stellate cells caused by removal of collagen matrix which triggers survival signal (PI3K/AKT/1KB) for stellate cells.

We are currently developing a new complex consisted of VA, biodegradable polymer and siRNAHSP47 for future clinical application of our modality.

S6-5
Hepatic stellate Cells in Liver Fibrosis

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Hepatic stellate cells (HSCs) are localized in perisinusoidal space of liver. In fibrotic liver, they lose lipid droplets containing vitamin A, change to myofibloblast-like phenotype and acquire increased proliferation activity. They also become synthesizing relatively large amount of matrix components including fibrillar collagens, what is called the "activated" state. In addition to type I and type III collagens, we detected that type IV and type XVIII collagens were synthesized by HSCs. These basement membrane collagens were reported to be the precursors of endogenous angiogenesis inhibitors.

We have investigated the treatment for liver fibrosis based on the concept of targeting "activated" hepatic stellate cells by introducing "non-activated" state or apoptosis. Vitamin E molecules are well known as antioxidants, however, recent research developments demonstrated that they possess powerful cholesterol lowering, platelet adhesion inhibition and anti-cancer properties. In this study, four tocopherols and tocol lacking methyl groups attached to the chromanol ring were applied to the "activated" hepatic stellate cells and examined the effects on proliferation activity of HSCs. Rat HSCs were prepared by collagenase perfusion and the "activated" state was induced by culture in vitro. Among four tocopherols and tocol, relatively high proliferation inhibition effects were detected in delta-tocopherol and tocol. Furthermore, cell detachment and apoptosis via anoikis were observed in delta-tocopherol treated and tocol treated cells in a dose response manner. The expression of alpha-smooth muscle actin, a marker for the activated HSCs, was significantly decreased in the treatment groups. These data suggest that vitamin E offers the promising treatment for liver fibrosis and cirrhosis.

Symposium VII:
S7-1
The Role of CD44-ECM Interactions in Tumor Invasion

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CD44 is a type-I membrane glycoprotein abundantly expressed in tumor cells and bind various extracellular matrix (ECM) components such as hyaluronan (HA) and chondroitin sulfates (CS). During tumor cell invasion, HA is degraded into small oligosaccharides by hyaluronidases produced by the tumor cells. We have shown that such HA oligosaccharides induce the proteolytic cleavage of CD44 from tumor cells and promote tumor cell migration in a CD44-dependent manner. We also found that chondroitin sulfate E (CSE), another component of the tumor ECM, strongly enhances CD44 cleavage and tumor cell motility when degraded into oligosaccharides. CSE can also be degraded by hyaluronidases, and CSE and its degradation products are detected in pancreatic ductal adenocarcinoma. In CD44-expressing pancreatic tumor cells, degraded forms of CSE but not intact CSE enhanced CD44 cleavage; enzymatic digestion of such low-molecular weight CSE (LMW-CSE) abrogated this enhancement. Among the LMW-CSE preparations examined, 3-kDa CSE most potently induced CD44 cleavage. NMR analysis showed that the 3-kDa-CSE bound to CD44 and that blocking such binding abrogated the CD44 cleavage induction. LMW-CSE also induced prominent filopodia formation and cytoskeletal changes in tumor cells; these effects were also abrogated by blocking the LMW-CSE binding to CD44. Chemically synthesized CSE hexasaccharides also enhanced the CD44 cleavage and tumor cell motility in a CD44-dependent manner. Thus, the degraded forms of CSE modulate cell adhesion and migration by interacting with tumor-cell CD44. These results strengthen the hypothesis that tumor cells and the surrounding ECM act on each other reciprocally, to promote tumor progression. They also indicate that tumor-cell CD44 plays a crucial role in these interactions by recognizing a non-HA ECM degradation product, LMW-CSE, directly implicating LMW-CSE in CD44-mediated tumor progression.

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S7-2
Maturation of Blood Vessels in The Tumor Environment

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Recent evidence suggests that cancers arise from cancer stem cells/initiating cells (CSCs/CICs). The niche for the maintenance of stemness has been identified in normal organs. Understanding the molecular mechanisms of how niche cells regulate stemness is very important for understanding the biology of stem cells. Although the niche in each organ is composed of different non-stem cell as well as stem cell types, it is likely that there is some commonality required for maintaining the slow-cycling, self-renewing, undifferentiated state of stem cells, as well as enhancing their resistance to stress; however, the molecular mechanisms supporting these behaviors are not clearly understood. As in normal tissue, it has been suggested that CSCs are maintained within peri-vascular niches. To study the localization of CSCs/CICs, we attempted to visualize them in tumor tissue by using a certain gene promoter tagged with LacZ or the EGFP gene. We found that CSCs/CICs are located near the blood vessels and form a cluster-like structure. Interestingly, the vascular niche for CSCs/CICs was mainly observed at the edge of the tumor mass, where the blood vessels are well matured, reflected by the adherence of mural cells to endothelial cells as frequently as observed in normal tissues. This implies that such mature blood vessels would be resistant against agents that disrupt angiogenesis. To destroy such vascular niches for CSCs/CICs, precise molecular mechanisms controlling the maturation of blood vessels at the edge of the tumor compared to the central region of tumor, where there are fewer mural cells adhering to endothelial cells, needs to be properly understood. In this session, we would like to present the association of Tie2/angiopoietin-1 [1-3] and APJ/apelin [4], receptor/ligand systems involved in the maturation process of blood vessel formation.

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S7-3
"Mouse Models for Colon Cancer Invasion and Metastasis"

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Most colorectal adenomas are initiated by the *APC* gene inactivation, and progress to malignant adenocarcinomas through additional mutations in the genes encoding RAS, TGF-β type II receptor, p53, etc.
To investigate the role of impaired TGF-β family signaling in colon cancer progression, we earlier constructed compound mutant mouse strain "*cis-Apc*$^{+/\Delta 716}$ *Smad4*$^{+/-}$ (*cis-Apc/Smad4*)" that carried a knockout allele of the *Smad4* gene on the same chromatid as that of *Apc* (*Apc*$^{\Delta 716}$) [1]. In the compound mutant, loss of the SMAD4-dependent TGF-β family signaling turns the intestinal adenomas into invasive adenocarcinomas, although SMAD4-independent signaling remains unaffected. Because polyp adenomas are initiated by loss of heterozygosity (LOH) of *Apc* that is caused by recombination at the centromeric rDNA cluster on chromosome 18, the tumor epithelial cells in the *cis-Apc/Smad4* mice carry homozygous mutations in both *Apc* and *Smad4* genes.
Focusing on the tumor-stromal interactions, we have investigated here the mechanism of intestinal tumor invasion in the *cis-Apc/Smad4* mice. We demonstrate here that a novel type of immature myeloid cells (iMCs) is recruited from the bone marrow to the tumor invasion front. These CD34$^+$ iMCs express MMP9/2 and CC-chemokine receptor 1 (CCR1), and migrate toward its ligand CCL9. In the adenocarcinomas, expression of CCL9 is increased in the tumor epithelium. By knocking out *Ccr1* gene in the *cis-Apc/Smad4* mutant mice, we further demonstrate that lack of CCR1 prevents the accumulation of CD34$^+$ iMCs at the invasion front and suppresses tumor invasion. Analysis of human colon cancer specimens that carried mutant TGF-β type II receptor showed similar iMCs expressing CCR1 and MMP9/2. These results indicate that loss of the TGF-β family signaling in tumor epithelium causes accumulation of iMCs that help tumor invasion [2], and show therapeutic implications in treating invasive colon cancer.

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S7-4

The BRAK Box Is Opening

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In order to find a suppressor(s) of tumor progression *in vivo* for head and neck squamous cell carcinoma (HNSCC), we searched for molecules down-regulated in HNSCC cells when the cells were treated with epidermal growth factor (EGF), whose receptor is frequently over-activated in HNSCC.

The expression of BRAK, which is also known as CXC chemokine ligand14 (CXCL14), was down-regulated significantly by the treatment of HNSCC cells with EGF as observed by cDNA microarray analysis followed by reverse-transcriptase polymerase chain reaction analysis. The EGF effect was attenuated by the co-presence of a MEK inhibitor, thus suggesting that BRAK down-regulation is controlled by the EGF Receptor (EGFR)-Raf-MEK-ERK pathway. The rate of tumor formation *in vivo* by BRAK-expressing vector-transfected tumor cells in athymic nude mice was significantly lower than that of mock vector-transfected ones. In addition tumors formed *in vivo* by the BRAK-expressing cells were significantly smaller than those of the mock-transfected ones. These results indicate that BRAK/ CXCL14 is a chemokine, having suppressive activity toward tumor progression of HNSCC cells *in vivo* [1].

Next we addressed whether inhibition of EGFR activity would affect BRAK expression and growth of tumor cell xenografts. Gefitinib (ZD1839, Iressa), which is an inhibitor specific for the EGFR tyrosine kinase, has been shown to be effective for tumor suppression in non-small cell lung carcinoma patients with over activation of EGFRs. Thus we investigated the relationship between BRAK expression and gefitinib efficacy for tumor suppression. We found that EGF inhibited BRAK expression through the MEK-ERK pathway and that this inhibition was reversed by gefitinib *in vitro* and that oral administration of gefitinib reduced the tumor growth of xenografts in athymic nude mice, which reduction was accompanied by increased BRAK expression specifically in tumor tissue. The introduction of a BRAK ShRNA vector into HNSCC cells reduced both the expression of BRAK in the cells and the antitumor efficacy of gefitinib *in vivo*. Our data indicate that the gefitinib-induced increase in BRAK expression is beneficial for tumor suppression *in vivo.* Our data also provide a new strategy for chemokine-mediated cancer therapy using gefitinib [2].

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Symposium VIII:

S8-1

Metabolic characteristics of cancer microenvironment and its implication in malignant progression of cancer

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Keywords: glucose deprivation, autophagy, hypoxia

Objective: Unlimited and unregulated cell proliferation is characteristics of cancer and to support this sufficient supply of oxygen and nutrients is regarded pivotal. Contrary to this, poor blood supply is often associated with poor patient outcome. An extreme example is pancreatic cancer. To understand how deprivation of nutrient is inversely correlated with progression, cancer microenvironment was analyzed from metabolic viewpoint.

Methods: Human materials were obtained after surgical treatments and the protocol was approved by Ethical committee of National Cancer Center. Metabolomic analysis was carried out mainly by CE-MS method established by Soga et al.

Results: Glucose concentration in many cancer tissues was found far less than blood glucose level being less than 0.1mg/ml in average. In contrary, amino acid concentrations were comparable to those of corresponding normal tissues. In vitro experiments using pancreatic cancer cell lines showed that they have a capacity to operate fumarate respiration, an energy production pathway by parasites without oxygen. Glucose deprivation has been shown to induce various types of proteases including cathepsin and MMPs. Immunohistochemical analysis revealed that cancer cells activates autophagy in early stage.

Conclusion: By intrinsic and environmental reasons, cancer cells acquire ability to survive glucose and oxygen deprivation by degrading protein to yield amino acid, leading to invasiveness.

S8-2

MT1-MMP as a potent modulator of Tumor Microenvironment

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Membrane-type 1 matrix metalloproteinase (MT1-MMP/MMP-14) is a potent modulator of cell physiology by degrading multiple proteins in the pericellular milieu. Expressed in tumor cells, MT1-MMP is shown to be involved in tumor growth, invasion, and metastasis. The roles of MT1-MMP are mediated by its proteolytic activity on the cell surface. Possible substrates include extracellular matrix (ECM) proteins, cell adhesion molecules, cytokines, and latent forms of proMMPs. However, our knowledge of the physiological substrates of MT1-MMP is still limited and identification of these substrates should enable a better understanding of the biological functions of MT1-MMP.

During the migration and invasion of cells, MT1-MMP localizes to the leading edges and invadopodia of cells. Although the exact mechanisms that determine the localization of MT1-MMP are not well understood, the binding of MT1-MMP to cellular proteins linked to the actin cytoskeleton, such as CD44 or integrin, is thought to be a factor determining its localization. Thus, identification of the proteins that interact with MT1-MMP on the cell surface provides us important clue to understand the mechanisms and functions of MT1-MMP.

We aimed to identify a catalog of proteins that associate either directly or indirectly with MT1-MMP. To do this, we purified MT1-MMP from cell lysate together with its associating proteins. A specific set of membrane proteins was co-purified with MT1-MMP. The purified proteins were analyzed by a nano-flow liquid chromatography-tandem mass spectrometry (nano-LC-MS/MS). We identified more than hundred proteins in the MT1-MMP complex obtained from human tumor cell lines. These are membrane proteins, cytoplasmic proteins, receptors, etc. including functionally unknown proteins. About half of the membrane proteins tested can be cleaved by MT1-MMP in cells. Analysis of the identified membrane proteins and cytoplasmic proteins will be reported.

S8-3

Macrophages, Microenvironment and Metastasis.

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Macrophages are abundant cells in the tumor microenvironment and in clinical studies their density is usually positively correlated with poor prognosis. This suggests that macrophages are tumor promoting. Consistent with this hypothesis studies in mouse models show that genetic or chemical ablation of macrophages results in a reduction in tumor progression and metastasis (1). In our mechanistic studies using genetic models of macrophage ablation as well as gain-of-function experiments we showed that tumor-associated macrophages regulate the angiogenic switch required for the malignant transition through the production of VEGF and that they also promote tumor cell invasion, migration and intravasation as a consequence of reciprocal EGF and CSF-1 signaling (2, 3). These macrophage-tumor cell interactions can also be visualized in mouse models that exploit florescent labeling through expression of fluorescent proteins from tissue specific promoters (4). In addition to these effects at the primary tumor site we have recently identified a sub-population of macrophages that are required for metastatic seeding and persistent growth at distant sites. These data together with that of others suggest that targeting macrophages and their unique signaling pathways could offer new therapeutic strategies against metastatic disease (5).

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Symposium IX:

S9-1
NHE1 (Na^+/H^+ exchanger 1) promotes invadopodia ECM degradation and invasion through the spatially restricted acidification of the peri-invadopodial space

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Degradation of the extracellular matrix (ECM) is a critical process of tumor cell invasion and requires membrane and released proteases focalized at membrane structures called invadopodia. While extracellular acidification is important in driving tumor invasion, the structure/function mechanisms driving it are still unknown. Invadopodia are very similar in structure and function to osteoclast podosomes responsible for bone degradation. Extracellular acidification is central to podosome action and, by analogy, could also be for invadopodial function. Here, we show that NHE1 and NHE1-dependent extracellular acidification are localized at invadopodia and are necessary for tumor cell matrix-degrading activity. Experiments were conducted in metastatic breast cancer cells seeded onto matrigel in which quenched BSA- or collagen-FITC was mixed and invadopodia activity evaluated microscopically. Focal proteolysis produces fluorescence which is used to quantitatively measure proteolytic activity, co-localization analysis of NHE1 expression and extracellular pH. Immunofluorescence showed that invadopodia-dependent focal ECM degradation is tightly associated with NHE1 expression and that NHE1 often co-localized with cortactin. Areas of focal ECM digestion had more acidic pH values compared to the edge of the cells where only pericellular digestion had occurred and the acidification was blocked by the specific NHE1 inhibitor, cariporide (HOE642). Stimulation with EGF increased both ECM degradation and NHE1-dependent proton secretion. Exposure of tumor cells to low medium pHe, low serum or hypoxia stimulated invadopodia-dependent ECM proteolysis. Exposure of tumor cells to low medium pH increased both NHE1 activity and invadopodial-dependent ECM proteolysis with a increase in invadopodial distribution, length and association with NHE1. Manipulation of the NHE1 expression level or activity by RNA interference, transport-deficient mutation or the specific inhibitor cariporide confirmed that NHE1 expression and activity are required for invadopodia-mediated ECM degradation. We conclude that NHE1 and its associated extracellular acidification are localized to cancer cell invadopodia and are necessary for invadopodial ECM digestion.

S9-2
Acidic pH signaling in metastasis

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The extracellular pH (pH_e) of tumor tissues has been well known to be often acidic. Although the acidification is believed to be mainly due to acidic metabolites, *e.g.*, lactate, caused by anaerobic glycolysis, recent studies have investigated that CO_2 from pentose phosphate pathway is also a major source of acidity. In earlier study, we reported that acidic pH_e up-regulated production of matrix metallo- proteinase-9 (MMP-9) / gelatinase B that plays an important role of type IV collagen degradation in tumor metastasis. Thereafter, other pro-metastatic factors, such as vascular endothelial cell growth factor (VEGF) and interleukin-8 (IL-8), have been reported to be acidic pH_e-regulated gene by several research groups. We further explored the intracellular signaling pathway for acidic pH_e signaling to induce MMP-9 expression as the target gene. Acidic pH_e significantly activated phospho-lipase D (PLD), but not phosphatidyl-inositol-specific phspholipase C. Proximal promoter assay for MMP-9 revealed that NFκB binding site was a major responsible element for the acidic pH_e. PLD activation conducted to mitogen-activated kinases followed by NFκB activation. Furthermore, we found that Ca^{2+}-influx triggered PLD activation and that acidic sphingomyelinase and protein kinase Cζ were associated with NFκB activation. In addition, RhoA, but not Rac1 and cdc42, was also involved in acidic pH_e signaling. Because hypoxia inducible factor 1α was induced by acidic pH_e, but not by hypoxia, cellular activity of tumor cells was regulated by acidic pH_e and hypoxia at different stage of extracellular microenvironment in the tumor tissue.

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S9-3

Role of Acid Microenvironment in Cancer-induced Bone Pain

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Bone pain is one of the major complications in bone metastases. The widely-known clinical observations that specific inhibitors of osteoclastic bone resorption such as bisphosphonates (BPs) effectively reduce bone pain suggest a potential role of osteoclasts that play a central role in bone metastases. Osteoclasts dissolve bone minerals by releasing protons through the vacuolar type proton pump (V-H^+-ATPase). Proton is a well-known cause of pain. Proton directly activates the acid-sensing nociceptors such as TRPV1 that converts pain signals into electrochemical signals and transduces them to CNS. Here, we studied the role of TRPV1 in the induction of bone pain associated with cancer colonization in bone using an animal model of bone cancer pain we established. TRPV1 was expressed on the calcitonin gene-related protein-positive sensory neurons in bone. Cancer-inoculated bones showed hyperalgesia and increased hind-limb lifting (flinching) compared with control bones, suggesting cancer colonization increased bone pain. The BP zoledronic acid and a specific inhibitor of the V-H^+-ATPase FR167356 significantly reduced the hyperalgesia and flinching, suggesting a critical role of protons released by osteoclasts. Ipsilateral dorsal root ganglion (DRG) showed increased Erk phosphorylation (pErk). In contrast, there were no differences in hyperalgesia and flinching between cancer-inoculated and control bones in TRPV1-/- mice. Acid (pH 5.5) increased pErk in WT DRG in organ culture. IRTX, a specific inhibitor of TRPV1, reduced pErk. However, acid failed to increase pErk in TRPV-/- DRG. These results suggest TRPV1 is responsible for elevated pErk. In conclusion, our results suggest that the activation of TRPV1 on the sensory neurons innervating bone by protons that are released by bone-resorbing osteoclasts plays a critical role in cancer-induced bone pain.

S9-4

Targeting MMP13 in Human Breast Cancer Metastasis to Bone

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Matrix metalloproteinases (MMPs) play important roles in cancer growth, invasion and metastasis, but to date have eluded therapeutic address in cancer. MMP-inhibition trials have been confounded by the emergence of cancer-inhibitory roles for some MMPs, and also the dose-limiting toxicity musculoskeletal syndrome (MSS). We reasoned that certain specific MMPs may not have such cancer inhibitory roles such that specific targeting of these may afford therapeutic benefit.

Our initial approach was to survey the MMPs produced by, and in response to, a series of human breast cancer xenografts. We found that MMP13 is dramatically upregulated in the stroma of xenografted human breast cancer cell lines in the primary site and in bone metastases developing after intracardiac inoculation (1). We hypothesise that MMP13 may represent an important MMP target if it can be targeted specifically.

To test this, we investigated the ability of a MMP-13-selective inhibitor (**cmpd-1**), to inhibit the osteolytic potential of human breast cancer cells *in vivo*. Treatment with **cmpd-1** for up to 55 days inhibited the occurrence of osteolytic lesions in the intra-cardiac mouse model and suppressed the growth of primary MDA-MB-231 human breast cancer cells tumours in the mfp.

Although the specific mechanism for MSS has not been reported, MMP13-selective inhibitors such as **cmpd-1** lack any evidence of MSS in animal models (2) and thus MMP13 appears not involved. Thus, MMP13 may represent an important **specific** MMP target. Further genetic studies with MMP13-deficient mice and MMP13-specific shRNA are ongoing to validate this possibility.

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S9-5

Quantitative Proteomics of Breast Cancer Identifies New Substrates and Roles for MMPs

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Proteomics technologies are revolutionizing the way in which drug targets can be identified, validated and assessed. Breast cancer is an interactive multi-cell, multi-tissue lesion dynamically linked to the stroma by signaling networks that regulate gene and protein expression, and post-translational modifications in the tumor and microenvironment. By the irreversible processing of bioactive proteins and signaling molecules, proteases modulate angiogenesis, growth, invasion, metastasis, and phenotypic evolution of cancer cells together with micro-environmental and host defense responses. To understand the role of proteases in breast cancer development and metastasis it is important to identify and characterize new proteases and their substrates involved in these processes. For this purpose we generated primary breast tumors in a syngeneic xenograft model in mice by mammary fat pad injection of breast cancer cells known to form either no metastasis (67NR cells), only lung metastasis (66cl4 cells) or multiple metastasis (4T1 cells). Subsequently, total RNA was isolated from the tumor tissue and hybridized to the CLIP-CHIP™, the most complete dedicated human and murine protease and inhibitor oligonucleotide microarray. From this analysis we found matrix metalloproteinases (MMPs) 10 and 13 to be highly expressed in primary tumors with highest expression in tumors derived from the most aggressive 4T1 cell line. MMP proteases were imaged spatially in 3D and temporally in these tumours by F18 coupled to Marimastat, a reversible nM MMP inhibitor drug, using a novel coupling strategy that allows for labeling under aqueous conditions at room temperature. To elucidate the function of these proteases in the establishment and progression of the tumor it is essential to identify their substrates. While this was previously mostly limited by the lack of appropriate techniques we overcame this limitation by the invention of Terminal Amine Isotope Labeling of Substrates (TAILS). CLIP-TAILS (Terminal Amine Isotope Labelling of Substrates) is a new proteomic approach we have developed to identify cleaved neo-termini of substrates after enrichment. MS/MS both identifies the substrate and sequence of the cleavage site in the same experiment. We incubated the secreted proteome of T-47D breast cancer cells that are deficient in MMP-10 and 13 expression with either MMP-10, MMP-13 or both proteases and subjected the samples to TAILS analysis. Thereby, we identified numerous known and novel MMP-10 and 13 substrate candidates including members of the insulin-like growth factor binding protein family that are known to play important roles in breast cancer development and progression. In related work proteome signatures that are hallmarks of proteolysis revealed cleavage of many known MMP substrates in the cellular context. Proteomic evidence of proteolytic processing of novel substrates was found including Insulin-like growth factor binding protein-4 and 6, follistatin-like 1 and cystatin C, heparin affin regulatory peptide (HARP/pleiotrophin) and connective tissue growth factor (CTGF), which released vascular endothelial growth factor (VEGF) from angiogenic inhibitory complexes. The cleaved HARP N-terminal domain increased HARP-induced cell proliferation, whereas the HARP C-terminal domain was antagonistic and decreased cell proliferation and migration. Hence the unmasking of cytokines, such as VEGF, by metalloproteinase processing of their binding proteins is a new mechanism in the control of cytokine activation and angiogenesis.

Workshop I-A

1W-01

ADAMTS1 is induced by hypoxia in endothelial cells and HIF-1 binds to the ADAMTS1 promoter

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Keywords: Hypoxia、endothelial cells、HIF1

Objective: ADAMTS1 (a disintegrin and metalloproteinase with thrombospondin motifs1) is a member of matrix metalloproteinase family. These molecules are involved in various biological events such as cell adhesion, cell fusion, cell migration, angiogenesis, metastasis and proteolysis. We have previously reported that ADAMTS1 was strongly expressed in myocardial infarction. In this study, we investigated whether hypoxia induced ADAMTS1 and investigated its regulatory mechanism. In hypoxia, the expression level of ADAMTS1 mRNA and protein rapidly increased in endothelial cells, but not in other cell types.
Methods: Human umbilical vein endothelial cells (HUVEC) and other cells were cultured in the hypoxia or normoxia. Expression of the ADAMTS1 analyzed by real-time PCR, CHIP assay, Reporter gene assay and Western blot analysis.
Results: In the promoter region of ADAMTS1, we found at least three putative hypoxia-inducible factor (HIF) binding sites, and the chromatin immunoprecipitation (ChIP) assay revealed HIF-1 binding to HIF binding sites in the promoter region of ADAMTS1 under hypoxia. Recombinant ADAMTS1 protein promoted the migration of HUVEC under hypoxic conditions.
Conclusions: ADAMTS1 is transiently induced by hypoxia in endothelial cells and its transcription is mediated by HIF-1 binding.

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ADAMTS1 Is A Unique Hypoxic Early Response Gene Expressed By Endothelial Cells,
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1W-02

Model Organism Approaches to understand the Role of WISP3, the Gene that is mutated in Progressive Pseudorheumatoid Dysplasia

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Key words: WISP3, PPD, Cartilage, BMP, Wnt, Zebrafish

Objectives: In humans, loss-of-function mutations in the gene encoding Wnt-1 inducible signaling pathway protein 3 (*WISP3*) cause the autosomal recessive skeletal disorder Progressive Pseudorheumatoid Dysplasia (PPD) [1]. PPD leads to joint failure by 20 years of age and requires joint replacement surgeries [2]. Therefore, understanding the patho-physiology in PPD is paramount to developing effective treatment. However, in mice there is no apparent phenotype caused by Wisp3 deficiency or Wisp3 over-expression with cartilage-specific or ubiquitous promoters [3, 4]. Consequently, the *in vivo* activities of Wisp3 have remained elusive.
Methods: Zebrafish are an excellent organism for evaluating the effects of gene and/or protein function on signaling pathways and developmental processes. The *in vivo* biologic activity of Wisp3 was investigated using loss-of-function and gain-of-function approaches in developing zebrafish. To better understand the mechanism by which Wisp3 functions, *in vitro* experiments using mammalian cells and biochemical assay were also performed.
Results: Inhibition of Wisp3 protein expression in developing zebrafish affected pharyngeal cartilage size and shape. Over-expression of Wisp3 protein inhibited bone morphogenetic protein (BMP) and Wnt signaling in zebrafish. Conditioned medium containing Wisp3 also inhibited BMP and Wnt signaling in mammalian cells by binding to BMP ligand and to the Wnt co-receptors low density lipoprotein receptor related protein (LRP) and Frizzled, respectively. More importantly, Wisp3 proteins containing disease-causing amino acid substitutions found in patients with PPD reduced activity in these assays.
Conclusions: These *in vivo* and *in vitro* data suggest that dysregulation of BMP and/or Wnt signaling contributes to joint failure in patients with PPD.

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1W-03

Suppression of Akt Activation on Collagen Gels (sAag); in the case of Cancer Cell Lines

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Keywords: Collagen, PKB/Akt, Cancer

Background: *"Just changing the way a cell interacts with its 3-D environment can radically alter its behaviors."* [1] As substrates for culture, the formation of supramolecular assembly of type I collagen (fibrils) or type IV collagen (meshwork structure) induces drastically different fates in cells. For example, both type I and type IV collagen molecule coated dish surfaces are good prolific substrates for human foreskin keratinocytes (HFKs) to maintain cells in the undifferentiated state. However HFKs cultured on fibril-formed type I collagen gels was induced apoptosis without differentiation [2]. On the type IV collagen meshwork formed gels, cell proliferation of HFKs was suppressed and differentiation was induced [3]. In both cases, HFKs cultured on gels was observed the suppression of Akt activation and focal adhesions component proteins activation. We pay attention to these common phenomena and describe them "suppression of Akt activation on gels (sAag)". We think that sAag occurs because of adhesion to collagen assembly form.

Objective: It is well-known that Akt activation plays important roles on cancer malignancy. We respected this matter and expected that sAag effects were observed in cancer cell lines.

Methods: To investigate sAag effects of type I collagen fibrils, we examined cell proliferation and Akt activation on type I collagen gels in five cancer cell lines.

Results: In four cell lines, Caco-2, MCF-7, MDA-MB-231 and HT1080, Akt activation and proliferation were suppressed on gels. Meanwhile independent the formation of supramolecular assembly, B16 scarcely adhered to collagen.

Conclusions: To culture some kinds of cancer cell lines on collagen gels could be suppressed PI3k/Akt signaling pathway activation.

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1W-04

A Quantitative Estimation System for Fibrosis in Non-alcoholic Steatohepatitis by Using Transgenic Collagen Promoter/Luciferase Reporter Mouse

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Keywords: Liver fibrosis, Non-alcoholic steatohepatitis, Methionine-choline deficient diet

Background & Aims: Non-alcoholic fatty liver diseases including its progressive form, non-alcoholic steatohepatitis (NASH), are often associated with metabolic syndrome. Early detection and treatment of NASH are very important for preventing its progression to liver fibrosis and cirrhosis. In order to seek pharmaceutical agents that suppress fibrogenesis at an early stage of NASH, here we report a quantitative estimation system to detect activation of type I collagen promoter in a murine NASH model.

Methods: Transgenic mice harboring α2(I) collagen gene (COL1A2) enhancer/promoter sequences linked to a firefly luciferase gene were fed with methionine-choline deficient diet (MCDD). Mice were sacrificed 2 or 4 weeks later to collect serum and liver tissue. Histopathological changes were evaluated by hematoxylin-eosin and Mallory-Azan staining, and activation of hepatic stellate cells, the major source of collagen in the fibrotic liver, was detected by α-smooth muscle actin (SMA) immunostaining. COL1A2 promoter activities in liver were determined by luciferase assays of tissue homogenates.

Results: The mean levels of serum alanine aminotransferase in mice fed with control diet and in those with MCDD are 25 U/L and 160 U/L after 2 weeks, and 41 U/L and 193 U/L after 4 weeks, respectively. Neutrophil infiltration and lipid droplets were observed in the liver parenchyma after 2 weeks of MCDD feeding. Although those histopathological findings became more evident, α-SMA expression and accumulation of collagen fibers were still limited after 4 weeks. On the other hand, luciferase activity in liver tissue was significantly increased up to 217 % in mice fed with MCDD for 4 weeks as compared with control animals.

Conclusions: By using transgenic reporter mice, COL1A2 promoter activation can be detected sensitively and quantitatively at an early stage of liver fibrosis associated with experimental NASH, which can be a good system to evaluate anti-fibrotic agents for the treatment of NASH.

1W-05

Versican expression is transient during wound healing but continues at high levels in keloid: Role of versican in keloid formation in a new mouse model

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Keloids are a refractory disease characterized by excessive deposition of extracellular matrices (ECMs). We previously found that increased expression of versican, a large chondroitin sulfate proteoglycan, was one of the key features that characterize keloid tissues as well as cultured keloid cells (KL cells). In this study, factors regulating versican expression were investigated with KL cells and normal dermal fibroblasts using RT-PCR and luciferase assay. KL cells showed two-fold higher transcription level. Screening of Wnt, β catenin, TGF-β, androgen, IL-1β and PI3K showed the latter two signals were involved in versican gene regulation.

Versican expression in normal skin wound healing process was also studied using C57BL mice. Immunohistochemistry showed transient versican expression which reached the maximum at 5 days post-wounding. To trace the destination of versican-expressing cells, we generated transgenic mice expressing versican promoter-Cre recombinase/rosa26. The number of LacZ positive cells reached the maximum at 5 days post-wounding, thereafter decreased and disappeared within 14 days. We hypothesized from these observations that persistent survival and proliferation of these versican-expressing cells might be involved in the keloid pathogenesis.

In order to establish the experimental model of keloids, we implanted KL cells with collagen sponge scaffolds in nude mice. Sponges with KL cells (KL sponges) appeared thicker and more opaque and weighed significantly heavier than those with normal fibroblasts (normal fb sponges) after 4 weeks. KL sponges deposited more versican than normal fb sponges did, which suggested that this model reflected the ECMs-producing characteristics of keloids. Administration of IL-1β and chondroitinase ABC to KL sponges for 4 weeks successfully suppressed the deposition of glycosaminoglycans as well as the increase in weight. Thus, this *in vivo* model could provide a valuable tool for the evaluation of therapeutic reagents targeted for keloids.

1W-06
ADAMTSL4 improves microfibril of Marfan syndrome derived cells

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Abstract
Marfan syndrome (MFS) is a systemic disorder affecting connective tissues that is caused by mutations of the FBN1 gene encoding fibrillin-1, a major microfibril component. Prior observation suggested that MFS is associated with increasing susceptibility to severe periodontitis which associated with irreversible damage of periodontal ligament (PDL). However, the molecular mechanisms of microfibrils assembly in PDL formation remain largely unknown. Here, we report that ADAMTSL-4β, a novel microfibril binding protein, not only promotes fibrillin-1 microfibril assembly in PDL but also improves microfibril disorganization in cultured PDL cells obtained from MFS patient (M-HPDL). Expression patterning analysis revealed that *adamtsl4β* mRNA is strongly expressed in the dental follicle, the origin of the PDL, and ADAMTSL-4β protein is colocalized with the fibrillin-1 microfibril in the course of microfibril maturation during PDL development. In contrast, mice homozygous for a targeted hypomorphic allele (mgR/mgR) of *Fbn1*, which served as a mice model of MFS, showed disorganization of PDL in association with progressive fragmentation of ADAMTSL-4β microfibrils. M-HPDL able to form insufficient fibrillin-1 microfibril, nevertheless overexpression of ADAMTSL-4β in M-HPDL markedly improved fibrillin-1 microfibril assembly. Our results suggest that ADAMTSL-4β regulates microfibril assembly of fibrillin-1 during PDL development, and could be a novel therapeutic target for the damaged PDL tissue in patients with MFS.

Workshop I-B
1W-07
Optimal Spaces for Bone Regeneration created by artificial ECM of Titanium Web

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Objective: To create a new dental implant system with biologically self-curing ability, we aimed improvement from the conventional 2-D bone-titanium connection into 3-D one, by which the osteoblast activity is able to extend into the spaces created by 3-D collaboration zone of fibrous titanium and active bone tissue.
Methods: The new device is composed of a titanium web layer of about 1 mm thickness that was vacuum-sintered with the rod of titanium bulk, named TWT (titanium web–equipped titanium rod). The web is an unwoven 3-D fibrous structure made of thin titanium fibers, whose cross-sections are square form of 50 microns in side. The web structure was designed to create the optimal spaces for bone ingrowth. Artificial roots of tooth of TWT (4 x 8 mm) were implanted into the created sockets of premolars region of Beagle mandibles, 2 months after the tooth extraction. As a control, commercial artificial roots (Astra Tech Co., USA, 8 mm in length and 4 mm) were implanted.
Results: After 20, 32 and 80 weeks, histological observations revealed that the bone ingrowth into web layers started at 20 weeks, and completed at 80 weeks, in the every areas where bone tissue contacted to the TWT implants.

Conclusions: TWT works as 3D living connection between bone and implants

1W-08

Versican/PG-M assembles hyaluronan into extracellular matrix and inhibits CD44-mediated signaling toward premature senescence in embryonic fibroblasts

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Versican/PG-M is a large chondroitin sulfate proteoglycan of the extracellular matrix which interacts with hyaluronan at the N-terminal G1 domain, composed of A, B, and B' subdomains. Recently, we generated knockin mice $Cspg2^{\Delta3/\Delta3}$, whose versican, without the A subdomain, has decreased HA-binding affinity, thereby exhibiting reduced deposition of versican in the extracellular matrix. Here, we show that the $Cspg2^{\Delta3/\Delta3}$ fibroblasts within 20 passages proliferate more slowly and acquire senescence. Whereas the extracellular matrix of the wild type fibroblasts exhibited a network structure of hyaluronan and versican, that of the $Cspg2^{\Delta3/\Delta3}$ fibroblasts exhibited ~35% and ~85% deposition of versican and HA, without such a structure. The $Cspg2^{\Delta3/\Delta3}$ fibroblasts showed a substantial increase of ERK1/2 phosphorylation and expression of senescence markers p53, p21, and p16. Treatment of wild type fibroblasts with hyaluronidase and exogenous hyaluronan enhanced ERK1/2 phosphorylation, and treatment with an anti-CD44 antibody that blocks HA-CD44 interaction inhibited the phosphorylation. These results demonstrate that versican is essential for matrix assembly involving hyaluronan, and that diminished versican deposition increases free hyaluronan fragments that interact with CD44 and increase phosphorylation of ERK1/2, leading to cellular senescence.

1W-09

Ovalbumin-induced Airway Hyperresponsiveness is increased in SHAP-hyaluronan complex deficient Mice

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Background: Serum-derived hyaluronan-associated proteins (SHAP), the heavy chains of inter-α-trypsin inhibitor, covalently bind to hyaluronan (HA) to form the SHAP-hyaluronan (SHAP-HA) complex. The SHAP-HA complex was found to be involved in the pathophysiology of inflammatory diseases such as arthritis and hepatitis. Thus, we sought the possibility that the complex is also involved in airway allergy.
Methods: The SHAP-HA deficient (KO) mice and wild type (WT) mice were used. The mice were immunized twice by intraperitoneal injection of ovalbumin (OVA), and exposed to aerosol OVA for 30 minutes each day for 2 weeks. Twenty-four hours after the final OVA challenge, airway responsiveness to inhaled methacholine (Mch) was measured, and analysis of bronchoalveolar lavage fluid (BALF) and lung histological studies were performed.
Results: Compared to WT mice, KO mice showed higher airway hyperresponsiveness (AHR) to inhaled Mch and higher late phase response to OVA, but the early phase response was comparable. In KO mice, total number of inflammatory cells in BALF was high, due to the increased number of macrophages and neutrophils as revealed by differential cell count. Furthermore, decreased concentrations of soluble tumor necrosis factor receptor-1 (sTNFR1) and interleukin (IL)-12p40 were found in BALF from KO mice, although levels of Th1 and Th2 cytokines were not different from WT mice.
Conclusions: The findings suggest that in murine model of asthma, the SHAP-HA complex plays an inhibitory role in the development of AHR and allergic airway inflammation, at least in part via negative feedback mechanisms by sTNFR1.

1W-10

Essential role of β3GnT7 for efficient KS-GAG production in cultured cells

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Keywords: Keratan sulfate, Sulfotransferase, Glycosyltransferase

Objective: Keratan sulfate (KS) glycosaminolycan (GAG) is one of the major carbohydrates in the corneal tissue and is suggested to have important role for biological function in the cornea. To study biosynthesis of KS-GAG in cultured mammalian cells, we constructed lentiviral vectors that express carbohydrate sulfotransferases. Using these vectors, we generated stably transfected cultured cells and analyzed production of highly sulfated KS-GAG by additional expression of glycosyltransferases.
Methods: We demonstrated that two carbohydrate sulfotransferases, KS galactose 6-*O* sulfotransferase (KSG6ST) and corneal GlcNAc 6-*O* sulfotransferase (CGn6ST, also known as GlcNAc6ST-5/GST-4β) are required for highly sulfated KS-GAG production. In this study, we constructed a lentiviral vector, which expresses both KSG6ST and CGn6ST, and infected the virus to two different human cell lines, HeLa and SV40-transformed human corneal epithelial (hCE) cells. Using 5D4 monoclonal antibody we analyzed highly sulfated KS-GAG in lentivirus-infected cells.
Results: On the infected HeLa cells by KSG6ST/CGn6ST-expression lentivirus, we found significant amount of 5D4-positive highly sulfated KS-GAG production, indicating that the lentivirus can induce production of highly sulfated KS-GAG in the infected cells. On the other hand, we observed much stronger 5D4-positive signal on hCE cells infected by the same virus than that on the infected HeLa cells, suggesting missing factor(s) for efficient production of highly sulfated KS-GAG in HeLa cells. Next we tested additional effect of β1,3 *N*-acetylglucosaminyltransferase-7 (β3GnT7) activity in the infected HeLa cells and found enhanced production of 5D4-positive KS-GAG in the lentivirus-infected HeLa cells that are transfected with β3GnT7-expression vector.
Conclusions: From these results we concluded that β3GnT7 expression is essential for efficient production of highly sulfated KS-GAG in cultured cells.

1W-11

Fractones: specialized extracellular matrix structures governing the stem cell niches

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Objective: Throughout life, stem cells and their immediate progeny proliferate and differentiate in restricted zones termed niches. However, the structural and functional characteristics that are specific of the stem cell niches are unknown. We have previously characterized fractones, specialized ECM structures that directly contact stem cells, in mammalian embryonic tissues and in the adult brain neurogenic niche. Fractones resemble basement membranes by their composition, laminins, collagen IV and heparan sulfate proteoglycans (HSPG), but differ by their localization and morphology. Our current objective was to determine whether fractones are the specific stem cell niches structures controling stem cell proliferation in the adult brain. Our mechanistic hypothesis was that fractone-HSPG are responsible for growth factor capture and activation at the stem cell surface. Our preliminary results indicated that FGF-2 binds fractones via HSPG.
Methods: To investigate the binding capabilities of fractones in vivo, we ICV injected adult mice with fluorescent-tagged FGF-2 (neurogenic stimulator), BMP-4 and BMP-7 (neurogenic inhibitors). To determine whether growth factor binding to fractones is responsible for growth factor activation at the stem cell surface, we injected heparitinase-1 (cutting growth factor binding via heparan-sulfate chains) prior to growth factors and analyzed the effect on neural stem cell proliferation in vivo.
Results: Fractones specifically bound FGF-2, BMP-4 and BMP-7 via HSPG in the primary adult neurogenic zone, the subventricular zone (SVZ) of the lateral ventricles. Binding of FGF-2 and BMP-7 to fractones was required to respectively stimulate or inhibit neural stem cell proliferation in the SVZ.
Conclusions: Our results demonstrate that fractones promote stimulatory and inhibitory growth factors at the stem cell surface. We anticipate that fractones are the stem cell niche structures responsible for most ECM/growth factor interactions that ultimately govern stem cell fate and the production of new specialized cells throughout life.

Workshop II-A

2W-01

Bone formation and ECM remodeling cease within a limited period regardless of completion of bone healing in the rat calvarial defect

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Healing of bone defects depends on a size of the defect, *i.e.* a bone defect larger than a certain size (a critical size) does not heal completely. There have been few reports on healing of bone defects, whereas numerous studies have investigated that of bone fracture. It has not been known how and why bone formation ceases in the course of healing of the large bone defect. Bone formation during development involves extensive remodeling of extracellular matrices (ECM), which is achived by both production and degradation of ECM. Our previous study suggested that osteoblasts and osteocytes secrete matrix metalloproteinases (MMPs) 2, 8 and 13 and play a role in ECM degradation as well as ECM production during bone development. The present study was designed to investigate the process of bone healing in the critical size defect focusing on the bone healing rate and the cellular activity of ECM production and degradation using the standardized rat calvarial bone defect model.

Twelve-week-old male Wistar rats were used. A full-thickness standardized trephine defect, 8.8 mm in diameter, was made in the rat parietal bone under anesthesia. The rats were fixed by perfusion through the aorta in days 1, 3 and weeks 1, 2, 3, 5, 8, 10, 12, 18, 24 and 36. The resected calvaria were radiographed for morphometric analysis of bone matrix appostion per week and then processed for in situ hybridization for type I collagen, osteocalcin and MMPs 2, 8 and 13. Alternatively, RNA was extracted from tissue that filled the original bone defect at the same time points and processed for quantitative analysis of expression of these bone matrix ECM proteins and MMPs using real-time PCR.

The bone healing rate (i.e. the rate of bone matrix apposition per week) was the largest in the fourth week and decreased thereafter. Little bone was apposed in the 36th week with leaving the defect unrepaired. The expression of type I collagen and osteocalcin as well as MMPs 2 and 13 increased towards weeks 2 and 3 and decreased thereafter. In contrast, the expression of MMP 8 was the highest in day 1 and decreased. The mRNA transcripts of type I collagen and osteocalcin were localized in osteoblasts and osteocytes. Some of those cells expressed MMPs 2, 8 and 13. Expression of the bone matrix ECM proteins and MMPs was no longer identified in week 24.

The results indicated that osteoblasts and osteocytes cease bone formation and ECM remodeling within 24 weeks regardless of completion of bone healing of the defect in the experimental model.

2W-02

Effect of Collagen Tripeptide of Type I Collagen on Proliferation, Migration and Collagen Synthesis in Human Aortic Smooth Muscle Cells

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Objectives: Collagen tripeptide (CTP) is a tripeptide fraction containing Gly-X-Y sequence which is a degrading product of type I collagen by a bacterial collagenase and showed many biological activities in recent studies. The objective of this study is to evaluate the effect of CTP on the proliferation, migration and the synthesis of type I and IV collagens in cultured human aortic smooth muscle cells (AoSMCs).

Methods: Three different concentrations of CTP (3, 30, 300ug/ml) were used as stimuli and the culture of human AoSMCs were performed, and then, immunohistochemistry, Western-blot, ELISA and Polycarbonate Membrane kit were used to investigate the effect of CTP.

Results: We found CTP can prominently inhibit the proliferation of AoSMCs by down-regulating PCNA protein expression level ($P<0.05$) and PCNA-positive cell ratio ($P<0.01$). The inhibitory effect of CTP on cell migration was also shown ($P<0.05$). The obvious dose-dependence has not been shown among these three concentrations. CTP also can accelerate the fibrillogenesis of type I collagen and promote the expression of type IV collagen as extracellular matrix around the AoSMCs.

Conclusion: These findings verified the effect of CTP on the proliferation, migration and synthetic activity of AoSMCs in vitro for the first time and suggested its pharmacologic value for some cardiovascular diseases such as atherosclerosis.

2W-03

Integrin-dependent Cell Adhesion to The Peptide-based Artificial Collagen

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Keywords: Collagen, Synthetic peptide, Self-assembly, Cell adhesion

Objective: Recently, we developed collagen-like supramolecules by means of the self-assembly of chemically synthesized peptides [1, 2]. The peptides are disulfide-linked trimers of collagen-like Gly-X-Y triplet repeats with self complementary shapes. In this paper, we tested the supramolecular material for *in vitro* cell culture system.

Methods: We designed and chemically synthesized peptide containing a cell adhesive Gly-Phe-Hyp-Gly-Glu-Arg (GFOGER) sequence found in the native collagen triple helix, which interacts with integrin $\alpha 1\beta 1$ and $\alpha 2\beta 1$. To examine specific cell adhesion, the synthetic peptide or collagen I were coated to multiwell plate and human dermal fibroblasts (HDF) were cultured on the plates.

Results: Significant adhesion of HDF to the peptide supramolecule containing GFOGER motif was observed. On the other hand, supramolecules lacking the motif did not show such effects.

Conclusions: The result indicated that HDF adhesion to the material is mediated by the interaction between integrin and GFOGER motif displayed on the collagenous supramolecule. Such functionalized artificial collagen will open new opportunity for the development of innovative biomaterials.

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2W-04

Proteomic characterization of cartilage matrix synthesis and breakdown

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Keywords: Cartilage, arthritis, proteomics

Objectives: Development of neo-cartilage *in vitro* has important applications in tissue engineering/ cartilage repair and analysis of the response to catabolic agents and mechanical injury, two key contributors to cartilage degeneration in arthritis. However, primary chondrocyte culture is challenging due to the inter-dependence of phenotype with chondrocyte morphology, ECM and environment. We aimed to develop a high-density chondrocyte culture system and evaluate phenotype and response to catabolic agents using proteomics.

Methods: Mouse P3 chondrocytes seeded at high density were maintained in DMEM/FCS/ascorbic acid. Independent 21-day cultures were then treated for 4 days (serum-free) with interleukin-1α (IL1) or all-*trans*-retinoic acid (RetA) to induce ECM breakdown. 7, 14 and 21-day and cytokine-treated cultures were harvested and proteins extracted sequentially using 1M NaCl then 4M GuHCl, followed by pepsin digestion of the GuHCl-insoluble fraction. Comparative analysis of media and sequential protein extracts of control and stimulated cultures was performed using SDS-PAGE, 2-DE and DIGE. Shotgun mass spectrometry was used for global profiling of the NaCl and GuHCl-soluble fractions.

Results: Over 21 days, levels of GuHCl-soluble components (matrilin-1, collagen VI, SLRPs) and collagen II increased ~2-fold, while NaCl extracts showed little change. Haptoglobin, MMP3, cartilage gp-39 and lipocalin-2 were detected in the media of IL1-treated cultures, whereas RetA caused COMP release, consistent with our experiments in intact femoral head cartilage [**1**]. In the protein extracts of IL-1 and RetA cultures we detected loss of link protein and the appearance of aggrecan fragments.

Conclusions: Our high-density chondrocyte cultures deposit a cartilage-like ECM, with further proteomic characterization of the NaCl and GuHCl-soluble fractions ongoing. Importantly, these cultures facilitate proteomic characterization of media *and* extracts, an improvement on previous analysis of intact cartilage degradation.

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2W-05
Collagen in Frozen Mammoths

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Key words: Frozen Mammoth, Collagen

Objective: In order to examine characteristics of extracellular matrix (ECM) components supporting sinusoidal wall (scaffolding function) of the liver, we analyzed livers of 2 frozen baby mammoths died about 40,000 years ago and buried in permafrost in Siberia.
Methods: We observed the livers of the 2 mammoths (kept in fixatives in Russian Academy of Sciences in St. Petersburg) by light and electron microscopy, scrutinized localization of ECM components by immunofluorescence, and analyzed amino acid contents.
Results: The livers were preserved at gross anatomical and histological levels. Sinusoidal walls of the liver were kept. Ultrastructure of ECM components, namely fibrillar structure having characteristic pattern of cross striation and basement membrane structure, was clearly demonstrated by transmission and scanning electron microscopy. Type I and type IV collagens were shown in ECM components by immunofluorescence.
Conclusions: These results indicate that ECM components including collagen were stable and preserved in the livers of these frozen mammoths for 40,000 years. These findings suggest that three-dimensional structure of ECM is important for maintaining gross and histological morphology of the sinusoidal wall in the liver.

Workshop II-B
2W-06
The role of fibulins in elastic fiber assembly of mouse aorta

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Keywords: DANCE, Fibulin, Elastogenesis

Objective: Elasticity is an important character of various human organs, such as aorta, lungs and skin. Loss of elasticity may cause aging-related signs, such as tortuous aorta, lung emphysema and loose skin. Elastic fibers, which are responsible for tissue elasticity, are known to be composed of polymerized elastin, microfibrils, and microfibril-associated proteins. However, precise mechanisms of elastic fiber assembly are still not elucidated.
We have previously reported that DANCE/fibulin-5 (Developmental Arteries and Neural Crest EGF-like, or fibulin-5) is indispensable for elastogenesis, by showing that elastic fiber formation is impaired in DANCE-deficient mice[1]. Recently, fibulin-4 deficient mice were also reported to show severely impaired elastic fibers and die perinatally [2]. It is interesting that knockout mice of these fibulins show elastic fiber-related phenotypes without compensating each other. In the present study, we investigate into the difference of Fibulin-4 and DANCE.
Methods: Genetically engineered mice were crossed each other to produce fibulin-4 and DANCE knockout mice. The elasticity of aortas of these mice was investigated.
Results: Disruption of both fibulin-4 and DANCE lead to severer phenotype than DANCE knockout mice.

Conclusions: These results suggest that fibulin-4 and DANCE plays distinct roles in elastogenesis.

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2W-07

The carboxyl-terminal region of laminin beta chains modulates the integrin-binding affinities of laminins

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Keywords: Laminin, Integrin,

Objective: Laminins are major cell-adhesive proteins in basement membranes that are capable of binding to integrins. Laminins consist of three chains (alpha, beta and gamma), in which three LG modules in the alpha chain and the Glu residue in the C-terminal tail of the gamma chain have been shown to be prerequisites for binding to integrins. Here, we report that the C-terminal regions of beta chains are also involved in the modulation of integrin binding affinities of laminins.
Methods: All laminins and integrins were generated as recombinants using 293-F cells. The binding activities of laminins to a series of laminin binding integrins (alpha3beta1, alpha6beta1, alpha6beta4, alpha7X1beta1, and alpha7X2beta1) were assessed by the solid-phase binding assay.
Results: Laminins containing beta2 chain (e.g., laminin-121, 221, and 521) were more potent than those containing beta1 chain (e.g., laminin-111, 211, and 511) in the binding affinity toward the "X2" region-containing integrins (alpha3beta1 and alpha7X2beta1). Such potentiation in integrin binding affinities was not observed with "X1" region-containing integrins (alpha6beta1, alpha6beta4, and alpha7X1beta1). Production of a series of swap mutants between the beta1 and beta2 chains revealed that the C-terminal 20 amino acids in the coiled-coil domain were responsible for the enhanced integrin binding by beta2-containing laminins.
Conclusions: Our results provide evidence that the C-terminal region of beta chains is involved in laminin recognition by integrins, and modulates the binding affinities of laminins toward X2-type integrins.

2W-08

Calcium influences hemidesmosome formation and processing of laminin332

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Keywords: Laminin, Hemidesmosome, Processing

Objective: Hemidesmosomes (HDs) are cell-matrix adhesion complexes connecting cytoplasmic intermediate filament network to the basement membrane. Analyses of the HD fraction isolated from bovine cornea revealed that there are five major hemidesmosomal constituents, plectin, BPAG1, integrin $\alpha6\beta4$ and type XVII collagen. It has also been expected that there might be minor unidentified hemidesmosomal components. However, further analysis using the HD fraction has been hampered by the limited availability of bovine materials because of the bovine spongiform encephalopathy epidemic. Therefore, we tried to obtain a HD-rich fraction from human cultured cells.
Methods: A human squamous cell carcinoma cell line (DJM-1) and a human keratinocyte cell line (HaCaT) were cultured in serum-free keratinocyte growth medium (KGM, Invitrogen) with or without supplemental $CaCl_2$ for 10 days. These cells were treated with 20 mM NH_4OH to isolate HD proteins and laminin332. Isolated polypeptides were analyzed by immunoblotting.
Results: The amount of hemidesmosomal polypeptides was most prominent when the sample was prepared from DJM-1 cells cultured for two weeks in serum-free keratinocyte growth medium (Invitrogen) without additional Ca^{2+}. The concentration of Ca^{2+} is of critical importance for the accumulation of HDs, since the amount of hemidesmosomal polypeptides in the fraction markedly decreased when the cells were cultured in the presence of 1 mM Ca^{2+}. Electron microscopy demonstrated that the cells cultured with calcium-free KGM contain a good many electron-dense plaque-like structures along the basal cell membrane, showing that they are not only rich in HD components but also superior in the formaiton of HD-like adhesion structures. We also found that processing of laminin $\alpha3$ and $\gamma2$ chains was suppressed in these HD-rich cells. The suppression may account for the accumulation of HD-like structures.
Conclusions: Our results demonstrated that the accumulation of HD was promoted in DJM-1 cells cultured with calcium-free KGM.

2W-09
TGFbeta-Dependent Localization of MT1-MMP Regulates Epithelial Tuburogenesis in 3D Collagen

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Objectives: Epithelial tubes are essential structures in multicellular organisms. However, the fundamental mechanism underlying the formation of such structures is still unclear. Previously it was reported that that MT1-MMP is essential for tube extension, but its activity needs to be regulated in order to form organised structures. In this paper, we investigated regulation of MT1-MMP during epithelial tubulogenesis using a Madin-Darby Canine Kidney (MDCK) cells as a model.
Methods: MDCK cells were cultured on collagen film in the trans-well chamber and FLAG-tagged MT1-MMP and its mutants were expressed, and their localization were analyzed by confocal microscopy. Tubulogenesis was studied by culturing MDCK cells in 3D collagen gel with stimulation of hepatocyte growth factor (HGF) and/or transforming growth factor beta (TGFbeta).
Results: Polarized MDCK cells cultured on fibrillar collagen exclusively localized MT1-MMP to the apical side of their plasma membrane. Upon treating cells with HGF, which stimulates tubulogenesis of MDCK cells in 3D collagen, MT1-MMP was localized to the basal side, resulting in efficient collagen degradation. In 3D collagen MT1-MMP localized to basal side of cells that were extending into the collagen gel, but cells in non-extending parts of the structure did not localize MT1-MMP to their basal side. TGFbeta is spontaneously expressed in MDCK cells and has been shown to inhibit tubulogenesis. We found that addition of TGFbeta at 10 ng/ml inhibited both tubulogenesis and basal localizaion of MT1-MMP, indicating TGFbeta is indeed a negative regulator. However, inhibition of TGFbeta signaling rather inhibited both tubulogenesis and MT1-MMP localization to the basal side. Interestingly, lower dose of TGFbeta at100 pg/ml enhanced both tubulogenesis and basal localization of MT1-MMP, indicating that action of TGFbeta is biphasic.
Conclusion: Taken together, differential local TGFbeta signaling may be a key to regulate local MT1-MMP levels at the basal side of the epithelial cells, resulting in formation of tube structure.

2W-10
A Prolonged Decrease in Phospholipase D Activity Modulates ECM Turnover by Increasing EGF Receptor Signal Transduction in Cultured Human Fibroblasts

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Objective: The turnover of extracellular matrix is tightly regulated by several cytokines and growth factors. We have previously reported that *N*-methylethanolamine (NME) suppress the collagen accumulation in dermal fibroblasts by decreasing phospholipase D (PLD) activity[1)]. In the study reported here, we found that a sustained treatment of dermal fibroblasts with NME enhanced the production of MMP-1 and hyaluronan (HA) after stimulation with EGF or bFGF. We investigated the contribution of PLD to the regulation of MMP-1 and HA production through EGF receptor (EGFR) signal transduction in dermal fibroblasts.
Methods: Normal human dermal fibroblasts were treated with 1 mM NME or PLD1 siRNA prior to stimulation with EGF. The mRNA expressions of *MMP-1* and HA synthase 2 (*HAS2*), and the production of MMP-1 and HA were quantified by real time PCR and ELISA, respectively. The levels of phosphorylated EGFR, ERK1/2 and MEK1/2 were evaluated by western blot analyses.
Results: PLD activity was decreased in fibroblasts after 6 days of NME treatment. Under these conditions, the mRNA levels of *MMP-1* and *HAS2* and the production of MMP-1 and HA in response to EGF were higher than those in non-treated cells. The levels of the phosphorylated EGFR and its downstream signaling molecules, phosphorylated ERK1/2 and MEK1/2, which are shown to be involved in EGF-mediated *MMP-1* and *HAS2* mRNA expressions, were also increased in NME-treated fibroblasts. Furthermore, the treatment of PLD1 siRNA was able to mimic the effects of NME: increased phosphorylations of EGFR, ERK1/2, and MEK1/2 were observed, followed by increased levels of *MMP-1* and *HAS2* mRNA expressions accompanied with MMP-1 and HA production.
Conclusions: PLD modulates MMP-1 and HA production by regulating the phosphorylation of EGFR in response to EGF in dermal fibroblasts.
Reference: 1) Yamamoto K., et al. (2004) *Eur. Heart J.,* 25, 1221-1229

Memo

Otaka Prize Lecture 1

Hyaluronan Oligosaccharides Inhibit Tumorigenicity of Osteosarcoma Cell Lines via Perturbation of Hyaluronan-Rich Pericellular Matrix of the Cells

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Numerous studies have demonstrated a correlation between hyaluronan expression and the malignant properties of various kinds of cancer, and inhibition of hyaluronan production causes decreased tumor growth. Hyaluronan oligosaccharides have been shown to inhibit several tumor cell types via disruption of receptor-hyaluronan interaction. However, few studies have addressed hyaluronan with respect to osteosarcoma. In this study, we examined the effects of exogenously added hyaluronan oligosaccharides on tumorigenicity of murine osteosarcoma cells, LM-8, and human osteoblastic osteosarcoma cells, MG-63. Moreover, the critical size of oligomers needed to inhibit malignant properties was defined. Fluorescent hyaluronan oligosaccharides accumulated both on the surface of cells and in the cytoplasm, and this retention was blocked by pretreatment with an anti-CD44 monoclonal antibody. Hyaluronan octasaccharides significantly inhibited cell viability and induced apoptosis as defined by cell proliferation and terminal deoxynucleotidyl transferase dUTP nick-end labeling assays, respectively. Octasaccharides also abrogated functional cell-associated matrices and significantly reduced the retention of endogenous hyaluronan. Further, octasaccharide treatment affected an inhibition of cell motility as well as cell invasiveness. Pretreatment of the cells with anti-CD44 antibody reduced the antitumor effect of the octasaccharides. In vivo, intratumoral injection of hyaluronan octasaccharides reduced the hyaluronan accumulation in local tumors, resulting in significant suppression of the formation of distant lung metastasis. Together these data suggest that hyaluronan oligosaccharides have potent antitumor effects functioning in part by the abrogation of hyaluronan-rich cell-associated matrices.

平成 20 年度大高賞受賞講演 1

ヒアルロン酸オリゴ糖は骨肉腫細胞株 MG-63 および LM-8 においてヒアルロン酸リッチな細胞周囲マトリックスを阻害することで抗腫瘍効果を発現する

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ヒアルロン酸（HA）蓄積の上昇はいくつかのの癌腫で確認されており，*in vitro* で HA 発現レベルは腫瘍細胞浸潤能，転移能に関与すると報告されているが，骨肉腫細胞において HA の関与を報告したものは少ない．骨肉腫は小児を含む若年齢者で最も多い悪性原発性骨腫瘍であり，その予後は化学療法導入後に改善しているとはいえ，多くの生命が失われており，骨肉腫患者に対する新たな治療選択肢の開発が必要である．近年,HA オリゴ糖が CD44 を代表とする HA レセプターを介して HA リッチなマトリックス形成を阻害することで様々な効果を発現することが報告され，実用化が期待されている．
本研究ではヒトおよびマウス骨肉腫細胞株 MG-63，LM-8 に対する HA オリゴ糖の抗腫瘍効果を CD44 とマトリックス形成阻害に着目して解析し，またその効果を発現するのに最適な HA オリゴ糖のサイズを解析した．
本研究の要約は
１．骨肉腫細胞において HA8 糖を投与することにより *in vitro* では細胞増殖抑制効果および浸潤能抑制効果が，*in vivo* では肺転移抑制効果が確認された．HA8 糖を投与することで内在性 HA 蓄積を阻害するとともに細胞周囲マトリックス形成を阻害が確認され，これが抗腫瘍効果に関与していると考えられた．さらに HA 蓄積阻害は細胞質においても確認されることから CD44 を介した HA の細胞内取り込みも阻害されていることが示唆された．
２．骨肉腫細胞において HA オリゴ糖は CD44 に特異的に結合することが示され，抗腫瘍効果発現の相違には CD44 のリガンドとしてのサイズが関連しており，HA8 糖以上のサイズが必要であると考えられた．
本研究は骨肉腫における HA オリゴ糖の抗腫瘍効果とそのメカニズムが解析され，臨床応用の可能性が示唆された．

Otaka Prize Lecture 2

Study of deposition and maturation of tropoelastin molecule on elastic fiber assembly

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Objective: Elastin is a highly insoluble extracellular matrix protein and the core protein of the elastic fibers that impart resilience to elastic tissues. Loss of elasticity is observed in a range of serious diseases or age-related lesions. However the mechanisms of elastic fiber formation remains unclear, it is believed that deposition onto microfibrils and maturation of tropoelastin (TE) is important. In this study, characterization of TE molecule was demonstrated on the deposition and maturation of TE in elastic fiber formation.
Methods: We demonstrated the deposition and maturation of TE using an *in vitro* model of elastic fiber assembly. Elastic fiber was evaluated by immunofluorescence staining, the quantitative analysis of cross-linked amino acids, and semi-quantitative analysis of matrix-associated tropoelastin.
Results: Our data showed that C-terminal of TE and whole molecule of TE is required for the deposition and maturation of TE, respectively. Moreover it is suggested that molecular interaction between TE and DANCE/fibulin-5 is important for the maturation of TE.
Conclusion: This study would provide new information for the mechanism of formation and regeneration of elastic fibers.

平成 20 年度大高賞受賞講演 2

弾性線維形成におけるトロポエラスチン分子の沈着と成熟化に関する研究

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【背景】弾性線維は、組織の弾性維持に大きく寄与しており、加齢や疾患などによるこれら組織の破綻は、組織や臓器の機能不全を引き起こすことが知られている。弾性線維形成の詳細な機序は未だ不明であるが、トロポエラスチン（TE）が足場タンパク質であるマイクロフィブリルに沈着し、分子間架橋による成熟化が重要であると考えられている。そこで、本研究では、弾性線維形成における TE 分子の沈着と成熟化に対する TE 分子の特性について検討した。【方法】ヒト網膜色素上皮細胞の培養上清に各種組換えトロポエラスチンを添加して培養した。蛍光免疫染色とデスモシンの定量によりエラスチン線維の確認を行い、TE の自己集合やエラスチン結合タンパク質との相互作用についても生化学的に解析した。【結果・結論】本研究結果より、TE の C 末端領域は TE の沈着に、そして TE 分子全体の構造はエラスチン線維として成熟するために必要であることを明らかにした。更に、エラスチン線維の成熟には、自己集合した TE と DANCE/fibulin-5 との分子間相互作用が重要であることが示唆された。本研究成果は、エラスチン線維形成機序の解明に新しい知見を与え、弾性線維再生の研究に役立つものと考えられる。

Poster Session I:

1P-01

Novel chondro-protective mechanisms of hyaluronic acid: down-regulation of ADAMTS-7 and ADAMTS-12, and reduced COMP release from articular cartilage

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Keywords: COMP, ADAMTS, hyaluronic acid

Objective: Hyaluronic acid (HA) is known to down-regulate matrix metalloproteinases (MMPs) and a disintegrin and metalloproteinases with thrombospondin motifs (ADAMTSs) in both chondrocytes and synoviocytes, and widely used for both osteoarthritis and rheumatoid arthritis patients in the clinical settings. However, it is not well understood whether HA could protects articular cartilage from proteolytic degradation. Cartilage oligomeric matrix protein (COMP) is a noncollagenous extracellular matrix protein consisting articular cartilage, and released from matrix by proteolytic degradation in the presence of MMPs and ADAMTSs. In this study, we examined whether HA could inhibit the proteolytic release of COMP from articular cartilage.
Methods: Bovine articular cartilage was cut into small pieces and incubated with RPMI in the presence of IL-1beta and synovium-derived SW982 cells, with or without HA. COMP levels were measured using ELISA. SW982 cells were also incubated with IL-1beta in the presence of HA, then mRNA was extracted, and the expression levels of MMP-3, ADAMTS-4, -5, -7, and -12 were examined using real-time PCR.
Results: Proteolytic release of COMP from bovine cartilage samples was up-regulated about 2-fold over the control level when incubated with SW982 for 3 days. This up-regulation was significantly inhibited in the presence of HA. In SW982 cells, the expression levels of MMP-3, ADAMTS-7 and ADAMTS-12 were increased about 3- to 5-fold over the control levels when stimulated with IL-1beta. HA also significantly inhibited these IL-1beta-induced up-regulation of proteases.
Conclusions: Chondro-protective effects of HA were observed in this study. HA suppressed proteolytic release of COMP from articular cartilage stimulated by IL-1beta and SW982 cells. This inhibitory effect is thought to be a result from down-regulation of MMP3.

1P-02

Ectopic bone formation after implantation of thermoreversible gelation polymer as a carrier of Bone morphogenetic protein-2

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Keywords: thermoreversible gelation polymer, carrier, Bone morphogenetic protein-2

Objective: Previous studies have reported that bone morphogenetic proteins (BMPs) induced ectopic bone formation after implanted with carrier materials in rats, and BMP-induced osteo-/ chondrogenesis was highly dependent upon the carrier. Thermoreversible gelation polymer (TGP) is characterized by its temperature-dependent dynamic viscoelastic properties. The sol-gel transiting temperature (SgTT) of TGP is 20°C. The purpose of this study was to investigate the ectopic bone formation on implantation of rhBMP-2 using TGP as carrier in a rat subcutaneous assay model and disposal of residual rhBMP-2/TGP after ectopic bone formation.
Methods: Twenty 8-week-old Wistar rats were used in the experiment. Subcutaneous pockets were created on the back of rats. The pockets were implanted with rhBMP-2/TGP, TGP alone, rhBMP-2/collagen, collagen alone. The rats were sacrificed at 10days, 4 and 8weeks for histological evalutation.
Results: Both rhBMP-2/TGP group and rhBMP-2/collagen group at 4 and 8 weeks after implantation, ectopic bone formation was found. In rhBMP-2/TGP group, the bone formation was found on the surface of the implanted carriers. In the TGP alone group, ectopic bone formation was not observed and connective tissue was found on the surface of the implanted carrier. In each case, TGP became hydrogel under 22°C and could be removed easily out of the implanted samples.

Conclusion: This study suggests that rhBMP-2/TGP could induce ectopic bone formation around the implanted samples. TGP could maintain in the same size as implantation without absorption and its thermoresponsive behavior even after it was implanted in vivo.

1P-03

Critical role of the TGF-β type I receptor ALK5 in skeletal development

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Keywords: ALK5, Perichondrium, Osteoblasts

Transforming growth factor-β (TGF-β) signaling initiates its diverse cellular responses by forming a specific cell surface complex consisting of a TGF-β ligand (TGF-β1, -β2, or -β3), and TGF-β type I (ALK5) and type II receptors. This activates downstream signaling through Smad-dependent and/or -independent pathways. The precise *in vivo* role of TGF-β signaling in skeletal development is not fully understood, primarily because of the redundant expression of TGF-β isoforms and diverse phenotypes ranging from early embryonic lethality to normal at birth of gene knockout (KO) mice for TGF-β signaling-related molecules. To circumvent these problems, we conditionally deleted *ALK5* ($ALK5^{CKO}$) in skeletal progenitors in mice using Dermo1-Cre, and identified the role of TGF-β signaling in skeletogenesis. We also used tamoxifen-inducible Cre-mediated ALK5 inactivation in primary neonatal calvarial cells. $ALK5^{CKO}$ mice were perinatally inviable, and $ALK5^{CKO}$ embryos exhibited severe defects in bone and perichondrium formation, with reduced mineralization, partial joint fusions, and abnormal cartilaginous ectopic protrusions formed from the resting/proliferative zones. We found that osteoblast proliferation and differentiation were decreased in $ALK5^{CKO}$ calvaria and limbs. Proliferation of ALK5-deficient calvarial cells was reduced, in part through reduced JNK-MAPK and Smad signaling. We also found that these calvarial cells showed reduced ability for osteogenic differentiation because of reduced activities of MAPK and Smad pathways. In contrast, adipocytogenesis was elicited even though the cells were cultured in osteogenic differentiation conditions, through the inhibition of p38-MAPK and Smad pathways. These results indicate that TGF-β signaling via ALK5 promotes the commitment of common osteoblast/adipocyte progenitors toward the osteoblast lineage. They also show that TGF-β signaling via ALK5 regulates osteoprogenitor proliferation and differentiation during skeletogenesis through Smad-dependent and -independent pathways.

1P-04

BMP-2 regulates expression of Gas6 during osteoblast differentiation

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Keywords: BMP-2, Gas6, osteoblasts

Objective: Bone morphogenetic proteins (BMPs) regulate many aspects of skeletal development, including osteoblast and chondrocyte differentiation, cartilage and bone formation, and cranial and limb development. Among them, BMP-2, one of the most potent osteogenic signaling molecules, stimulates osteoblast differentiation, but inhibits myogenic differentiation in C2C12 cells. To evaluate important genes for BMP-2-induced osteoblast differentiation, we performed cDNA microarray analysis between BMP-2 treated and untreated C2C12 cells, and identified growth arrest-specific 6 (Gas6) as a gene that was clearly induced during osteoblast differentiation. Gas6 is a ligand for Axl, Sky, and Mer among the receptor tyrosine kinases, and its interactions with these receptors have been implicated in cell proliferation and differentiation. The aim of this study was to investigate the mechanism that governs the regulation of Gas6 gene expression by BMP-2.
Methods: During BMP-2-induced osteoblast differentiation, C2C12 cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM) containing 2.5% fetal bovine serum (FBS). 4 days after the addition of BMP-2 (400 ng/ml), total RNAs were extracted and subjected to GeneChip® analysis. Gene knockdown experiment was performed by Stealth™ siRNA according to the manufacturer's protocol. Treatment with the p38 MAPK inhibitor, SB203580 abolished BMP-2-induced activation of p38 MAPK.
Results: The cDNA microarray analysis revealed that expression of Gas6 was significantly up-regulated during BMP-2-induced osteoblast differentiation. BMP-2 enhanced Gas6 gene expression in a time- and dose dependent manner. The osteogenetic activity induced by BMP-2 is mediated by both canonical Smad signaling and p38 MAPK activation. BMP-2-induced Gas6 expression was blocked by Smad4 siRNA and p38 MAPK inhibitor, SB203580.

Conclusions: Our results indicate that the expression of Gas6 was enhanced by BMP-2 through the mechanism dependent on Smad signaling and p38 MAPK activation. It will now be important to study whether Gas6 induction by BMP-2 is involved in osteoblast differentiation.

1P-05
Role of Carbonic Anhydrase IX in Chondrocyte Differentiation

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Keywords: Carbonic anhydrase, Differentiation, Chondrocyte

Objective: Carbonic anhydrase (CA) IX is one of the membrane-bound isoforms of CAs which catalyze the reversible hydration of carbon dioxide to bicarbonate. Recently CA IX attracts attention for its high expression in tumor. It is also known that the expression of this enzyme is induced under hypoxic conditions, suggesting its possible distribution in cartilage. Here we investigated the expression and biological roles of CA IX in mouse chondrocytes *in vitro*.
Methods: Real-time RT-PCR and Western blot analysis were employed to assess the mRNA and protein levels of CA IX in mouse primary chondrocytes as well as in mouse chondrogenic ATDC5 cells. ATDC5 cells transfected with siRNA for CA IX gene or its control were treated with insulin and/or bone morphogenetic protein-2 (BMP-2) to promote their chondrogenic differentiation. Expression of the mRNAs for the marker genes of chondrocyte differentiation, such as type II collagen, type X collagen, aggrecan, Sox9, Sox6, and Sox5 were quantitatively analyzed to examine the role of CA IX in chondrocyte differentiation.
Results: Expression of mRNA and protein for CA IX increased in the primary chondrocytes after treatment with BMP-2 as well as in ATDC5 cells cultured in the presence of insulin. In the same conditions, the gene expression of type II collagen, type X collagen, aggrecan, and Sox6 were also up-regulated in these cells. In ATDC5 cells of which CA IX was knocked down by siRNA, insulin- or BMP-2-induced expressions of type II collagen, type X collagen, aggrecan, and Sox6 were significantly suppressed.

Conclusions: The present results indicate that CA IX functions as a positive regulator of the differentiation of chondrocytes.

1P-06
Reactive Oxygen Species reduce the Eexpression of BRAK/CXCL14 in Human Head and Neck Squamous Cell Carcinoma Cells

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Keywords: ROS, BRAK, HNSCC

Objective: It has been previously reported that oxidative stress stimulates gene expression of IL-8/CXCL8 (IL-8), which is ELR-motif angiogenic CXC chemokine in human squamous cell carcinoma (SCC). We recently demonstrated BRAK, which is also known as non-ELR motif angiostatic CXC chemokine ligand 14 (CXCL14) to have anti-tumor activity in human head and neck squamous cell carcinoma (HNSCC) cell. Here we investigated the effects of oxidative stress induced by reactive oxygen species (ROS) such as hydrogen peroxide (H_2O_2) and hydroxyl radical ($HO^{\bullet}$), on the expression of both *CXCL8* and *CXCL14* in HNSCC.
Methods: HNSCC cells were cultured in DMEM-10; and after serum starvation, nearly confluent cells were cultured in the presence or absence of ROS with or without addition of N-acetylcysteine (NAC) or MAPK inhibitors. Messenger RNA levels were measured by quantitative PCR method and protein levels by western blotting. By use of electron spin resonance, we confirmed $HO^{\bullet}$ generation by Fenton's reaction ($H_2O_2/FeSO_4$).
Results: When the HNSCC cells were cultured in the presence of ROS, the expression of *CXCL14* was significantly decreased; whereas that of *CXCL8* was increased. Interestingly, the effects on the expression of both genes in HNSCC cells were much greater with $HO^{\bullet}$ than with H_2O_2. The effects of ROS on both *CXCL8* and *CXCL14* expression were attenuated by the pretreatment with NAC or MAPK inhibitors.
Conclusions: Oxidative stress induced by ROS stimulates not only an increase in the expression of *CXCL8* but also a decrease in that of *CXCL14* in HNSCC cells. These results indicate that oxidative stress induces angiogenesis of tumor progression by regulating the gene expression of both angiogenic and angiostatic factors in HNSCC cells.

1P-07

Optical imaging of mouse articular cartilage using the glycosaminoglycans binding property of fluorescent-labeled octaarginine

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Keywords: Optical imaging, Articular cartilage, Arthritis

Objective: The aim of the current study was to examine the cartilage-specific binding property of polyarginine peptides (R4, 8, 12, and 16) and specifically to test octaarginine peptides for the optical imaging of articular cartilage in experimentally-induced arthritis in mice.

Methods: Four rhodamine-labeled polyarginine peptides each with a different-length arginine chain were injected into the knee joints of mice.

Results: Fluorescent signals were specifically detected in the cartilage pericellular matrix from the surface to the tide mark but were completely absent in the calcified layer or bone marrow. The number of arginine residues significantly influenced peptide accumulation in articular cartilage, with R8 accumulating the most. The fluorescent signal in the femoral condylar cartilage diminished when it was treated with Ch'ase ABC. R8 accumulation was significantly decreased in the degenerative cartilage of CAIA mice, and this was demonstrated both histologically and in 3D-reconstruction image by OPT.

Conclusion: R8 may be a useful new experimental probe for optical imaging of normal and arthritic articular cartilage.

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1P-08

The application of elastin haploinsufficiency mice on lung disease with aging

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Keywords: Elastin, lung, Aging

Objective: Elastin (ELN) is a highly insoluble extracellular matrix (ECM) protein and the core protein of the elastic fibers that impart resilience to elastic such as skin, lungs, ligaments, and arterial walls. Loss of elasticity is observed in a range of serious diseases or age-related lesions, such as arteriosclerosis, emphysema, or chronic obstructive pulmonary disease (COPD). However, COPD is predisposed the increase of incidence, experimental model of COPD with aged mice is not still established. The purpose of this study was to investigate the expression of elastic fiber related protein with experimental lung disease in elastin haploinsufficiency mice.

Methods: ELN+/+ or ELN+/- was treated with elastase in nose to induce lung disease. The elastic fiber related mRNA and protein expression in lung was determined by RT-PCR and Western blot assay, respectively. Tropoelastin expression in BALF was also determined by Western blot assay.

Results: The mRNA expression of senescence maker protein 30 (SMP 30) significantly reduced in retire mice and ELN +/-. The mRNA and protein expression of tropoelastin decreased and increased on the 3rd day after treatment with elastase in ELN +/+ and ELN +/-, respectively. Increase of tropoelastin expression in BALF was observed in both ELN +/- treated with elastase and human lung disease such as COPD.

Conclusions: In this study, our data show that the heterozygous mutation mouse in elastin gene showed phenotype same as aged mouse and experimental model of lung disease with elastase showed similar data with human lung disease such as COPD. These results suggest that ELN +/- is very useful for the study of novel diagnostic procedures or new therapeutic approaches for the patients with lung disease.

1P-09

Phenotype of vascular smooth muscle cells and aortic calcification in elastin haploinsufficiency mice

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Keywords: Elastin, Calcification, Phenotype

Aim: It is known that elastin knockout mice (ELN-/-) die of an obstructive arterial disease, which results from subendothelial cell proliferation and reorganization of smooth muscle and ELN+/- occur hypertension and decrease blood flow in kidney such as aging mice. In the present study, we examined arterial obstruction with phenotypic change from smooth muscle cells (SMCs) in elastin knockout mice (ELN+/-) and aging mice.
Methods: The mRNA expressions were determined by RT-PCR. Moreover, ELN+/+ and ELN+/- mice were induced vascular calcification by treatment with activated Vit.D_3. The aortic calcium deposition was determined by Calcium C-test Wako.
Results: The mRNA of SM22α, smooth muscle lineage markers, and *h*-Caldesmon, smooth muscle contraction markers, decreased in aging mice and elastin knockout mice ELN+/-. Aortic calcification induced by activated Vit.D_3 also significantly increased in the ELN+/- mice compared with ELN+/+ mice. Phenotypic change from VSMCs to osteogenic cells promoted in ELN +/- treated with 1α, 25-$(OH)_2$ vitamin D_3.
Conclusion: These results suggest that male Eln+/- mice are useful for aging model. Our present data would provide beneficial information on the development of therapeutic approach for arterial calcification, especially aged patients.

1P-10

Involvement of Lipid Raft-associated Signaling in EMMPRIN Gene Expression and Secretion

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Keywords: EMMPRIN, Lipid raft, Phosphorylated Akt, Methyl-b-cyclodextrin

Objective: Extracellular matrix metalloproteinase inducer (EMMPRIN) is a membrane-bound glycoprotein with two extracellular loop domains and has been characterized as a tumor invasion stimulator. Although EMMPRIN has been expressed on the cell surface of various tumors, recent reports show that soluble EMMPRIN exists in some tumors. However, the regulatory mechanisms of EMMPRIN expression and secretion are not fully understood. Since EMMPRIN has been reported to be anchored in lipid raft, we here examined whether or not lipid raft signaling may influence the expression and secretion of EMMPRIN in human uterine cervical carcinoma SKG-II cells.
Methods: Preconfluent and confluent SKG-II cells were pretreated for 1 h with methyl-b-cyclodextrin (MBCD), an inhibitor of lipid raft formation, and then treated for up to 24 h in a fresh medium without MBCD. The gene expression of EMMPRIN was analyzed by real-time PCR. The production and secretion of EMMPRIN and phosphorylated Akt (pAkt) were investigated by Western blot analysis.
Results: Western blot analysis showed that the expression of pAkt was detectable in SKG-II cells, indicating that a lipid raft signaling pathway, phosphatidylinositol 3-kinase (PI3K)/Akt, was constitutively activated. In addition, MBCD-treatment was found to decrease the level of pAkt. Both gene expression and secretion of EMMPRIN were enhanced in SKG-II cells under low-cell density culture conditions rather than the confluent culture. Furthermore, the level of pAkt was concomitantly augmented in the pre-confluent cells. The gene expression and secretion of EMMPRIN were dose-dependently inhibited by MBCD in preconfluent SKG-II cells.
Conclusion: These results provide novel evidence that the regulation of gene expression and secretion of EMMPRIN is dependent on lipid raft formation in SKG-II cells, in that a PI3K/Akt signaling pathway contributes to the augmentation of both EMMPRIN transcription and secretion.

1P-11

Suppression of EMMPRIN-mediated Tumor Cell Migration by Syndecan-1

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Keywords: EMMPRIN, Cell migration, Syndecan-1, Heparan sulfate

Objective: EMMPRIN (extracellular matrix metalloproteinase inducer) is a membrane-bound glycoprotein with two extracellular loop domains. We have previously revealed that EMMPRIN enhances tumor cell migration through an active site in the second loop domain (termed EM9). On the other hand, EMMPRIN has been reported to bind to various functional molecules through the extracellular loop domains, which is closely associated with the physiological and pathological functions of EMMPRIN. However, the molecular mechanism of EMMPRIN-mediated tumor cell migration is still unclear. In the present study, we examined the association of Syndecan-1, a membrane-bound heparan sulfate proteoglycan, with EMMPRIN-mediated tumor cell migration in human uterine cervical carcinoma SKG-II cells.

Methods: The expression of EMMPRIN and Syndecan-1, and their interaction on the cell surface of SKG-II cells was investigated by immunocytochemical, Western blot, and co-immunoprecipitation analyses. Cell migration was measured by scratch-wound assay.

Results: EMMPRIN and Syndecan-1 were co-localized on the cell surface of SKG-II cells. Syndecan-1 was found to form a complex with EMMPRIN through its heparan sulfate, whereas the enzymic deletion of *N*-glycosylation in EMMPRIN did not alter the interaction between Syndecan-1 and EMMPRIN. A synthetic EM9 peptide was found to interfere with the EMMPRIN-Syndecan-1 interaction. In addition, the deletion of heparan sulfate in Syndecan-1 by heparinase, derived from a Gram-negative bacteria, was found to facilitate SKG-II cell migration. Furthermore, the cell migration of SKG-II cells was dose-dependently augmented by administering an antibody against the EM9 peptide.

Conclusion: These results provide novel evidence that Syndecan-1 negatively regulates EMMPRIN-mediated tumor cell migration by a heterogeneous complex formation in that heparan sulfate of Syndecan-1 interacts with the EM9 region in the second loop domain of EMMPRIN.

1P-12

Release of emmprin as glycocalyceal bodies

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Keywords: Matrix metalloproteinases, emmprin, tumor invasion, sarcoma

Objective: Emmprin is involved in tumorigenesis via stimulating production of matrix metalloproteinases (MMP), hyaluronan, and vascular endothelial growth factor (VEGF) by stromal fibroblasts and tumor cells. In human tumors, peritumoral fibroblasts that are not in direct contact with emmprin-expressing tumor cells are frequently stimulated to produce MMP. Here we investigated the mechanisms of emmprin release [1].

Methods and Results: Conditioned medium (CM) of human epithelioid sarcoma cell line FU-EPS-1 stimulated MMP-2 production by dermal fibroblasts, and this stimulation was inhibited by anti-emmprin antibody. Fractionation of CM by ultracentrifugation revealed selected presence of highly glycosylated form of emmprin (60 kDa) in the small vesicle fraction (glycocalyceal body fraction) compared with the larger vesicle fraction. This 60 kDa form of emmprin reacted with antibodies raised against N- and C-terminal portions of emmprin, indicative of the release of full length emmprin. Localization of emmprin on glycocalyceal bodies was also identified by immunoelectron-microscopical techniques. Biochemical and biological characteristics of emmprin-positive glycocalyceal bodies were studied.

Conclusions: Our results indicate that emmprin is released as glycocalyceal bodies from tumor cells and may stimulate peritumoral fibroblasts.

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1P-13

Primary Culture of Hepatocytes on A13 Peptide derived from Laminin Alpha1 Chain

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Keywords: laminin, hepatocyte

Primary hepatocytes are widely considered to be ideal for liver tissue models that are useful in fundamental biological studies, bio-artificial liver devices, and drug screening. However the isolated cells rapidly lose viability and phenotypic functions upon isolation from in vivo microenviroments of the liver. Primary hepatocytes are often cultured on laminin-111, heterotrimer composed of alpha1, beta1, and gamma1 chains, to maintain hepatic functions. Of three laminin type chains, laminin alpha1 is a candidate subunit that regulates cell behavior. Our previous studies have reported that synthetic peptides derived from laminin alpha1 exhibit biological functions such as cell adhesion, migration, angiogenesis, and tumor metastasis. In this study, we screened hepatocyte attachment peptides using the twenty-five biologically active peptides and examine the maintenance of hepatic function on peptides. Rat hepatocytes were isolated by two-steps collagenase perfusion and used for cell attachment assay. Of the peptides, A13 (RQVFQVAYIIIKA), mouse laminin alpha1 chain residues 121-133 exhibited the strongest activity. Furthermore, primary heptocytes on A13 peptide maintained the gene expression of hepatic differentiation markers such as Tyrosine aminotransferase, Tryptophan-2,3-dioxygenase, and Cytochrome P450. We also determined the active core sequence of A13, using systematically truncated N- and C-terminal peptides. The results indicated that the nine-amino acid sequence RQVFQVAYI was critical for A13-hepatocytes adhesion activity. However, the truncated peptides could not interact with beta1-intgerin and maintain the gene expression of hepatic differentiation markers. The amino acid sequence of A13 peptide was required for regulating hepatocyte behavior. Our results showed that the synthetic peptide regulates not only cell attachment but also hepatic functions. The hepatocyte adhesive peptides may be utilized in tailoring synthetic bio-surfaces in order to achieve a specific cellular response.

1P-14

Evaluation of dermal degeneration in photoaged skin using polarization-sensitive optical coherence tomography

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Photoaging is the extrinsic aging caused by sun exposure. Photoaged skin is observed in exposed areas, such as exterior forearm, face, and neck. UV irradiation causes dysplasia of the keratinocytes in the epidermis and dermal degeneration of the extracellular matrix component, which leads to the formation of wrinkles. Collagen, a main dermal extracellular matrix component, is a major target for photo-damage. UV irradiation impairs the metabolism and the collagen fibers. Photoaging should be diagnosed by non-invasively evaluating the collagen structure.

Polarization-sensitive optical coherence tomography (PS-OCT) permits non-invasive three-dimensional visualization of dermal birefringence, mainly due to the highly aligned and packed structure of the collagen fiber. The purpose of this study is to evaluate the dermal degeneration of photoaged skin using PS-OCT.

We began by measuring the dermal birefringence of cheek skin (exposed area) and the skin of the interior upper arm (protected area) on old and young volunteers. Our algorithm automatically produces a transverse dermal birefringence map from a scan of the skin, which allows quantitative comparison and visualization of the transverse distribution of the dermal birefringence.

We found that the old group had significantly less dermal birefringence of the cheek skin than the young group, whereas the interior upper arm showed no age-dependent difference. This suggests that degeneration of the upper dermal collagen structure is caused by photoaging, not intrinsic aging.

We then examined the relationship between dermal birefringence and the wrinkle morphology in the eye corner area using subjects in their 70's. The average upper dermal birefringence showed a significant depth-dependent correlation with the wrinkle-morphological parameters. This suggests that degeneration of the reticular dermis promotes wrinkle formation.

Analyses of dermal birefringence using PS-OCT are useful for diagnosing photoaged skin and investigating the wrinkle-formation mechanism.

1P-15
ADAMTS-4 and ADAMTS-5 in Degradation of Inner and Outer Zones of the Meniscus

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Objectives: Meniscal degeneration is a significant predictor of osteoarthritis (OA) development. Little is known about the mechanisms of meniscal degeneration compared with articular cartilage (AC).
Methods: Ovine AC and inner and outer zones of menisci were cultured for 4 days in serum free media ± IL-1α (10ng/ml) or TNFα (100ng/ml). Proteoglycan (GAG) and collagen (HyPro) content and release (% total), and mRNA expression of matrix proteins and enzymes were quantified.
Results: Meniscus HyPro > AC (16.5-21±2.6-3.1 versus 9.7±1.4μg/mg) with no difference between zones. Only outer meniscus released HyPro in response to cytokines. GAG content (μg/mg) of AC (49.3±21.0) > inner (25.8±9.0) > outer (7.7±8.8) meniscus. All meniscal zones released more GAG (% total) than AC in all cultures. Inner meniscus was more responsive than outer to IL-1α and TNFα. Tissue GAG content was consistent with relative aggrecan mRNA expression *ex-vivo.* Aggrecan mRNA increased in unstimulated AC but not meniscus, but was decreased by IL-1α and TNFα in all tissues. ADAMTS-5 but not -4 mRNA was increased in unstimulated cultures of meniscus but not AC compared with *ex-vivo*. ADAMTS-4 mRNA was up regulated in all tissues by IL-1α and TNFα, meniscus > AC and inner more responsive to IL-1α than TNFα. Cytokines increased ADAMTS-5 mRNA in all tissues, meniscus > AC, both zones more responsive to TNFα than IL-1α.
Conclusions: Our results highlight topographical differences in aggrecan and collagen turnover in the meniscus compared with AC. In meniscus, ADAMTS-5 mRNA levels correlated with higher basal GAG loss, while increased GAG release with IL-1 and TNF correlated with elevated ADAMTS-4 and -5 mRNA, respectively. High levels of ADAMTS enzymes secreted by the meniscus could contribute to AC degradation in the knee, and their differential regulation in meniscus compared with AC has important implications for potential anti-ADAMTS therapy in OA.

1P-16
MIG-17/ADAMTS interact with nidogen and UNC-6/netrin to guide the reader cell of organ

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Keywords: Nidogen, ADAMTS, Netrin

Objective: Remodeling of the extracellular matrix is an important process for organ morphogenesis in animal development. In *C. elegans*, the U-shape of the gonad is determined by the migration path of the gonadal leader cells. We previously demonstrated that a secreted metalloprotease of the ADAMTS family, MIG-17, regulates the directional migration of the leader cells. *mig-17(null)* mutation enhance the leader cell migration defects of *e78*, a weak allele of *unc-6/netrin* mutamt [1]. We also found that gain-of-function (*gf*) mutations in the gene *fibulin-1 (fbl-1)* can suppress the leader cell migration defects of *mig-17* mutants, suggesting that the FBL-1 protein interacts with MIG-17 in the gonadal basement membrane to control leader cell migration. Recently, we found that the suppression by *fbl-1(gf)* depends on NID-1/nidogen. The basement membrane localization of NID-1 was reduced in the loss-of-function mutants *mig-17* [2].
Methods: We checked whether *nid-1* is involved in *unc-6*-dependent guidance of the leader cell migration.
Results: Overexpression of NID-1 in *mig-17(null); unc-6(e78)* mutants substantially rescued the leader cell migration phenotypes.

Conclusions: Our results indicated that the interactions of *mig-17* and *nid-1* are required in the context of the *unc-6*-dependent guidance of the leader cell migration.

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1P-17

Analysis of the role of caspase-14 in ameloblast differentiation

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Keywords: Caspase-14, Ameloblasts, Cell differentiation

Objective: Epithelial-derived cells of the enamel organ, ameloblasts, synthesize enamel. Little is known about the characteristics of ameloblasts themselves or the regulatory mechanism of ameloblast differentiation. Here we analyzed the gene expression profile with DNA microarray to identify the genes responsible for ameloblast differentiation.
Methods: The enamel epithelium was isolated from the lower incisors of 7-day old mice. After 3 days culture, ameloblasts were purified by EDTA, and the time point of this separation was considered to be day 0 of the ameloblast culture. cRNA prepared from these cells was applied to the DNA microarray system to analyze the gene expression profile. RT-PCR and real-time PCR analysis were performed to investigate time-dependent changes in the expression level of genes that are expressed specifically in ameloblasts.
Results: In DNA microarray analysis, we found that several genes related to keratinocyte differentiation were specifically expressed at high levels in the ameloblasts. Among them, the expression level of caspase-14 was markedly increased during the culture of ameloblasts, in parallel with the up-regulation of kallikrein 4 (KLK-4), a marker for mature ameloblasts, and down-regulation of amelogenin, a marker for immature ameloblasts. Furthermore, the expression level of caspase-14 was strongly up-regulated by vitamin D_3. The expression level of amelogenin was suppressed by vitamin D_3, whereas the expression of KLK-4 was enhanced by vitamin D_3. Caspase-14 is a nonapoptotic caspase family member whose expression in the epidermis is confined to the suprabasal layers, which consist of differentiating keratinocytes. As reported previously, vitamin D_3 treatment results in inhibition of proliferation and the induction of caspase-14 expression in keratinocytes. As ameloblasts are derived from undifferentiated epithelial cells, the differentiation of ameloblasts will likely behave identically to that of keratinocytes.

Conclusions: These results suggest that caspase-14 is likely to be involved in the differentiation and the cellular functions of ameloblasts.

1P-18

In Vitro Calcification by Dentin Phosphoprotein and Effects of Cationic Peptides

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Keywords: Dentin phosphoprotein, Calcification, Dentin

Objective: Matrices of bone and tooth contain various acidic proteins that can induce and control nucleation of hydroxyapatite crystals. For example, dentin phosphoprotein, the major acidic protein of dentin, is a potent inducer of the crystal nucleation. In the present study we attempted to use cationic and anionic peptides to investigate the role of acidic groups of the protein on calcification.
Methods: Dentin phosphoprotein was extracted and purified from bovine dentin. *In vitro* calcification was performed by using a system composed of a nylon membrane spotted with the acidic proteins. The membrane was sandwiched with filter papers containing calcium or phosphate ions. The calcium phosphate precipitated on the membrane was stained with Alizarin red.
Results: In the calcification system, dentin phosphoprotein enhanced the deposition of calcium phosphate at low concentration. The protein inhibited the deposition at higher concentration. Phosvitin, a phosphoprotein of egg, and casein phosphopeptide also had positive effect on the calcification. Poly (glutamic acid) had week positive effect, although poly (aspartic acid) had negative effect. An anionic dendrimer had even more week positive effect. The effect of dentin phosphoprtotein was inhibited by cationic peptide such as protamine or poly arginine. These cationic peptides can interfere with phosphate groups of the protein.
Conclusions: Clusters of the phosphorylated amino acids may be essential for induction of the crystal nucleation.

1P-19

Differential expression of basement membrane type IV collagen α chains as a prognostic factor in extrahepatic bile duct carcinoma

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Keywords: basement membrane, type IV collagen, extrahepatic bile duct carcinoma

Objective: The destruction of the basement membrane (BM) is the first step in cancer cell invasion and metastasis. Type IV collagen is a major component of the BM, and is composed of six genetically distinct α(IV) chains: α1(IV) to α6 (IV). The loss of α5/α6(IV) chains from the epithelial BM at the early stage of cancer cell invasion has been reported in several types of cancers of alimentary tract [1-3]. However, the expression of α5/α6(IV) chains in extrahepatic bile duct carcinoma (EBDC) remains unclear.

Methods: The expression of α(IV) chains, p53, and Ki-67 in 36 resected EBDC specimens were immunohistochemically examined.

Results: In EBDC, α5/α6(IV) chains disappeared partially or completely earlier than α1/α2(IV) chains around the tubular cancer cells. The expression of α5/α6(IV) chains were related to T classification and TNM staging, and inversely with p53 expression, but not with Ki-67 index. The patients with α1/2(IV) chains-negative and α5/6(IV) chains-negative around the invasive cancer cell nests on EBDC had significantly poorer prognosis than those with α1/2(IV) chains-positive and α5/6(IV) chains-positive/negative.

Conclusions: The loss of α1/2(IV) and α5/α6(IV) chains might be a useful prognostic factor in EBDC.

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1P-20

Crucial effect of ultraviolet radiation on mammalian skin under the Antarctic ozone hole

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Keywords: Collagen, Ultraviolet, Antarctica

Objective: The ozone holes, appearing in Antarctica, cause an increased ultraviolet B (UVB) irradiation. In this research, we examined the qualitative change of bovine skin exposed to UV in Antarctica aiming to elucidate the influence of the ozone holes on the mammalian skin.

Methods: Bovine skin tissues were exposed to sunlight including UV for 25 days at the Syowa station, Antarctica {December (The amount of UV is the maximum); February (There is no ozone hole; as a control against September); September (There is ozone holes)}. Collagen was extracted with pepsin from exposed skin and analyzed by SDS-PAGE. In order to examine the damage of exposed skin, collagen fiber was observed by scanning electron microscope (SEM). Moreover, acid soluble collagen (ASC) was irradiated *in vitro* with UVB lamp. We consider this experiment as a model to understand the influence of UVB on skin collagen.

Results: At all examined seasons, the amounts of collagen extracted from exposed skins apparently decreased compared to that from shaded control skins. However, destruction of collagen fiber was not observed by SEM observation. When ASC was irradiated to UVB *in vitro*, cross-links between collagen molecules were formed in proportion to the exposure time, and then, cross-linked collagen was gradually degraded. Therefore, it is suggested that the formation of cross-links decreased the amount of solubilized collagen from the exposed skin. Although short wavelength UVB is known to reach the ground in September more than in February and December, higher solubility of collagen was found in the exposed skin in September than that in February. Moreover, collagen was hardly extractable from the skin exposed in December.

Conclusions: These results suggest that the total amount of UVB energy is more effective on the cross-links formation of collagen than the amount of short wavelength UVB due to the presence of ozone holes.

1P-21

Clinical and genetic features of Japanese patients with the vascular-type of Ehlers-Danlos syndrome

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Objectives: Vascular-type Ehlers-Danlos syndrome (EDS) is the most serious among the major types of EDS recognized. Vascular-type EDS (vEDS) is an autosomal dominant inherited disorderresulting from mutations within the $\alpha 1$ type III collagen gene (*COL3A1*)[1]. Recently, we analyzed the clinical characteristics, type III collagen production levels from cultured dermal fibroblasts, and identified mutations of *COL3A1* in 16 Japanese patients with vEDS.

Methods: For quantification of the type III collagen production, fibroblasts were cultured with ^{3}H-proline and the radio-labeled proteins were separated by SDS-PAGE, and the radioactive bands were detected by fluorography. Mutations in the *COL3A1* were detected by cDNA analysis and subsequently by genomic DNA analysis.

Results: As for mutations of the *COL3A1*, glycine-substitution mutations were demonstrated in 8 patients (50%), and splice-site mutations of exon junctions, such as exon skips, in the remaining 8 patients (50%). The type III collagen production level in the cultured fibroblasts was 12.05% of the normal value, on average. In regard to the clinical manifestations, thin, translucent skin in 92.8% of the patients, extensive bruising in 87.5%, the characteristic facies in 78.5%, acrogeria in 40.0%, hypermobility of the small joints in 92.8%, pneumothorax in 50.0%, a positive family history in 46.6%, arterial rupture or dissection in 18.7%, and rupture of the gastrointestinal tract in 25.0%.

Conclusions: Half of mutations in the *COL3A1* were splice-site mutations of exon junctions and the rest of those were glycine-substitution mutations. The analysis in the present series revealed a low frequency of cases presenting with serious clinical findings, such as rupture of the arteries or gastrointestinal tract. As these serious complications have been shown to increase with advancing age[2], future development of these serious complications among the patients of this series is very possible, because the mean age of the patients was relatively low (27.2 years) at the time of the analysis.

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1P-22

Expression and localization of lysyl oxidase in the presumptive dermis of chick limb bud

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Keywords: Lysyl oxidase, collagen, chick limb bud

Objective: Lysyl oxidase (LOX) activity is involved in the formation of crosslinking between and within the molecular units of elastin and of collagen. The enzyme converts their lysine side chains into allysines, which stabilize macromolecular assembly of extracellular fibers. We showed temporospatial progress of elastic system fiber formation and the coordinated accumulation of collagen fibers in the dermis of the developing chick leg bud [1]. Because this developmental process must be closely related to LOX activity in the dermis, we have investigated its temporospatial expression by immuno-histochemistry and real-time quantitative RT-PCR.
Methods: Cryosections of chick leg bud were used for immunohistchemistry and RNA extraction for RT-qPCR. Laser capture microdissection system was used for isolating tissue in specific regions of the dermis precisely.
Results: The results of PCR analysis showed that the expression of LOX mRNA became apparent at ED13 and increased considerably at ED17. Intensity of immunohistochemical staining for LOX in the dermis was very weak at all stages of development. Accumulation of collagen fibers was seen in the dermis at ED10 and longer wavelengths of birefringence became evident by ED13.

Conclusions: Our findings suggested that temporal pattern of LOX mRNA expression is correlated to collagen fiber accumulation in the dermis of developing chick limb bud. LOX expression was relatively constant at the protein level, and it appeared plausible that degradation and/or translational control of LOX mRNA may occur.

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1P-23

Culture conditions affecting cellular clump formation accompanying intercellular accumulation of type V collagen fibrils

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Keywords: Type V collagen; collagen fibril; clump

Introduction: Human fibroblasts form clumps when cultured on a dish coated with reconstituted type V collagen fibrils. This phenomenon involves intercellular accumulation of the type V collagen fibrils and cellular dynamism that have eventually led to formation and detachment of the clump during the culture. In this study, we investigate culture conditions affecting the phenomenon to elucidate the mechanism.
Methods: Human diploid fetal lung fibroblasts, TIG-1, were cultured on plastic dishes coated with type V collagen fibrils. Numbers of plated cells, incubation time for fibril formation and coating, and concentration of type V collagen fibrils to coat were varied and examined about the duration to need the clump formation.
Results: More cells plated resulted in faster detachment of the clumps but the detachment occurred after the culture reached to confluency. The incubation time for fibril formation and coating less than 4 hrs resulted in failure of the detachment. This indicates that type V collagen fibrils but not molecules is required for the phenomenon since it takes roughly 24 hrs for the fibril formation. More coated fibrils delayed the detachment. It would take time for residual fibrils to be accumulated in the intercellular space and to establish rigid connection via the accumulated fibrils between cells.
Conclusions: Culture conditions affected the cellular clump formation. The results indicate involvement of type V collagen fibrils in the tissue remodeling.

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1P-24

Intercellular accumulation of type V collagen fibrils in accordance with cell aggregation

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Keywords: Type V collagen fibrils; clump formation; cell cementing; accumulation of collagen fibrils; cell-collagen interaction

Introduction: We reported previously that human fibroblasts form clumps when cultured on a dish coated with reconstituted type V collagen fibrils. Essentially all the type V collagen fibrils initially coated on the dish were recovered in the cell clumps that had eventually formed during the culture. We interpreted that type V collagen fibrils adhere to cells more strongly than to the dish and are detached by cell movements. In this study, type V collagen was suspended with fibroblasts to examine the fate of the type V collagen fibrils and to determine whether the fibrils affect the behaviour of the cells directly adherent to the dish.

Results: The added type V collagen accumulated in the intercellular space concomitantly with the local aggregation of fibroblasts. SEM examination indicated that type V collagen fibrils were found in the vicinity of cells in cultures without ascorbic acid where essentially no collagen secretion takes place. These results indicate that type V collagen forms fibrils and the fibrils are accumulated in the intercellular spaces.

Conclusions: The accumulated type V collagen fibrils work as a cementing material for cell clump formation. This phenomenon is discussed in relation to the possible involvement of type V collagen fibrils in tissue organization.

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Kihara T., Imamura Y., Takemura Y., Mizuno K., Adachi E., Hayashi T. (2008). Intercellular accumulation of type V collagen fibrils in accordance with cell aggregation. J. Biochem., 144, 625-633.

1P-25

Substrate recognition of collagen-binding domains derived from bacterial collagenases

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Keywords: Collagen-Binding Domain, Structure-Function Relationship, Preclinical Applications

Objective: Histotoxic clostridia produce collagenases responsible for tissue destruction in gas gangrene. The C-terminal collagen-binding domain (CBD) of these enzymes is capable to bind to collagen fibrils. We have demonstrated that growth factors fused to CBD remained at the sites of injection much longer than growth factors alone to induce extended cell proliferation. To obtain insight into the molecular mechanism to recognize triple helical substrate, we set out to a structural study.

Methods: A ^{1}H-^{15}N HSQC NMR titration with collagenous peptides was carried out to identify the binding site. NMR titrations with nitroxide spin-labeled analogues of collagenous peptide were performed to identify the binding direction. The structures of CBD-collagenous peptide complex were analyzed by small angle x-ray scattering.

Results: The NMR titration study mapped a saddle-like binding cleft on CBD. That with three nitroxide spin-labeled collagenous peptides unambiguously demonstrated unidirectional binding of CBD to the tropocollagen analogues. Small angle x-ray scattering data revealed that CBD binds closer to a terminus for each of the five different tropocollagen analogues, which in conjunction with NMR titration studies, implies a binding mode where CBD binds to the C-terminus of the triple helix.

Conclusions: Though CBD targets the least ordered region of the peptides, CBD could also target partly unwound regions even in the middle of a tropocollagen. Preclinical applications to anchor a hormone or growth factors will be also discussed.

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1P-26

Physical and biological functions of soluble elastin from Pisces

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Keywords: Soluble elastin, Pisces, Functions

Objective: There are so far few reports about functions of soluble elastin from Pisces. Here we present the temperature profiles of coacervation and circular dichroism (CD) spectra of soluble elastin from Pisces and cell migration and proliferation in response to piscine soluble elastin. In addition, these functions were compared with those of soluble elastin from Mammalia.

Methods: Histoligical studies of bonito bulbus arteriosus were performed by electron microscopy. Temperature profiles of coacervation were obtained by following the light scattering at 300 nm with a temperature at a rate of 0.5°C per minute. CD spectra were obtained in H_2O at temperatures of 5°C to 45°C. Cell migration assays were conducted in 48-well microchemotaxis chamber and cell proliferation assays were performed in cultured human skin fibroblasts.

Results: Elastin in bonito bulbus arteriosus after alkali- and collagenase-treatments showed a meshwork structure consisted of fine fibrils. The onset temperature of coacervation of soluble elastin from bonito was lower than that from Mammalia. Soluble elastin from bonito exhibited a lower content of α-helix than that of Mammalia did. A higher responsiveness of migration and proliferation of human skin fibroblast to soluble elastin from bonito than that from Mammalia was observed.

Conclusions: Soluble elastin from Pisces differs from that from Mammalia in physical functions such as temperature profiles of coacervation and secondary structural features and biological functions such as cell migration and cell proliferation.

1P-27

Protective Effect of the Fibronectin-Derived Peptide PHSRN in Cultured Human Corneal Epithelial Cells

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Purpose: Corneal epithelial cells are subject to various insults and turnover continuously through apoptosis. We have previously shown that the peptide PHSRN, which corresponds to the second cell-binding domain of fibronectin, stimulates both migration of the corneal epithelium and corneal epithelial wound healing. We have now investigated the effect of the PHSRN peptide on apoptosis in cultured human corneal epithelial cells.

Methods: Simian virus 40–transformed human corneal epithelial (HCE) cells were incubated with sodium nitroprusside (SNP), a nitric oxide donor and inducer of apoptosis, in the absence or presence of the PHSRN peptide. Apoptosis was detected by flow cytometric analysis of cells stained with annexin V and propidium iodide. Phosphorylation of the protein kinase Akt, which plays an important role in antiapoptotic signaling, was assessed by immunoblot analysis. Cell death was also quantified by measurement of the release of lactate dehydrogenase (LDH) into culture supernatants, and cell proliferation was evaluated by measurement of [^{3}H]thymidine incorporation.

Results: SNP (1 mM) induced the release of LDH from HCE cells as well as an increase in the proportion of apoptotic (annexin V^+, propidium iodide$^-$) cells, and both of these effects were inhibited by the PHSRN peptide (1 μg/ml). The inhibitory effects of the PHSRN peptide on SNP-induced LDH release and apoptosis were blocked by LY294002 (10 μM), an inhibitor of phosphatidylinositol 3-kinase. The PHSRN peptide also induced the phosphorylation of Akt, but it had no effect on HCE cell proliferation.

Conclusions: The fibronectin-derived peptide PHSRN suppresses the induction of apoptosis in cultured human corneal epithelial cells, and this effect may be mediated by the activation of phosphatidylinositol 3-kinase and Akt.

1P-28

The Production and Purification of Recombinant Human Laminin-332 in *Leishmania tarentolae* Expression System

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Keywords: Laminin, Basement membrane, LEXSY

Objective: An understanding of the biological and physiological functions of each laminin isoform is very important for considering them as resources for tissue engineering. However, it is difficult to obtain large amounts of trimeric laminin molecules except for laminin-111 (α1β1γ1), which is available in nearly gram-order quantities from murine Engelbreth-Holm-Swarm (EHS) tumor. Here we report the use of *Leishmania talentolae* (Trypanosomatidae) expression system (LEXSY) for production of human laminin-332 (α3β3γ2) in active form.

Methods: Plasmids containing cDNA encoding full-length β3 and γ2 subunits and truncated α3 subunit with N-terminal FLAG-tag were sequentially transfected into *L. tarentolae* strain by electroporation. Recombinant strain harboring three constructs was analyzed for expression of laminin-332 subunits.

Results: Secreted heterotrimer was recovered from culture medium and purified by anti-FLAG affinity gel. The eluted fraction contained three subunits as confirmed by immunoprecipitation and western blotting. It showed similar cell adhesion activity as laminin-332 purified from mammalian expression system.

Conclusions: Our results demonstrate that laminin heterotrimer can be secreted from *L. tarentolae*. Because the yield of the purified laminin was not so high, this system must be improved by optimization of the cultivation and purification conditions.

1P-29

Expression of laminin α3B chain in vascular and epithelial basement membranes and its possible functions

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Keywords: Laminin, Extracellular matrix, Basement membrane, Vascular

The basement membrane (BM) proteins laminins, which consist of α, β, and γ chains, play critical roles in the maintenance of tissue structures and cellular functions. In blood vessels, the α4- and α5-laminins, e.g. laminin-411 (laminin-8) and laminin-511 (laminin-10), are thought to play major-roles, and there are few studies reporting other laminins. The present study demonstrates the presence of a novel α3B-containg laminin in vascular BMs. Laminin α3 chain has two isoforms, the truncated form α3A and the full-sized form α3B. Although the α3A-containing laminin, laminin-3A32 (laminin-5A), has been extensively studied, there is little information about α3B-containing laminins. RT-PCR analysis indicated the wide distribution of the α3B chain in normal human tissues. To show the histological distribution of the laminin α3B chain, we prepared α3B-specific monoclonal antibodies. Immuno-histochemical analysis showed that the α3B chain was colocalized with α3A, β3, and γ2 chains in the epithelial BMs of the skin, esophagus, breast and lung, suggesting the presence of laminin-3B32 (laminin-5B) and laminin-3A32. In the lung alveoli, laminin-3B32 was dominant over laminin-3A32, but vice versa in other epithelial BMs. In contrast, the BMs of blood vessels including capillaries and venules in these tissues were strongly positive for the α3B, almost negative for α3A, and completely negative for β3 and γ2. The α3B chain was colocalized with β1 and γ1 chains in these BMs. These results strongly suggest that the laminin α3B chain is widely expressed in vascular BMs of normal tissues, probably as laminin-3B11/3B21 (laminin-6B/7B). To express laminin-3B11, HEK-293 cells were sequentially transfected with the expression vectors of the laminin β1, γ1, and α3B chain. A HEK293 cell clone highly expressing the three Lm3B11 chains was used as Lm3B11-HEK. The novel laminin laminin-3B11 was purified from the conditioned medium of Lm3B11-HEK. Properties of this laminin are presented.

1P-30

G1 domain of versican in transitional granulation tissue in pressure ulcer

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Objective: Granulation tissue formation is an essential process for wound healing of pressure ulcer. In the tissue remodeling, extracellular matrix (ECM) restructuring is required. However, ECM in the granulation tissue has not been characterized yet. Since we previously showed that the amino-terminal fragment (G1 fragment) of versican recruit hyaluronan (HA) to fibrillin-microfibrils through versican/versican self interactions, we focus the role of the G1 fragment of versican in the granulation tissue of pressure ulcer.
Methods: Protein samples were obtained from the wound surface using absorbent cotton. ECM molecules in wound surface and granulation tissues were extracted with 6 M guanidine hydrochloride buffer. We performed western blotting analysis using antibodies for versican and the relating molecules including fibrillin-1, fibulin-2, fibulin-5, fibronectin, collagen V, IV, I and decorin. We also tested tissue overlay assay using versican G1 and G3 expressed by mammalian cells. Immunofluorescence microscopic study using the anti-versican was also employed in the tissue specimen of granulation tissue and normal dermis. Real time observation for assembly of the G1 domain was investigated by cell culture utilizing normal skin fibroblast.
Results: The biochemical profiles of the wound surface ECM and from granulation tissue were comparable. By the multiple approaches, we characterized two functionally different versican fragments from wound, HA binding and non-HA binding. The HA binding G1 enhanced the HA recruitment, however, non-HA binding versican inhibited HA recruitment. Self-aggregation of versican was observed at the interface of granulation tissues and fibrotic matrices. Spatio-temporal interaction between digesting ECM exists around condensation cells and transitional interface of matrix phase shift.
Conclusions: The functionally different G1 fragment in wound healing process play significant roles for the regulation of HA rich environment and viscoelastic matrices.

1P-31

Regulation of fibrillin-1 fiber formation and tropoelastin deposition in Rho-ROCK signaling pathway

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Background & Aims: Recently it has been reported that fibronectin fiber formation is closely related to Rho and Rho-associated coiled-coil forming kinase (ROCK) signaling pathway. Our purpose of this study was to investigate the regulation fibrillin-1 fiber formation and tropoelastin deposition in Rho-ROCK signaling pathway.
Methods: Human retinal pigment epithelial cells (ARPE-19 cells) were treated with Y-27632, Rho kinase inhibitor, or lysophosphatidic acid (LPA), Rho kinase activator.Fibrillin-1 fiber formation and tropoelastin deposition was evaluated by immunofluorescence staining and semi-quantitative ELISA. Moreover, mRNA expression was determined by RT-PCR.
Results: Immunofluorescence staining showed that fibrillin-1 fiber formation and tropoelastin deposition was decreased and was increased by treatment with Y-27632 and LPA, respectively in ARPE-19 cells. Moreover, mRNA expression of microfibril-associated glycoprotein (MAGP) and fibrillin-1 was suppressed and was accelerated by treatment with Y-27632 and LPA, respectively.
Conclusions: In this study, our data revealed that fibrillin-1 fiber formation and tropoelastin deposition is regulated via Rho-ROCK signaling pathway. Our present study would be useful for understanding of mechanisms of elastic fiber regeneration.

1P-32

Cyclosporin A suppresses up-regulated matrix metalloproteinase (MMP)-9 expression together with caspase-3/7 activity from keratinocyte in high calcium condition

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Keywords: gelatinase, keratinocyte, differentiation

Objective: Cyclosporin A is now administrated for not a few inflammatory conditions such as psoriasis and atopic dermatitis in dermatology. Among a variety of inflammatory conditions on which MMP-9 (gelatinase B) plays the role, we have focused on the apoptotic ones including abnormal keratinization in the epidermis, and the association between caspase-3/7 acitivity and gene regulatory mechanism of MMP-9 expression has been recently elucidated. This study presents the effect of cyclosporin A on the expressions of these enzymes from cultured keratinocyte in high calcium condition, which is considered to reflect hyperkeratois, high degree of epidermal differentiation, seen in many inflammatory skin diseases.
Methods: Human primary keratinocytes were cultured either in low or in high calcium concentration. In addition, cyclosporin A was added or not for each condition, and gelatinase activities together with caspase-3/7, -8, and -9 activities were analyzed.
Results: High calcium stimulation up-regulated both MMP-9 and caspase-3/7 expressions, which were suppressed by the addition of cyclosporine A in a dose-dependent manner.

Conclusions: These results indicate that the effect of cyclosporin A on inflammatory skin conditions is at least partly from the suppressions of MMP-9 and caspase-3/7 activities in hyperkeratosis.

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1P-33

IL-1 beta stimulates activin βA mRNA expression in human skin fibroblasts through MAP kinase pathways, NF-κB pathway and prostaglandin E2

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Objectives: Accumulating evidences indicate that activin, a member of the transforming growth factor-β superfamily, plays important roles during skin wound healing. A noticeable increase in activin βA mRNA occurs in the injured skin and interleukin-1 (IL-1), which increases in the early phase of wound healing, is thought to be a probable factor inducing the increase in activin expression. The aim of this study is to reveal the mechanisms responsible for the IL-1β-induced activin βA mRNA expression in human skin fibroblasts.
Methods: Human skin fibroblasts were stimulated with IL-1β for 6 h, and levels of activin βA mRNA were examined by realtime PCR. To examine signaling pathways responsible for the IL-1β-induced activin expression, fibroblasts were pretreated with a JNK inhibitor SP600125, a p38 MAPK inhibitor SB202190, a MEK1/2 inhibitor U0126, an IKK2 inhibitor SC-514, a PKA inhibitor H-89, or a cyclooxigenase inhibitor indomethacin.
Results: Stimulation of fibroblasts with IL-1β considerably increased activin βA mRNA expression (more than 20-fold vs control at 1 ng/ml). SB202190, U0126 and SC-514 significantly suppressed the IL-1β-stimulated activin βA mRNA expression while SP600125 and H-89 failed to suppress it. Especially, SB202190 almost completely suppressed the IL-1β-stimulated activin βA mRNA expression. Because these effective inhibitors also suppressed IL-1β-stimulated expression of cyclooxygenase 2, a key enzyme for prostaglandin E2 (PGE2) synthesis, contribution of PGE2 to the IL-1β-stimulated activin βA mRNA expression was examined. Indomethacin significantly suppressed the IL-1β-stimulated activin βA mRNA expression to 40% of cells treated with IL-1β alone. Furthermore, stimulation of fibroblasts with 1 μM PGE2 for 6 h significantly increased activin βA mRNA.
Conclusion: The present study revealed that p38 MAP kinase, Erk1/2 and NF-κB pathways mediated the effect of IL-1β on activin βA mRNA expression. Furthermore, present results indicate that PGE2 is, at least in part, involved in the IL-1β-induced increase in activin expression.

1P-34

Gadolinium promotes osteogenic differentiation in MC3T3-E1 cells and human adipose tissue-derived mesenchymal stem cells: a possible role of gadolinium on ectopic calcification of nephrogenic systemic fibrosis

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Keywords: Gadolinium, nephrogenic systemic fibrosis, osteoblasts, mesenchymal stem cells, calcification

Objective: Recent studies have suggested a close association between the administration of gadolinium (Gd)-based contrast agents and the development of nephrogenic systemic fibrosis (NSF), an acquired disorder characterized by systemic fibrosis and ectopic calcification in patients with severe renal dysfunction. However, causative roles of Gd has remained unknown. The aim of this study is to investigate the effect of Gd on the development of fibrosis and calcification in cultured cells.
Methods: MC3T3-E1 cells (pre-osteoblastic cells), human adipose tissue-derived mesenchymal stem cells (AMSCs), human osteoclasts, human preadipocytes and human dermal fibroblasts (HDFs) were cultured in each differentiation medium with or without gadolinium chloride ($GdCl_3$). Osteogenic differentiation of MC3T3-E1 cells and AMSCs was determined by Arzarin Red staining. Adipogenic differentiation of human preadipocytes and AMSCs was determined by Oil Red O staining. Osteoclast differentiation was determined by TRAP stainig. Fibrogenesis of HDFs was determined by real time PCR for the mRNA expression of type I collagen.
Results: $GdCl_3$ promote osteogenic differentiation and osteoclast differentiation, but not adipogenic differentiation. In addition, gadodiamide also promote osteogenic differentiation in MC3T3-E1 cells. $GdCl_3$ did not increase the mRNA expression of type I collagen in HDFs.

Conclusions: We have demonstrated a direct relationship between Gd and osteogenic differentiation that may be involved in the development of ectopic calcification of NSF patients.

1P-35

Decorin Regulates Osteoblastic Differentiation of Mesenchymal Stem Cell

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Keywords: Cell differentiation, Decorin, Osteoblasts

Objective: The aim of this study was to clarify the role of decorin in osteoblastic differentiation of mesenchymal stem cell (MSC) *in vivo.*
Methods: SiRNA for decorin (siDecorin)-expressing plasmid was transfected into KUSA-A1 cell (mouse MSC) and succeed to establish siDecorin-transfected cells (siDT) line. Some siDT were transplanted into abdominal cavity within diffusion chamber (DC) and another butch of siDT were transplanted in subcutaneous site with or without collagen gel for 1-8 weeks. Both transplanted cells were induced ectopic ossification and were analyzed the cellular response in osteoblastic differentiation.
Results: KUSA-A1 (control) cell transplanted within DC strongly expressed alkaline phosphatase (ALP), also calcium (Ca) was deposited intercellular space of A1. The siDT within DC showed low level of ALP and Ca throughout the harvesting period, and possessed lipid drops in cytoplasm. Moreover the activity of glycerol 3-phosphate dehydrogenase (adipocyte specific marker) of siDT was much higher than that of A1. In subcutaneous site, the transplanted siDT without collagen gel slightly formed connective tissue structure surrounded by fatty tissue. As for this structure, the sectional area was extremely small compared with the tissue formed by A1, and levels of ALP activity and osteopontin was low. The A1 transplanted with collagen gel formed large bony tissue showing high activity of ALP and osteopontin, and high contents of Ca. While the siDT transplanted with collagen gel formed significantly small-sized tissue with low level of Ca content and osteoblastic markers.
Conclusion: Our results indicated that decorin controls distinctly in the differentiation process of MSCs to osteoblast. Thus, decorin would regulate cell-lineage decisions and cell fate, differentiating from the MSCs to osteoblasts or adipocytes.

Poster Session II:

2P-01

Production of BRAK knockout mice

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Keywords: chemokine, BRAK, knockout mice

Objective: To clarify the physiological function of the chemokine BRAK, we generated mice with targeted disruption of the *BRAK* gene.
Methods: The Cre/loxP system was used to delete a genomic fragment containing exon 2 of the *BRAK* gene, which encodes the chemokine CXC motif of BRAK. The conditional targeting vector was introduced into C57BL/6 ES cells. Homologous recombinant ES cell clones were injected into blastocysts to produce chimeric mice. These chimeras were crossed with beta-actin Cre transgenic mice and crossed further with C57BL/6 mice to obtain heterozygous knockout mice (BRAK+/-). BRAK+/- mice were further crossed to generate homozygous knockout mice (BRAK-/-). Genotypes were confirmed by PCR and southern blotting.
Results: To date, we obtained male BRAK-/- and female BRAK+/- mice and they are crossed to build a reproductive colony.

Conclusions: We obtained BRAK-/- mice. These mice were fertile but their birth ratio was lower than the expected Mendelian frequency, suggesting BRAK/CXCL14 to be important for normal development of the fetus.

Reference

Ozawa, S, Kato, Y, Komori, R, Maehata, Y, Kubota, E, Hata, R. (2006) BRAK/CXCL14 expression suppresses tumor growth *in vivo* in human oral carcinoma cells. *Biochem. Biophys. Res. Commun.* 348, 406-412,

2P-02

Suppression of growth of Lewis lung carcinoma cell xenografts in BRAK transgenic mouse: Production of cancer resistant mouse

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Objective: Previously we reported that forced expression of BRAK (CXCL14) in tumor cells increased significantly rejection rate of xenografts as well as suppressed the growth rate of the remaining tumor cells. [1]. Here we addressed whether BRAK/CXCL14 over expressed transgenic mice are resistant or not to tumor cell xenografts.
Methods: To investigate the tumor growth suppressive effect of BRAK, we produced transgenic (TG) mice by introducing a BRAK expression vector. Lewis lung carcinoma cells were injected into both sides of these TG mice and of the same strain (C57BL/6J) of wild-type (WT) mice. Tumor volume was determined following 4 weeks, and animals were sacrificed to dissect kidney, tumors and blood serum. RNA was extracted from kidney and tumor xenografts, and subjected to real-time PCR after production of cDNA. Concentrations of BRAK protein in plasma were determined by ELISA. Tumors were processed for pathological investigation and stained for immunohistochemical determination of the expression of CD31, a marker for endothelial cells.
Results: Levels of BRAK mRNA and serum protein were higher in TG mice. The sizes of tumor xenografts were significantly smaller in TG than in WT mice and staining pattern for CD31 was different between WT and TG mice.
Conclusions: The data suggest that BRAK plays a role in tumor suppression *in vivo*.

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2P-03

Chemokine BRAK stimulates apoptosis elicited by gefitinib in oral squamous cell carcinoma

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Objectives: The chemokine BRAK/CXCL14, a non ELR-motif chemokine, is expressed in many normal tissues, but absent or down regulated in transformed cells and cancerous tissues including oral carcinoma. We reported previously that BRAK had suppressive activity toward tumor progression of oral carcinoma *in vivo* when over-expressed in tumor cells. In this study, we investigated whether BRAK expression is associated with the tumor suppression by gefitinib, an inhibitor of the epidermal growth factor receptor (EGFR).

Methods: To examine the mechanism of the tumor suppression *in vivo*, we xenografted nude mice with HSC-3 cells that had been transfected with control Sh-scrambled vector or ShRNA of BRAK to down-regulate BRAK mRNA expression. In order to investigate the cell proliferation and/or apoptosis with regard to the suppression of tumorigenicity, we prepared paraffin sections and used them for immunohistochemical detection of Ki-67, a marker of cell proliferation and for the TUNEL method to detect apoptosis.

Results: As to the cell proliferation, the number of Ki-67-positive cells in both Sh-Scrambled-treated control tissue sections and Sh-BRAK-treated one was decreased, when the animals were treated with gefitinib. There was no difference between Sh-Scrambled vector-treated tumor cells and Sh-BRAK vector-treated ones with respect to the responsiveness to gefitinib. On the other hand, with respect to apoptosis, we found a significant increase ($P<0.05$) in the number of apoptotic cells in the Sh-Scrambled vector-treated control tumor cells concomitant with the suppression of tumor mass after the mice had been treated with gefitinib. In contrast, gefitinib affected neither the number of apoptotic cells nor tumor volume suppression in the case of Sh-BRAK vector-treated tumor cells.

Conclusions: These results suggest that a BRAK dependent signal(s) was essential for the stimulation of apoptosis by gefitinib and reduction in tumor volume *in vivo*.

2P-04

Basic study on prescription of effective conservative combined therapy for malignant tumor using quantitative imaging analysis for vascular structure

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Keywords: Radiotherapy, Hyperthermia, Chemotherapy, Micro-angiography

Objective: The aim of this study was the development of a detailed and quantitative X-ray imaging evaluation method for analyzing the vascular structures of malignant tumor in experimental small animals like mouse, and to develop effective conservative combined therapy for malignant tumor by using our analysis method.

Methods: The digital X-ray imaging was used to quantitatively analyze the vascular structures in mice. The vascular structures were quantitatively analyzed as various parameters, after the vascular structure patterns were extracted from the digital X-ray image data. The therapeutic effects of various conservative combined therapies were evaluated by in vitro and in vivo experiments.

Results: The vascular structures in the mice were indicated as various parameters by our analyzing method [1]. The combined therapy of radiotherapy plus chemotherapy or radiotherapy plus hyperthermia sensitized therapeutic effect of each therapy. Furthermore, the combined therapy of radiotherapy plus chemotherapy plus hyperthermia remarkably sensitized therapeutic effect. KB tumors in the mice showed complete response with the dose of less than half of conventional treatment.

Conclusions: The results of investigation suggest that our quantitative X-ray imaging evaluation method is useful for the analysis of the vascular structure of tumors in experimental small animals. It is also indicated that our conservative combined therapy is very effective for the treatment of malignant tumor, and our evaluation method proved it.

Reference

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2P-05

Expression of BRAK/CXCL14 is associated with antitumor efficacy of gefitinib in head and neck squamous cell carcinoma

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Objectives: The clinical efficacy of gefitinib (ZD1839, Iressa), which is an inhibitor specific for the epidermal growth factor (EGF) receptor tyrosine kinase, has been demonstrated in non-small cell lung carcinoma patients with EGF receptor mutations, and so these mutations are a useful marker(s) to find responders to this drug. However recent studies showed that the EGF receptor gene mutation is rare in squamous cell carcinomas of the esophagus and head and neck regions. In the present study we investigated the relationship between BRAK expression and gefitinib efficacy for tumor suppression.

Methods: HNSCC lines were cultured Dulbecco's Modified Eagle's Medium (DMEM) containing 10% fetal bovine serum. Nearly confluent cells were cultured overnight in serum-free DMEM. After starvation, they were incubated with or without EGF (10 ng/ml) and/or gefitinib (1 mM). HSC-3 cells were subcutaneously injected into athymic nude mice. HSC-3-xenografted mice were daily administered gefitinib (50 mg/kg) orally.

Results: Gefitinib attenuated the effect of EGF, or even stimulated BRAK mRNA expression of HNSCC cell lines *in vitro*. Oral administration of gefitinib reduced the size of the tumors formed by HSC-3 cells in the nude mice concomitantly increased BRAK mRNA expression *in vivo*.

Conclusions: Our results indicate that oral administration of gefitinib reduced tumor size, at least in part, through elevation of BRAK expression. Thus, the use of gefitinib for treatment of patients with HNSCC in whom there is an inducing effect of the drug on the BRAK expression of their cancer cells may be advantageous. Furthermore, BRAK may be a promising molecule for gene therapy of HNSCC.

2P-06

Functional analysis of promoter region of human BRAK/CXCL14, a tumor progression suppressor

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Objectives: CXCL14/BRAK, a non-ELR motif chemokine, is highly expressed in all normal cells, but is not expressed or expressed at a negligible level in most of head and neck squamous cell carcinomas examined. Earlier we reported that the BRAK expression level is inversely related to tumor size (Ozawa *et al.,* Biochem. Biophys. Res. Commun. 348: 406-412, 2006). However, the mechanisms by which the gene is regulated are still unclear. Thus, to elucidate the mechanisms regulating BRAK gene expression, we determined the transcriptional start site and promoter motifs of the gene.

Methods: For determination of the transcriptional start-site, the 5' Rapid Amplification of cDNA End (5'-RACE) method was employed by use of a 5'-RACE CORE SET (TAKARA). For determination of the promoter region of the gene, we constructed vectors containing presumptive promoter regions for the BRAK gene connected to the luciferase reporter gene and introduced them into HSC-3 cells. Promoter activities were determined by use of the Dual-GloTM Luciferase Assay System (Promega). Cells were cultured in the presence or absence of okadaic acid (20 nM).

Results: The transcriptional start site was found to be in the previously reported exon 1 region (+284) of the gene. Determination of luciferase activities by use of deletion and/or mutation constructs clarified that a TATA-like sequence, TATTAA was essential for the transcription of the gene. Also an AP-1 binding sequence was necessary for stimulating the expression of the gene. Okadaic acid up regulated the expression level of BRAK. When HSC-3 cells were transfected with the control and mutated luciferase constructs and treated with okadaic acid, only the cells transfected with the mutated AP-1 binding sequence or deletion construct lost sensitivity to okadaic acid.

Conclusions: Our data indicate that the TATA-like sequence forms an essential part of the promoter of the BRAK/CXCL14 gene and that an AP-1 binding sequence is responsible for the stimulation of BRAK transcription by okadaic acid.

2P-07
ADAMTS1 as a hypoxia sensing biomarker

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Keywords:ADAMTS1,Hypoxia, metalloproteinase

Objective: The ADAMTSs (a disintegrin and metalloproteinase with thrombospondin motifs) are a group of extracellullar, multidomain proteases that are found both in mammals and invertebrates whose known functions include: (i) collagen processing as procollagen N-proteinase; (ii) cleavage of matrix proteoglycans; (iii) inhibition of angiogenesis: and (iv) blood coagulation homoeostasis [1]. We have previously reported that ADAMTS1 was strongly and transiently expressed in the infarcted heart. Recently we have reported that ADAMTS1 in induced by hypoxia [2]. These data indicated that ADAMTS1 is a hypoxia-inducible gene. Interestingly, its expression is induced by a few hours` hypoxia (i.e., acute ischemia). The aim of this study is to test the hypothesis that whether ADAMTS1 promoter can be used for detecting acute hypoxia.
Methods: We cloned the human ADAMTS1 promoter region. To determine which region is responsible for hypoxic induction of ADAMTS1, we prepared several different constructs with different length of promoter region of ADAMTS1. We made green fluorescence protein (GFP) expressing construct under the control of the ADAMTS1 promoter. We transfected GFP construct into human umbilical vein endothelial cells (HUVEC), and examined GFP production under 3,6, or 24 hours of hypoxia. After incubation, cells were fixed using 4% paraformaldehyde (PFA) and observed under the fluorescence microscope.
Results: When GFP–transfected HUVEC were cultured in normoxic condition, GFP was very slightly observed. In contrast, a considerable number of GFP-positive HUVEC was observed when exposed to 3h hypoxia. GFP fluorescence and nearly returned to the normoxic level at 24h.

Conclusions: The ADAMTS1 promoter may be used for detecting acute hypoxia.

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2P-08
Matrix array as a novel research tool for analysis of cell-ECM interactions

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Keywords: Matrix array, Collagen, Keratinocyte

Extracellular matrix (ECM) does not only provide structural support for the cell, but it also regulates cellular activities via cell surface receptors. The interaction of cells with the ECM has been shown to be important for the regulation of many fundamental cellular processes including proliferation, migration, and survival.
Here, we developed a novel biological research tool (Matrix array) based on a concept of analyzing the cell behavior on various ECMs at the same time. Matrix array is cell-culture device made of glass slide, plastic slide or culture plate, which have multiple kinds of ECM-coated wells. We can freely arrange the various matrix proteins coated on glass slide or plate at any request. Using this device, you can investigate multi-cellular processes on different ECM proteins such as cell-proliferation, matrix metalloproteinases (MMPs) expression, ECM synthesis and localization of intracellular proteins.
In this study, we prepared collagen (type I, II, IV and V), laminin (111 and 332), fibronectin, gelatin and hyaluronic acid as matrix substrata in order to examine the responses of human keratinocyte cell-line (FEPE1L-8) to ECM proteins (cell-adhesive capacity and MMPs production). When FEPE1L-8 cells were seeded onto the matrix array and cultured for 1 hour, we found that cells adhered to and spread on type I, IV and V collagens and laminin 332, but not on gelatin, laminin 111 and hyaluronic acid. We next examined the MMPs expression by real-time zymography. Although we detected MMP-2 and -9 secreted from all cells cultured on each substrata, expression levels of MMP-2 and -9 did not differ significantly between substrata conditions.
By using the Matrix array, we can examine the cell-ECM interaction and determine the optimal culture-condition for cell growth or cell differentiation in various cells.

2P-09

Osteogenesis-mimicking Matrices as models of remodeling extracellular matrix in osteogenesis

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Keywords: Mesenchymal stem cell, osteogenesis,

Objective: Cellular microenvironment including extracellular matrices (ECM) is an important factor to regulate stem cell differentiation. During tissue development, ECM are remodelled dynamically to regulate stem cell differentiation *in vivo*. Here, we developed a novel kind of "stepwise osteogenesis-mimicked matrices" that were supposed to mimic the *in vivo* developmental ECM by decellularizing serially differentiated mesenchymal stem cells (MSC).
Methods: The MSC were cultured in proliferation or osteogenic induction medium to control their osteogenic differentiation at different levels. The ECM derived from non-differentiated, and differentiated MSC at early and late stages were prepared by the decellularization treatment and were referred as stem cell matrices, early stage matrices and late stage matrices, respectively.
Results: MSC cultured on the early stage matrices were more positively stained by alkaline phosphatase staining than were cells on the late stage matrices and stem cell matrices. And the expressions of *ALP* and *osteopontin* genes on the early stage matrices were higher than those on the stem cell matrices and late stage matrices. These results indicate that the early stage matrices enhanced osteogenesis of MSC. The results could be explained by the expression of osteogenesis-related transcription factors. MSC cultured on the early stage matrices and late stage matrices expressed a higher level of *RUNX2* than did those on stem cell matrices, suggesting that the stem cell matrices might reduce osteogenic differentiation by directly suppressing the transcription factor expression. MSC cultured on the late stage matrices expressed a high level of *PPARG*, an adipogenic transcription factor which inhibit the Runx2 activity, suggesting that the late stage matrices might reduce osteogenic differentiation by up-regulation of *PPARG*.

Conclusions: The stepwise osteogenesis-mimicked matrices could regulate osteogenic differentiation of MSC. They will provide a new model for the exploration of ECM on osteogenesis and be useful for tissue engineering.

2P-10

Sp1 and CBF/NF-Y transcription factors up-regulate the proximal promoter of mouse α3(V) collagen gene in osteoblasts

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We previously reported that mouse α3(V) collagen is expressed in bone and its basic N-terminal peptide adheres to osteoblasts (*Matrix Biol. 2005*). In this study, we analyzed the transcriptional regulation of this gene in osteoblasts. Oligo-Cap Race indicated that major transcriptional start site was located at 102 bp upstream from the initiating ATG codon. Cell transfection experiments with a series of *Col5a3* promoter-luciferase constructs demonstrated that the fragment from -337 to +1 is necessary for the proximal transcriptional activity in osteoblasts. In this region, two transcription factor binding sites (BS1: -194/-185 and BS2: -134/129) were identified by electrophoretic mobility shift assays. Interference assays using consensus oligonucleotides and specific antibodies indicated that Sp1/3 and CBF/NF-Y were bind to BS1 and BS2, respectively. Chip assay showed that these transcription factors bind the same region of the endogenous *Col5a3* gene. Moreover, the absence of these binding using deletion and/or mutation luciferase constructs, and interferes of these binding with Mithramycin A and/or dominant negative CBF/NF-Y suppressed *Col5a3* promoter activity. Furthermore, overexpression of Sp1 was increased this promoter activity, whereas Sp3 was not. These results suggest that Sp1 and CBF/NF-Y up-regulate the proximal promoter of mouse α3(V) collagen gene in osteoblasts.

2P-11

Distinct mechanisms in maintaining calvaria and long bone mass in adult mouse

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Keywords Osteocyte, SOST/sclerostin, Wnt/β-catenin, Mechanical stress, Bone formation, Osteoblast

Objective: Among diverse anabolic mechanical stimuli, which bones may experience, we have previously reported *in vitro* that signals downstream of stretching were processed in osteocytes resulting in bone formation while those downstream of low intensity, high frequency pulsed ultrasound (LIPUS) were processed in osteoblasts resulting in differentiation. It has been reported by others that disuse osteoporosis by bed rest affects long bones but not skull bones. To evaluate distinct mechanisms in the different responses, we isolated osteoblasts and osteocytes and analyzed mechanotransduction pathways**.**

Methods: Osteogenic cells were isolated from 16-week-old C57BL/6J mouse lower leg and calvarial bone chips by sequential treatments with collagenase and EGTA: osteoblasts from repeated collagenase digestion and osteocytes, after EGTA treatment. Cells were exposed to mechanical stimuli after one-week culture either by stretching in a FlexCell strain unit (osteocytes) or by exposing to LIPUS (osteoblasts).

Results: In both isolated long bone osteoblasts and osteocytes, mechanical stimulation resulted in upregulated message levels of component molecules in mechanotransduction pathways such as Wnt 1 and 3a, FZD, and COX-2. By stretching, upregulation of DMP-1 and downregulation of SOST/sclerostin, two reported mechanosensitive osteocyte markers, reproduced the response in loaded long bone. On the other hand, mouse long bone osteoblasts responded to LIPUS with elevated levels of DMP-1 and SOST/sclerostin, suggesting that LIPUS accelerated differentiation of osteoblasts to osteocytes. The above mentioned machinery molecules in mechanotransduction as well as SOST/sclerostin, a Wnt/β-catenin-pathway inhibitor, behaved similarly in the stimulated calvarial cells. Basal expression levels in osteoblasts, however, are generally much higher in calvaria than in the long bone.

Conclusions: Our results suggested that maintaining long bone mass in adult mouse requires mechanical stimuli but that calvarial bone relies on some other pathway(s) as a default mechanism.

2P-12

Sequential remodeling and loss of epithelial basement membrane type IV collagen α chains in the intraepithelial neoplasia (CIN) and squamous cell carcinoma of the uterin cervix

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Keywords: basement membrane, type IV collagen α chains, uterine cervical cancer

Objective: The destruction of the basement membrane (BM) is the first step in cancer cell invasion and metastasis. Type IV collagen is a major component of the BM and is composed of six genetically distinct α(IV) chains: α1(IV) to α6(IV). The loss of α5/α6(IV) chains from the epithelial BM at the early stage of cancer cell invasion has been reported in several types of cancer (1-3). However, the sequential remodeling or loss of α(IV) chains in the BM of intraepithelial neoplasia (CIN) and squamous cell carcinoma (SCC) of the uterine cervix remains to be unknown.

Method: The expression of α(IV) chains were immunohistochemically examined in 60 cases of biopsy and resected samples with CIN and SCC of the uterine cervix.

Results: In CIN 1-2 (mild to moderate dyspasia), both α1/α2(IV) and α5/α6(V) chains were linearly expressed in the BM of the squamous epithelium. However, in CIN 3 (severe dysplasia/carcinoma in situ), sequential remodeling in the BM of the squamous epithelium were observed that severe dysplasia expressed both α1/α2(IV) and α5/α6(IV) chains in the BM, and that carcinma in situ expressed only α1/α2(IV) chains in the BM. Interestingly, these transitional zone of the sequentially remodeled BM from α1/α2(IV) and α5/α6(IV) to α1/α2(IV) chains was confirmed in the BM of CIN3 lesion.

Conclusions: The sequential remodeling of type IV collagen α chains of the BM of the uterine cervical cancer seems to be closely related to cancer development preceded by cancer cell invasion.

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2P-13

Etracellular Matrix in frozen Mammoths-Protein Profile and Amino Acid Sequencing using LC/MS

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Objective: We attempt to make clear the characteristics of extracellular matrix (ECM) components of the frozen baby mammoths died and buried in Siberian permafrost about 40,000 years ago. We report here the result of collagen analysis from the mammoth lung and liver (kept in fixatives in Russian Academy of Sciences in St. Petersburg).

Methods and results: First we attempted to extract collagen molecules from the tissues by 3 different ways. But, full length or fragments of collagen could not be extract by acid, pepsin nor alkali treatment. It may indicate the occurring of heavy crosslink formation between collagen molecules during preservation in frozen soil or after excavation. The differential scanning calorimetry analysis showed existence of components with higher denaturation temperature. Next we analyzed protein by the LC/MS after digestion with trypsin. Type I and type III collagen were detected as a major component and also minor content of complement may be from the blood. We could not detect type IV collagen by this method. We achieved the partial sequencing of collagen I and III and it covered 1/3 of collagen sequence. We also sequenced collagen from Indian elephant bone as a control. Its sequence showed homology to elephant and human collagen

Discussion: For the analysis of lineage of extinct animals, usually mitochondrial DNA is used. We showed here collagen could be used for evolutional analysis of some fossil samples.

2P-14

Cell-cell Contacts Differently Regulate Alpha-Smooth Muscle Actin Expression and Collagen Production in Hepatic Stellate Cells

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Key Words: Hepatic stellate cells, Collagen, Alpha-smooth muscle actin

Background & Aims: Transformation from hepatic stellate cells (HSC) to alpha-smooth muscle actin (SMA)-positive myofibroblast-like cells is a central event in liver fibrogenesis. It has recently been shown that cell-cell contacts regulate HSC activation. Here we examined how cell density affects alpha-SMA expression and collagen production in cultured HSC.

Methods: HSC were isolated from wild-type mice or transgenic animals harboring tissue-specific enhancer/promoter sequences of alpha 2(I) collagen gene (COL1A2) linked to an enhanced green fluorescent protein (EGFP) gene, using a modification of the collagenase-pronase perfusion method. Cells seeded at different densities were grown and observed under a phase-contrast microscopy. HSC from transgenic reporter mice were stained with anti-alpha-SMA antibodies, and the expression of EGFP and alpha-SMA was examined using a confocal laser-scanning microscopy.

Results: A remarkable loss of lipid droplets and robust alpha-SMA expression were observed after 7days of culture of HSC seeded at a low cell density. In contrast, lipid droplets still remained in the cytoplasm and alpha-SMA expression was suppressed in cells seeded at higher confluency. On the other hand, activation of COL1A2 promoter occurred in HSC after 7days of culture irrespective of cell densities. Expression of alpha-SMA and EGFP was observed mostly in different cells from each other, and activation of COL1A2 promoter was detected in a very limited number of alpha-SMA-positive HSC.

Conclusions: The results indicate that the cell density has dramatic impacts on HSC activation as estimated by loss of lipid droplets and alpha-SMA expression. However, alpha-SMA expression and collagen production are not the common features of activated HSC, and cell-cell contacts may differently regulate those two major events in HSC activation.

2P-15

Little Contribution of Epithelial-to-mesenchymal Transition of Biliary Epithelial Cells to the Progression of Experimental Biliary Fibrosis

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Key Words: Epithelial-to-mesenchymal transition, Liver fibrosis, Collagen

Background & Aims: It has recently been reported that portal fibroblasts play a central role in the progression of biliary fibrosis, and that epithelial-to-mesenchymal transition (EMT) of biliary epithelial cells might be a source of those collagen-producing cells. Here we examined possible contribution of EMT to the development of experimental biliary fibrosis by using transgenic collagen promoter reporter mice.

Methods: Transgenic mice harboring tissue-specific enhancer/promoter sequences of alpha 2(I) collagen gene (COL1A2) linked to either firefly luciferase or enhanced green fluorescent protein (EGFP) gene underwent ligation of the common bile duct (BDL) to introduce biliary fibrosis. Activation of COL1A2 promoter was quantified by luciferase assays from day 0 to day 14 after BDL. The localization of EGFP-positive cells was determined by a laser-scanning confocal microscopic examination.

Results: A number of alpha smooth muscle actin (SMA)-positive myofibroblasts appeared around the dilated bile ducts on day 2 following BDL, where accumulation of collagen fibrils was observed. Prior to those histopathological changes, COL1A2 promoter was already activated 3-fold on day 1, and further increased thereafter. EGFP-positive cells were detected in the fibrous tissue underneath the dilated biliary epithelial cells as early as 2 days after BDL. Most of them were positive for alpha SMA. Biliary epithelial cells did not express EGFP, nor were they stained positive for alpha SMA throughout the observation period.

Conclusions: By using transgenic reporter mice which detect COL1A2 promoter activation with high sensitivity and specificity, we exclude collagen production by biliary epithelial cells, which indicates a limited role of EMT in the development of biliary fibrosis.

2P-16

Autophagy eliminates misfolded procollagen aggregates in the endoplasmic reticulum for cell survival

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Keywords: Collagen, protein degradation, autophagy, quality control, osteogenesis imperfecta

Objectives: Type I collagen is a major component of the extracellular matrix, and mutations in the collagen cause several matrix-associated diseases. These mutant procollagens are misfolded and often aggregated in the endoplasmic reticulum (ER). Although the misfolded procollagens are potentially toxic to the cell, little is known about how these misfolded procollagens are eliminated from the ER.

Methods: We examined two collagen degradation pathways, ERAD and autophgy, using two models: one is Hsp47-null chaperone-deficient cells and the other is Mov13 cell lines, which produce disease-causing collagen mutants. Furthermore, we analyzed the role of autophgy for cell survival against the cytotoxicity of ER-accumulated misfolded collagen by RNAi-mediated knockdown of autophagy proteins.

Results: Procollagen trimers aggregated in the ER are eliminated by an autophagy-lysosome pathway, but not by ERAD. Inhibition of autophagy by specific inhibitors and RNAi-mediated knockdown significantly stimulated accumulation of aggregated procollagen trimers, and treatment with an autophagy activator resulted in reduced amount of aggregates. In contrast, monomer procollagen mutant, which is deficient in trimer formation, is degraded by ERAD. The autophagic elimination of aggregated procollagen occurs independent of ERAD system. Moreover, we found that autophagy plays an essential role in cell survival against toxicity of the ERAD-inefficient procollagen aggregates.

Conclusions: Our study demonstrates that autophagic degradation of misfold procollagen aggregates in the ER is strictly dictated according to their conformation, and autophagic activity is essential for the cell survival by eliminating the ERAD-untreatable procollagen aggregates.

2P-17

Interaction of hemidesmosome protein and focal contact protein in healing wound

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Key words: β4 integrin, α-actinin, cell migration, live cell imaging

Objective: Keratinocytes (KCs) have two anchoring devices, hemidesmosomes (HDs) and focal contacts (FCs). From previous reports, HDs and FCs have been predicted to interact each other through laminin-332, CD151, plectin and signaling molecules. However, there have been no direct evidence of interaction between HD protein and FC protein in KCs. Therefore, we investigated the interaction between HD protein and FC protein in KCs.

Methods: To observe dynamics of HD protein and FC protein at the same time, we expressed YFP-tagged β4 integrin, and CFP-tagged a-actinin, respectively, in live HaCat cells at wound edges under several conditions and observed their dynamics by time-lapse video microscope.

Results: At the leading edge of scraped wound, FC protein assembled rapidly and regularly in the direction of the wound. Subsequently, HD protein followed and filled into the "FC protein-rich" region where FC protein disassembled. FC protein disassembled together with the appearance of HD protein and new FC protein assembled at the newly formed leading front of KCs. KCs repeated this cycle until KCs no longer moved. Under conditions that affect FCs, the HD protein dynamics became highly stable and HaCat cells ceased migration. Under conditions that affect HDs, the velocity of FC protein became more rapid and the direction of the assembly of FC became irregular. The migration of KCs was not in alignment. Under other conditions (treatment of anti-laminin-332 antibodies, transfection of CD151 siRNA, plectin siRNA, FAK and Shc siRNA), the dynamics of HD protein and FC protein were affected and the migration of KCs became irregular.

Conclusions: The interaction between HDs and FCs does occur at at least in protein level in KCs at the wound edge and that this interaction is mediated by the fine tuning of each constituent of HD, FC, signal molecules and in-between proteins.

2P-18

Inflammatory Alveolar Bone Resorption in Mouse Model of Marfan Syndrome

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Keywords: *Marfan syndrome,* severe periodontitis, fibrillin-1

Objective: Marfan syndrome is a systemic disorder of connective tissue, such as, skeletal, cardiovascular, and ocular systems. In addition, severe periodontitis is frequently seen in this disorder. *FNB1* encoding fibrillin-1, which is a microfibrillar protein in elastic system fibers, is one of the responsible genes for this disorder. We hypothesized that abnormal fibrillin-1 expression might relate to the pathogenesis of the severe periodontitis in this disorder. In order to clarify the mechanism of the periodontitis, the mouse model of this disorder (hypomorphic *Fbn-1* mouse) was challenged by *Porphyromonas gingivalis (P.g.)* in this study.

Methods: Hypomorphic *Fbn-1* mouse (6-week-old heterozygous MgΔ mice; n=6), which have 5 times lower *Fbn-1* expression than age-matched wild-type mice (WT; n=6), were infected with *P.g.* after one week of the antibiotic treatment. At 2 and 8 weeks after the infection, the distance and area were measured between the cementoenamel junction and alveolar bone crest. The blood sample and were also collected from mice. The alveolar bone resorption was examined by μCT analysis (Shimadzu InspeXio SMX), and the level of TNF-α was examined by ELISA.

Results: The *P.g.* infection induced the alveolar bone resorption both in hypomorphic *Fbn-1* and WT mice. The amount of bone resorption was significantly higher in hypomorphic *Fbn-1* mice than in WT mice. This was accompanied by the higher level of TNF-α.

Conclusion: The severe periodontitis in Marfan syndrome was reproduced in mice. Findings suggest that the decreased *Fbn-1* expression induces the increased level of TNF-α.The results suggest that the normal fibrillin-1 expression is indispensable for the integrity and maintenance of the periodontal tissues.

2P-19

Eosinophil Cationic Protein (ECP) Protects hearts against myocardial infarction

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Keywords: Heart, Myocardial infarction, Remodeling

Objective: ECP is a basic protein secreted from activated eosinophils. Recently, we found that ECP accelerates cardiomyocyte differentiation. Here we examined the protective effects of ECP against myocardial infarction (MI).
Methods: Adult male Sprague-Dawley rats were anesthetized, and the left anterior descending coronary artery was ligated. First, ECP or saline (PS) was injected directly to the heart. Next, we administered ECP or PS systemically using osmotic pump (Alzet model 2ML2). After 7 days, cardiac function was examined using ultrasound and recorded LV systolic and diastolic parameters. Then the heart was taken and embedded in paraffin, and embedded sections were cut (5 μm) and stained with hematoxylin and eosin or Masson Trichrome. Ventricular remodeling after MI was calculated as a width of left ventricle divided by a width of septum.
Results: ECP injection attenuated ventricular remodeling after MI compared with control rats. Echocardiography demonstrated an improvement of cardiac function after MI in ECP-treated rats compare with PS-treated rats.

Conclusions:
Our results indicate that ECP has protecting effects on hearts against myocardial infarction.

2P-20

Recombinant α1 chain of human type I collagen in the silkworms *Bombyx mori*: production of human gelatin as a novel biomaterial

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Keywords: Recombinant, Gelatin, Silkworm

Objective: Most of the marketed collagens and gelatins are currently derived from animal skins or bones. Recently we generated the transgenic silkworms producing a recombinant α1 chain of human type I collagen (rα1) into their cocoons. Because of the absence of hydroxyprolines, rα1 does not possess the triple helical structure. In the present study we analyzed biochemical and cell biological properties of rα1 to demonstrate its usability as a novel biomaterial.
Methods: Purified rα1 was characterized on amino acid composition, N-terminal sequence, and CD spectra. We also analyzed the attachment and spreading of human skin fibroblasts on dishes coated with rα1. Cynomolgus monkey ES cells were cultured with murine embryonic fibroblast feeder cells on dishes coated with rα1.
Results: Analysis of amino acid composition and N-terminal sequence showed that the primary structure of rα1 was identical to that of native type I collagen except for the absence of hydroxyprolines and hydroxylisines. CD spectra of rα1 showed that the secondary structure was similar to denatured type I collagen, confirming the absence of the triple helical structure in rα1. rα1 was also shown to be the useful substrata to promote the attachment and spreading of fibroblasts at appropriate concentrations. ES cell colonies cultured on the dishes coated with rα1 expressed markers for the undifferentiated state after seeding 30 passages. The cells implanted into immunodeficient mice formed teratomas, demonstrating that the ES cells actually possessed pluripotency after culturing in this condition.
Conclusions: This study showed that the biochemical property of rα1 was similar to that of denatured collagen. Cell biological analyses suggested that rα1 may be used as an alternative to gelatins derived from animal tissues. Since rα1 has a very low risk of contamination of animal-derived materials, rα1 promises to be useful as a novel biomaterial for regenerative medicine.

2P-21
A 384-well format screening of the compounds that inhibit collagen-protein interactions

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Keywords: Collagen, Screening, Collagen-binding protein

Objective: Collagen is a multifunctional protein that exhibits diverse biological activities. These functions are elicited by interactions between dozens of collagen-binding proteins (CBPs) and the collagen triple-helix. Since interactions of CBPs with collagen are often related to pathophysiological events in human body, some CBPs are currently regarded as targets for drug development. In this paper, we developed a high-throughput turbidmetric assay system to obtain inhibitors of collagen-CBP interactions.
Methods and Results: Our assay system is based on the finding that CBPs retard spontaneous collagen fibril formation *in vitro*, and the fibril formation is restored in the presence of compounds that disrupt the collagen-protein interaction. In this paper we show results of 384-well format screening of a set of test compounds against five recombinantly expressed CBPs, such as heat-shock protein 47, pigment epithelium derived factor, von Willebrand factor, glycoprotein VI and bacterial collagenase.
Conclusions: Using the system, the inhibitory activity of a set of test compounds were effectively evaluated for the five target CBPs in the same assay platform. Moreover, the use of the common assay platform will also bring us information about the specificity in the inhibitory action of a compound. The rapid screening system is a powerful tool for obtaining inhibitors for disease-related CBPs.

2P-22
APC-induced MMP Activation in Human Diseased Chondrocytes Requires EPCR and Thrombomodulin

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Objectives: Activated protein C (APC) is derived from its inactive precursor Protein C (PC) through binding to endothelial protein C receptor (EPCR). We have previously shown that in cytokine-stimulated ovine cartilage, APC leads to collagenolytic MMP activation and cartilage degradation. This study investigates if APC can activate MMPs in human articular chondrocytes and the mechanisms/pathways whereby APC has its effects.
Methods: Chondrocytes were isolated from knee joints of normal young (6-12 month old) sheep, and patients undergoing joint replacement surgery. Cell monolayers were cultured serum free ± IL-1 and APC for 3 days. MMP-2, -9 and –13 activity was measured using gelatin zymography and fluorogenic substrate assays, and expression of enzymes and matrix components analysed using real-time RT-PCR. Activation of human MMP-2, -9 and -13 proenzymes by APC was examined in vitro.
Results: APC was unable to directly activate recombinant human proMMP-2, -9 or -13 in solution in vitro. In contrast, APC led to the activation of MMP-2, -9 and –13 in IL-1-stimulated cultures of normal ovine chondrocytes. APC also activated MMPs in IL-1-stimulated but not control human chondrocyte cultures. Interestingly, this activation only occurred in half of the OA-human patients, even though they all synthesised pro-MMPs that could be activated by APMA. Patients in which MMP-activation by APC was observed were distinguished by upregulation of EPCR, thrombomodulin and MMP-9 mRNA by IL-1 + APC.
Conclusions: These results suggest that APC is a physiologically relevant activator of chondrocyte MMPs implicated in cartilage breakdown in arthritis in humans. The differentiation of human OA patients into two sub-populations suggests that MMP activation by APC requires chondrocyte EPCR and TM and could be important in disease progression and as a therapeutic target in OA.

2P-23

Development of ELISA Measurement for Urinary 3-Hydroxyproline containing Peptides and its Preliminary Application to Community Healthy Persons and Cancer Patients

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Keywords: 3-hydroxyproline; cancer; basement membrane

Introduction: As basement membrane is degraded by cancer cell invasion to blood vessels and/or lymph vessels, the increased excretion of endogenous 3-hydroxyproline (3-Hyp) is expected in cancer patients because 3-Hyp is the unique component of type IV collagen in basement membrane. We developed ELISA method to measure 3-Hyp containing peptides in urine and report preliminary application for cancer screening.

Methods: Polyclonal antibodies were made against a synthetic peptide of 10 amino acids including putative prolyl 3 hydroxylation product in collagen sequence. Competitive ELISA method using the antigen peptide was developed to measure urinary 3-Hyp containing peptides and applied to healthy controls and cancer patients.

Results: The ELISA assay detected the antigen peptide mixed in urine in the range of 0.1μg/ml to 80 μg/ml. One hundred and eighty healthy controls and 22 cancer patients samples were assayed by this method. The values in controls were 2.44 ± 1.90 (SD) mg peptide /gm creatinine for 52 men (with a range from 0.65 to 10.51) and 2.87 ± 2.01 (0.94 to 17.31) for 128 women. The values in 22 cancer patients unexpectedly showed the very low value, 0.110 ± 0.137 ($p<0.001$). As reported previously, endogenous urinary excretion of 3-Hyp measured by an amino acid analysis showed very low levels in healthy controls and high levels in cancer patients, but this ELISA study showed the opposite results. This suggested that cancer tissues have high levels of MMPs and/or peptidase activities that could degrade 3-Hyp-containing polypeptides.

Conclusions: The competitive ELISA assay to measure urinary 3-Hyp containing peptides showed the difference between healthy control and cancer patient samples.

2P-24

Insulin–like growth factor binding protein-related protein 1 (IGFBP-rP1/TAF) synergistically modulates tumor cell adhesion with laminin-332 (laminin-5)

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Keywords: IGFBP-rP1, Laminin-5, cell adhesion activity

Objective: Insulin–like growth factor binding protein-related protein 1 (IGFBP-rP1), a member of IGFBP superfamily, was originally identified as a tumor-derived, cell adhesion factor (TAF) that interacts with heparan sulfate proteoglycans. This protein is highly accumulated in blood vessels of tumor tissues and has recently been reported to have tumor-suppressing activity. However, exact function of IGFBP-rP1 remains unknown. On the other hand, laminin-332 (Lm5) is an important basement membrane protein which has potent cell adhesion and migration activities. Lm5 is also involved in tumor growth and invasion. In this study, we attempted to characterize the cell adhesion activity of IGFBP-rP1 and its functional interaction with Lm5.

Methods: The cell adhesion activities of purified IGFBP-rP1 and Lm5 were analyzed using human colon adenocarcinoma cell line DLD-1. DLD-1 cells introduced with a control vector or an IGFBP-rP1-expression vector were also used.

Results: When IGFBP-rP1 alone was coated on plastic plates, it scarcely supported adhesion of DLD-1 cells to the substrate. However, when IGFBP-rP1 was co-coated with Lm5 at 0.2 μg/ml, where Lm5 alone did not support cell adhesion, the cell adhesion and spreading were strongly promoted depending on the amount of IGFBP-rP1. This synergistic cell adhesion activity of IGFBP-rP1 was efficiently blocked by heparin, anti-integrin-α3 or β1 antibody, whereas heparin did not inhibit the cell adhesion to Lm5 (0.5 μg/ml) alone. This suggests that IGFBP-rP1 efficiently promotes the integrin-mediated cell adhesion to Lm5 by binding heparan sulfates on cell surface. We also found that IGFBP-rP1-expressing DLD-1 cells attached on plates more efficiently than the control cells.

Conclusions: These data indicate that IGFBP-rP1 modulates tumor cell adhesion to Lm5 and possibly other integrin-dependent substrates. It seems likely that IGFBP-rP1 produced by tumor cells or stromal cells affects tumor growth by the synergistic action with Lm5 at invasion fronts.

2P-25

Effects of nicotine and lipopolysaccharide on the expression of MMPs, PAs, and their inhibitors in human osteoblasts

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Keywords: osteoblast, MMPs, PAs, PAI-1, LPS, nicotine

Objective: Lipopolysaccharide (LPS) from periodontopathic bacteria can initiate alveolar bone loss through the induction of host-derived cytokines. Smoking increases the risk and severity of periodontitis. We examined the effects of nicotine and LPS on the expression of matrix metalloproteinases (MMPs), plasminogen activators (PAs), and their inhibitors, including tissue inhibitors of metalloproteinases (TIMPs) and PA inhibitor-1 (PAI-1), in human osteoblasts.

Methods: The cells were cultured with or without 10^{-4} M nicotine and 100 ng/ml LPS for 12 days or with 100 μg/ml polymyxin B, 10^{-4} M D-tubocurarine, 10^{-5} M NS398, or 10^{-6} M celecoxib in the presence of either nicotine or LPS for 12 days. The gene and protein expression levels for MMPs, PAs, TIMPs, and PAI-1 were examined using real-time PCR and ELISAs, respectively. PGE_2 production was determined using an ELISA.

Results: The addition of nicotine and/or LPS to the culture medium increased the expression of MMP-1, -2, and -3 and tissue-type PA (tPA); decreased the expression of TIMP-1, -3, and -4; and did not affect expression of TIMP-2 or PAI-1. In the presence of D-tubocurarine or polymyxin B, neither nicotine nor LPS stimulated the expression of MMP-1. In the presence of NS398 or celecoxib, the stimulatory effects of nicotine and LPS on MMP-1 expression were unchanged, but they were unable to stimulate PGE_2 production.

Conclusion: These results suggest that nicotine and LPS stimulate the resorption process that occurs during turnover of osteoid by increasing the production of MMPs and tPA and by decreasing the production of TIMPs. Furthermore, they suggest that the stimulatory effect of nicotine and LPS on PGE_2 production is independent of their stimulatory effect on MMP-1 expression.

2P-26

Immobility-induced Cartilage Degeneration differed at Three Specific Areas

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Keywords: Immobilization, Cartilage, Degeneration

Objective: Joint immobilization induced cartilage degeneration. In our previous report, the changes of the articular cartilage were different at the three specific areas as follows; atrophic changes in *the non-contact* area, hypertrophic differentiation of chondrocytes in t*he transitional area*, and decreased number of chondrocyte in *the contact area* [1]. The purpose of this study was to investigate the mechanism of articular cartilage degeneration after immobilization at the three specific areas.

Methods: Adult male Sprague-Dawley rats' knee joints were immobilized at 150° of flexion by rigid internal fixator (3 days to 16 weeks). After fixed with 4% paraformaldehyde and decalcified, specimens were embedded in paraffin. Expression of collagen I and II, matrix metalloproteinase (MMP)-8 and -13 was evaluated by in situ hybridization or immunohistochemistry. Total RNA was extracted from the articular cartilage and expression levels of these mRNA were measured by quantitative PCR.

Results: Expression of collagen II and MMP-8 was decreased after 3 days in the three areas, but increased after 2 weeks at hypertrophic differentiated chondrocytes in the transitional area. Immunostaining of collagen II at the transitional and contact areas was decreased. Immunostaining of collagen I was increased at hypertrophic differentiated chondrocytes in the transitional area and superficial chondrocytes in the non-contact area. Immunostaining of MMP-13 was observed at the hypertrophic differentiated chondrocytes in the transitional area. Expression levels of collagen II mRNA was decreased, however, MMP-8 and -13 mRNA was increased by quantitative PCR.

Conclusions: The mechanism of the articular cartilage degeneration after immobilization differs at the three specific areas [2, 3].

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2P-27
The role of type I collagen in full-thickness articular cartilage repair

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Keywords: Articular cartilage repair, Collagen, Mesenchymal stem cells

Objective: Type I collagen is well used for cartilage repair. However its own role is not understood and it has been used simply as 'scaffold'. Our objective is to demonstrate the role of type I collagen itself for the cartilage repair by detail histological evaluation.
Methods: 5mm-diameter full-thickness articular cartilage defect was created at patellar groove of rabbit knee joint. 1) Defect with no implant, 2) Defect with collagen gel were made and evaluated. Toluidine blue staining, type I and II collagen IHC for qualitative analysis, BrdU for detect of proliferating cell, moreover triple staining of BrdU, CD44, and CD45 using CLSM and TEM were performed for cell type defection.
Results: Articular cartilage repair was promoted in defect with collagen gel. There are many proliferating cells in the peripheral area of defect with collagen gel. Many of these cells were mesenchymal stem cells [1].

Conclusions: Type I collagen gel actively enhance recruitement of mesenchymal stem cells from bone marrow. Utilizing this function, we try new approach for cartilage repair using only type I collagen gel [2].

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2P-28
Over-stress of cyclic compressive load on human synovium-derived cells in three-dimensional cultured tissue induces prolonged MMP-3 gene expression

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Keywords: 3D tissue, Cyclic compressive loading, Synovium-derived cell

Previous our studies revealed that human synovium-derived cells in three-dimensional collagen-based cultured tissue express MMP genes after cyclic compressive load for five days.
Objective: The objective of this study was to examine the time course of mRNA expression levels of MMP genes after cyclic compressive load for one hour.
Materials and methods: Human mesenchymal cells were isolated from knee synovium and cultured in monolayer. Collected cells (5.0×10^5/scaffold) were suspended in 0.5% atellocollagen gel and incorporated into a collagen scaffold(diameter 5mm×3mm) by centrifugal force to construct three-dimensional tissue. After 3 days incubasion, unconfined uni-axial cyclic compressive load was applied for 1 hour at 0, 20 or 40kPa in the frequency of 0.5 Hz using a custom-made cyclic load bioreactor. Histological analysis was performed by hematoxylin-eosin staining and DAPI-phalloidin staining. mRNA expression levels for MMPs and inflammatory cytokines genes were analyzed at pre-load and 0, 3, 6, 12, 24 hours after loading by real time RT-PCR.
Results: mRNA expression levels for MMP-1, MMP-9 and IL-8 increased up to 6 hours after loading, and then resumed after 12 hours. mRNA expression level for IL-6 increased until 3 hours after load, and then decreased. mRNA expression level for MMP-3 increased and prolonged up to 24 hours after loading. mRNA expressions for MMP-13, TIMP-1 did not change after cyclic compressive load.
Conclusions: The time course of MMP-3 gene expression level was different from those of other MMPs or ILs and its gene expression increased and prolonged after one hour cyclic compressive load on human synovium-derived cells in three-dimensional collagen-based cultured tissue.

2P-29

Molecular profiles of basement membranes during early stages of mouse embryogenesis

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Keywords: Basement Membrane, Laminin, Mouse Embryo

Objective: Basement membrane (BM) plays indispensable roles during embryogenesis. Molecular composition of BMs are critical for the cellular microenvironment which regulates cell behavior and tissue organization. We have investigated the distribution of BM proteins in early mouse embryos and reported overall profiles of the BM protein composition [1]. To obtain further insight into relationships between BM composition and embryonic development, we focused on the BM proteins expressed in developmental stage- and tissue-dependent manners and compared their distributions with tissue specific proteins.
Methods: Whole-body sections of early mouse embryos (E5.5 ~ E10.5) were immunostained for various BM or tissue specific proteins.
Results: Among 20 BM proteins including individual subunits of laminins and type IV collagens, 11 showed spatiotemporally specific expressions. Laminin α1 and α5 were detected at the ealiest stage, i.e. E5.5. The complexity of BM compositions increased as embryonic development advanced. The lung bud of E10.5 embryos showed unique and highly complex BM protein composition. Several characteristic expression patterns of BM proteins were also observed. For example, localizations of laminin α4 were mostly coincident with PECAM-1, a blood vessel endothelial protein. However, in the liver of E10.5 embryos, laminin α4 was hardly detected despite that the distribution of PECAM-1 was similar to several other BM proteins such as laminin α1 and α3, indicating that the blood vessel BM in the developing liver has a unique laminin composition.
Conclusions: The comprehensive study of BM composition through developmental stages provides an integral view of the role of BMs and cell-BM interactions in organogenesis.

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2P-30

Xenopus dicalcin, a novel mediator of sperm-egg interaction in the extracellular egg-coating membrane in *Xenopus laevis* eggs

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Keywords: Fertilization, *Xenopus laevis*, egg

Objectives: In the sexually reproducing organisms, fertilization is crucial to produce a zygote. The success of fertilization completely depends upon appropriate sperm-egg interaction beginning with species-restricted recognition of sperm and egg coating membrane, a relatively thick extracellular coating membrane, called zona pellucida (ZP) in mammal and vitelline envelope (VE) in other species including amphibian. Despite identification of several candidates for ligands and their receptors in sperm and eggs, molecular mechanisms of sperm-egg interaction remain elusive. To contribute to the study of sperm-egg interaction, we have characterized *Xenopus* dicalcin, recently isolated by us in *Xenopus* eggs.
Methods: We examined localization of dicalcin in *Xenopus* eggs by standard immunohistochemical procedures, where *Xenopus* eggs were dejellied, fixed, embedded, and cut into serial sections, and then reated with specific antibody. To examine effect(s) of dicalcin on sperm-egg interaction. we performed *in vitro* fertilization assay where dejellied eggs were pretreated with BSA or dicalcin, followed by addition of sperm suspension, and the fertilization rate was scored.
Results: *Xenopus* dicalcin is localized prominently in the VE, and dicalcin exhibits a Ca^{2+}-dependent binding to two glycoproteins that constitute a polymeric framework of VE. Since these VE glycoproteins are considered to function as sperm-receptors, we tested the effect of dicalcin on sperm-VE binding, sperm-VE penetration, and fertilization *in vitro*. Preincubation of eggs with recombinant dicalcin reduced the number of sperm that bound to VE as well as the efficiency of fertilization. In contrast, inhibition of intrinsic dicalcin by preincubation with anti-dicalcin antibody increased sperm-binding to VE and the efficiency of fertilization. Furthermore, in our *in vitro* penetration assay, recombinant dicalcin inhibited sperm-VE penetration significantly.
Conclusions: These results strongly suggested that dicalcin dampens sperm-egg interaction both in sperm-VE binding and sperm-VE penetration, causing an inhibitory action during fertilization. Molecular mechanism of dicalcin’s action will also be discussed.

2P-31
Sustained Activation of β1-Integrins induces Proliferative Arrest or Apoptosis in Fibroblasts

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Objectives: Cells require not only a signal from growth factor receptor but also an additional signal from a family of adhesion receptor, integrin, for their survival and proliferation. We previously found that a peptide derived from tenascin (TN)-C, termed TNIIIA2, strongly activates β1-integrins [1]. TNIIIA2 is capable of protecting normal mouse fibroblast NIH3T3 from anoikis-like cell death, but of inducing apoptosis in human sarcoma-like WI38VA13cells. It is interesting to verify whether TNIIIA2 can induce apoptosis preferentially in malignant tumor cell types. In this study, we investigate the cellular responses of human fibrosarcoma-like cell line WI38VA13 and its parental normal cell line WI38 to β1-integrin activation.

Methods Results and Conclusions: TNIIIA2 induced β1-integrin activation also in WI38 normal cells. When WI38 normal cells were stimulated with TNIIIA2 on the fibronectin (FN)-coated culture plate, the proportion of cells spreading was increased. This spreading on the FN-substratum was retained with TNIIIA2 for a long time, resulting in proliferative arrest in WI38 normal cells, as evaluated by the BrdU assay. Cell cycle inhibitor proteins, $p21^{cip1}$ and $p16^{INK4a}$, became expressed in WI38 normal cells after treatment with TNIIIA2. Under the same conditions, WI38VA13 malignant cells underwent apoptosis, as judged by DNA fragmentation, cleavages of caspase-9 and -3 and PARP. The small G-protein Ras was spontaneously activated in WI38VA13 malignant cells, but not in WI38 normal cells. The status of Ras activation might be one of the determinants as to whether β1-integrin activation leads cells to proliferative arrest or apoptosis.

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2P-32
Promotion of PDGF-dependent cell proliferation through β1-integrin activation

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Objective: Cross-talk between integrin and receptor tyrosine kinase (RTK) is essential for the anchorage-dependent regulation of cell proliferation. We recently found that NIH3T3 cell proliferation stimulated with PDGF is markedly promoted through β1-integrin activation by a peptide derived from tenascin-C, termed TNIIIA2 [1]. Integrin-mediated adhesion is generally considered to activate synergistically the Ras/MAP-kinase pathway in concert with the RTK signaling. As expected, the TNIIIA2-induced activation of β1-integrin induces a synergistic activation in not only the MAPK (ERK1/2) but also the small G protein Ras. Surprisingly, β1-integrin activation by TNIIIA2 also generates a conspicuous increase in the autophosphorylation of PDGF receptor (PDGFR) stimulated with PDGF. In this study, we investigate the signaling pathway relevant for promotion of the PDGF-dependent cell proliferation through β1-integrin activation by TNIIIA2.

Methods and Results: NIH/3T3 cells were seeded on a culture plate coated with increasing concentrations of FN, stimulated with PDGF, and then examined for detection of tyrosine phosphorylation of PDGFR. Autophosphorylation of PDGFR was remarkably increased depending on the coated concentration of FN. To clarify the signaling route responsible for TNIIIA2-induced promotion of the PDGFR autophosphorylation, we established the stable transfectants of NIH3T3 cells (NIHdnfak-shc) expressing dominant negative FAK and Shc, both of which are known to transduce a signal from β1-integrin to the Ras. Although the signal pathway from β1-integrin to the Ras was partially blocked in NIHdnfak-shc cells, neither autophosphorylation of PDGFR nor cell proliferation were influenced by TNIIIA2 treatment. Co-immunoprecipitation of β1-integrin with RTKs is an important approach to identify biochemical interaction between those receptors. Results showed that physical association of β1-integrin with PDGFR became detectable by treating cells with TNIIIA2.

Conclusions: These results suggest that TNIIIA2 promotes PDGF-dependent cell proliferation by inducing the direct association of β1-integrin with PDGFR, independent of the intracellular signaling pathways, such as the FAK-Src and Shc-caveolin.

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2P-33

Chemosensitization of Malignant Tumor Cells to Anticancer Drugs through β1-integrin Activation

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Objectives: Adhesion receptor integrin plays an essential role in fundamental cellular processes, including cell growth and survival. We previously found that a peptide derived from tenascin-C, termed TNIIIA2, has a potent ability to induce β1-integrin activation. When B16-BL6 mouse melanoma cells are enforced to adhere to fibronectin through a potent activation of their β1-integrins by TNIIIA2, cells undergo apoptosis. We reported last year that chemosensitivity of B16-BL6 cells to doxorubicin (DOX, an anti-cancer drug,) was remarkably increased by stimulation with TNIIIA2. Here, we investigate the molecular mechanism underlying this chemosensitization of B16-BL6 cells to DOX.
Methods, Results and Conclusion: TNIIIA2 was capable of promoting B16-BL6 cell spreading on the FN substrate through β1-integrin activation. B16-BL6 cells underwent apoptosis when kept spreading of B16-BL6 cells with TNIIIA2 for 2 days. When B16BL6 cells were treated with the DOX in conbination with TNIIIA2, cell apoptosis was synergistically increased. Flow cytometric analysis showed that treatment with TNIIIA2 caused a significant increase in the intracellular accumulation of DOX. TNIIIA2 also induced the intracellular accumulation of Rh123, which is known as a substrate of MDR1. TNIIIA2mut, control peptide of TNIIIA2, which is lacking in proadhesive activity due to its point mutation of the amino acid sequence, was inactive in the intracellular accumulation of DOX. Another integrin activator, 9EG7, also induced the accumulation of DOX in B16-BL6 cells. Moreover, siRNA-based down regulation of talin, which plays an indispensable role in the β1-integrin activation, resulted in loss of the Rh123 accumulation within B16-BL6 cells in responce to TNIIIA2. These results suggest that β1-integrin activation may cause a functional prevention of MDR1, resulting in intracellular accumulation of DOX.

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2P-34

The Expression and the Distribution of Epiplakin on Wound Healing

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Keywords: Epiplakin, Wound healing, Cell migration, Keratinocyte

Objective: Epiplakin (EPPK) belongs to the plakin family of cytolinker proteins. EPPK is mainly expressed in the outer layer of epidermis. In EPPK -/- mice, wounds on the backs closed more rapidly than those on the backs of wild type and heterozygous mice. We propose that EPPK might be linked functionally with keratin 6. Here we studied the relationship between the expression of EPPK, the change of shapes of the keratinocytes, and the network of the keratins on wound healing.
Methods: In the back skin of wild type and EPPK -/- mice, wounds were made and the keratin networks and shapes of keratinocytes were observed with immunofluorescence and electron-microscope.
Results: EPPK expressed in the suprabasal keratinocyte, especially in the outer layers of the wound edge. EPPK did not expressed at the tip of the leading edges during wound closure. After injury, EPPK, keratin10 and 6 were colocalized in the hypertrophic keratinocytes on 4 to 6 days wound. The expression reduced on day 8, and the staining pattern of EPPK returned to normal on day 10. In 4 days wound of EPPK -/- mice, keratin expressions were similar to those of wild-type mice wound. But in electron microscopy, the keratin fibers were loose, and the connections to desmosomes were not recognized remarkably. Keratin fibers were thinner than those of wild-type mice.
Conclusions: The migration speed of keratinocytes seemed to be modified by interaction between these keratin fibers and EPPK.
Ref Goto et al Mol Cell Biol 26: 548-58, 2006

2P-35

The study of fibrogenesis using a wound healing model

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The excess production of collagens and other ECM components causes tissue fibrosis. It is therefore important to elucidate the mechanism of production of collagen molecules in order to obtain a better understanding of fibrogenesis.

We examined the expression of collagen subtypes, the kinds of fibers and the phenotypes of fibroblasts in wounded skin using a mouse model. Two full-thickness wounds, which measured 5-mm diameter, were made on the dorsal skin of the ICR mouse. Thereafter, the wound and the surrounding areas thereof were cut at different time points, namely at 2, 4, 6, 10 and 15 days and 1 year after the wounds had been made. The excised tissue specimens were then used for *in situ* hybridization and electron microscopy analyses. The major types of collagen, namely types I and III, were initially expressed at 2 days in the post-wound granulation tissue speciens. Interestingly, the fibroblasts derived from the superficial fascia of connective tissue in body wall muscle, and not the dermal skin fibroblasts, were thus found to play a major roles in the production of collagen and wound closer. The former one had a lot of vesicles and long filopodiums, which did not appear to be fibroblastic. However, after making the wound, the cells showed a fibroblastic form that had an abundant rough endoplasmic reticulum and cytoskeleton fibers and which also induced collagen production. For cell culture, we obtained morphologically uniform fibroblastic cells from the subcutaneous superficial fascia in the connective tissue of the non-injured skin. When these cells were stimulated with a medium containing 10% FCS, morphological changes which were similar to those in the wounded tissue specimens were thus observed. These cells were different from the circulating fibrocytes in the peripheral blood that have recently been reported in regard to their presence in connective tissues, while they have also been observed to be CD13 negative. These fibroblastic cells express a high ratio of Type V collagen in comparison to type I collagen and they also produce rather thin fibers. As a result, they may be newly categorized as fibrolastic cells. Therefore, further investigation is called for to find new specific marker and to elucidate biological function of these cells.

2P-36

Monitoring of Pressure Ulcer Detecting ECM Fragments From Wound Surface

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Objective: Although pressure ulcer is defined as pressure induced skin ulcer, its clinical appearance is heterogeneous. The heterogeneity of pressure ulcer makes the treatment and prevention difficult. Therefore, we investigate the wound surface ECM in order to categorize pressure ulcer and to develop the biomarker for the wound. In this study, we focus dermal matrix molecules such as versican, decorin, fibulin-2, latent TGF-beta binding protein-1 (LTBP-1) and fibronectin,

Methods: We sampled ECM from wound surface using absorbent cotton. Then the samples were extracted with 6 M guanidine hydrochloride buffer. We mainly employed dot blotting analysis using specific antibodies for the ECM molecules, since high molecular ECM aggregates do not enter the SDS-PAGE gel. Immmuno histochemical study was also performed.

Results: Versican G3 fragments were detected from transitional granulation tissue between hydrated fragile matrix and stabilized epithelial connective tissue. Versican G3 fragments were also detected from granulation tissue with friction. Fibronectin was detected from wound surface in epithelial formation. Decorin was detected from almost all wound surfaces.

Conclusions: Combination of detecting these matrix molecules by dot blot assay can clarify the pathogenesis of pressure ulcer.

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平成 17 年度　永井　裕　（東京医科歯科大学名誉教授）
　　　　　　森　陽　（東京薬科大学名誉教授）

平成 18 年度　新海　浤　（千葉大学大学院医学研究院教授）
　　　　　　早川 太郎　（愛知学院大学名誉教授）
　　　　　　林　利彦　（帝京平成大学薬学部教授）

平成 19 年度　木全 弘治　（愛知医科大学分子医科学研究所所長・教授）

平成 20 年度　受賞者なし

日本結合組織学会　功労賞受賞者

平成 17 年度　三共株式会社
　　　　　　生化学工業株式会社

平成 18 年度　受賞者なし

平成 19 年度　受賞者なし

平成 20 年度　受賞者なし

日本結合組織学会　論文賞受賞者

（　）内は受賞時の所属

平成 17 年度

- Kikuji Yamashita, Satoru Eguchi, Hiroyuki Morimoto, Takao Hanawa, Tetsuo Ichikawa, Nobuyoshi Nakajo and Seiichiro Kitamura：
Extracellular matrix formed by MC3T3-E1 osteoblast-like cells cultured on titanium. Connective Tissue 36(1)1-8, 2004.
- 久保 孝利、能勢 卓、岩本 昭英、笹栗 靖之、森 陽、伊東 晃：
ウサギ軟骨関節の細胞外マトリックスおよびマトリックスメタロプロテアーゼ産生に及ぼす加齢の影響 Connective Tissue 36(4)197-205.

平成 18 年度　受賞者なし

CTR 誌移行に伴い平成 18 年度で廃止

日本結合組織学会　優秀演題賞

（　）内は受賞時の所属

平成 16 年度

- 岡崎　賢　（九州大学整形外科学教室）
軟骨特異的蛋白 CD-RAP の組織特異的転写調節領域の解析
- 輪千　浩史　（星薬科大学臨床化学教室）
新たな in vitro エラスチン繊維形成モデルの確立
- 望月　早月　（慶應大学医学部病理学教室）
ADAM28 の MMP-7 による活性化と IGFBP-3 切断による乳癌細胞増殖促進作用
- 福士　純一　（九州大学整形外科学教室）
NG2 プロテオグリカンはガレクチン 3 と a3b1 インテグリンを介して血管新生を促進する

- 竹澤　俊明　　（独立行政法人農業生物資源研究所♂動物細胞機能研究チーム）

Ⅰ型コラーゲンゲルの物性と繭糸の形状を改善した新しい培養担体の開発と強度のある　結合組織の再構築

平成 17 年度
- 渡邉　淳　　（日本医科大学医学部第２生化学）

血管型 Ehlers-Danlos syndrome（EDSIV）に対する遺伝子治療方略の検討
- 都甲　武史　　（京都大学大学院医学研究科形成外科）

耳介軟骨膜による耳介軟骨再生：ドナーとしての資質
- 村井　純子　　（大阪大学大学院医学系研究科器官制御外科学）

Rxrb/Col11a2 locus における CTCF タンパク結合領域の同定とその機能解析
- 松村　紳一郎　　（慶應義塾大学医学部病理学）

MMP-2 の遺伝子欠損と薬物的阻害はマウスの心筋梗塞後の心破裂を抑制する
- 加藤　靖正　　（神奈川歯科大学分子生物学）

酸性細胞外 pH は PKCζ-NFkB を介して matrix metalloproteinase-9 発現を誘導する

平成 18 年度
- 廣畑　聡　　（岡山大学大学院医歯学総合研究科分子医化学）

IV 型コラーゲン NC1 ドメインの腫瘍特異的発現は内皮細胞の管腔形成とマウスでの腫瘍発育を阻害する

平成 19 年度
- 松本　嘉寛　　（九州大学医学部整形外科）

脊髄発生時のアクソンガイダンスにおけるヘパラン硫酸の役割
- 岡田　愛子　　（慶應義塾大学医学部病理学教室）

変形性関節症（OA）関節軟骨における膜型 ADAM12 の発現と OA 軟骨細胞増殖への関与
- 鳥越　清之　　（九州大学医学部整形外科）

軟骨特異的 TGF－βⅠ型受容体の欠損マウスにおける軸骨格形成異常
- 佐藤　隆　　（東京薬科大学薬学部生化学分子生物学）

分泌型 EMMPRIN によるガン細胞の移動活性促進作用とその活性部位の同定
- 平川　聡史　　（愛媛大学医学部皮膚科）

VEGF-A,-C トランスジェニックマウス皮膚発癌モデルにおけるリンパ節転移とリンパ管　新生の促進機序
- 高坂　一貴　　（大阪大学大学院歯学研究科）

ADAMTSL-4 と Fibrillin-1 はオキシタラン線維形成を介して歯根膜発生に協調的に働く

平成 20 年度
- 荒木　絵里　　（京都大学医学部皮膚科）

皮膚創傷におけるパーシカン発現：ケロイド発生病理との関連
- 江口　真由　　（東京理科大学大学院薬学研究科分子病態学研究室）

β１インテグリン活性化による悪性腫瘍細胞のアポトーシス誘導とその分子機構の解明
- 小倉　有紀　　（株式会社資生堂　ライフサイエンス研究センター）

偏光分解 SHG イメージによる真皮コラーゲンの光老化の解析
- 佐藤かおり　　（東京都医学研究機構東京都臨床医学総合研究所蛋白質代謝研究分野）

ケモカイン BRAK/CXCL14 は Rap1 の活性化により舌癌由来細胞のコラーゲンへの接着を増強する
- 澤田　賢志　　（東京薬科大学薬学部生化学・分子生物学教室）

関節リウマチにおける滑膜 EMMPRIN の関節破壊への関与
- 澤地　恭昇　　（ケネディーリウマチ研究所，インペリアル大学）

線維芽細胞増殖因子(FGF)-2 の軟骨破壊における役割
- 塩野　智康　　（東京薬科大学薬学部生化学・分子生物学教室）

EMMPRIN を介して細胞表層に局在する間質プロコラゲナーゼ/proMMP-1 の活性化とガン細胞浸潤機能の促進

- 田中　啓友　　（株式会社ニッピ　バイオマトリックス研究所）

線維芽細胞株における UVB 感受性の違い

- 二木　杉子　　（大阪大学蛋白研究所）

マウス胚発生初期における基底膜蛋白質の局在プロファイル

- 東山　礼一　　（東海大学医学部肝線維化研究ユニット）

皮膚創傷治癒ならびに線維化過程における骨髄由来細胞のコラーゲン合成への関与

- 堀口　真仁　　（京都大学医学研究科循環器内科）

弾性線維形成における DANCE/fibulin-5 プロセッシングの役割

日本結合組織学会　法人会員

エーザイ株式会社
〒112-0002　文京区小石川５－５－５

カネボウ株式会社 化粧品研究所
〒250-0002　小田原市寿町５－３－２８

塩野義製薬株式会社
〒553-0002　大阪市福島区鷺洲５－１２－４

株式会社資生堂
〒224-8558　横浜市都筑区早渕２－２－１

生化学工業株式会社 中央研究所
〒207-0021　東大和市立野３－１２５３

平成 20 年度　日本結合組織学会理事会議事録

日　　時：平成２０年５月２９日(木)　午前１１時より
場　　所：こまばエミナース　３階　「孔雀」
出 席 者: 岡田保典(理事長)、安達栄治郎、伊東 晃、稲垣 豊、清水 宏、妹尾春樹、二宮善文、　畑 隆一郎、林 利彦、藤原作平（以上 理事)、石川 治、鍋島一樹、西田輝夫、野水基義、上野 光(以上 新理事候補者)、渡辺秀人（監事）
欠席者：岩本幸英、木村友厚、多島新吾、中村耕三、安井夏生（以上 理事)、澤井高志（新理事　候補者)、高岸憲二（監事）

岡田理事長より挨拶があった。
平成２０年度就任新理事の紹介がなされた。

- 内科系　石川　治　（群馬大学皮膚科）
  上野　光　（産業医科大学生化学）
- 外科系　西田　輝夫　（山口大学眼科学）
- 形態学系　鍋島　一樹　（福岡大学病理部病理学）
  澤井　高志　（岩手医科大学病理学）
- 生理機能系　野水　基義　（東京薬科大学病態生化学）

I.　報告事項

岡田理事長より以下の報告がなされた。

１．学会活動報告

１）学術大会の実施
第３９回日本結合組織学会学術大会・第５４回マトリックス研究会大会　合同学術集会
会長：　岡田　保典　会期：　２００７年５月９日(水)～５月１１日（金）　北とぴあ（東京）
参加者：約２５０名

２）学術誌ＣＴＲの発行　（６号　３６３ページ）

３）学会ホームページの刷新　（平成１９年８月１日より運用開始）

４）会員への電子メール配信の実施　（同上日開始）（メールアドレス登録率は現在６８％）

５）各種情報を掲載したニュースレターの配信を開始（平成１９年８月１日より１１通）
編集委員：伊東　晃、服部俊治、今村保忠、林　利彦、（上野　光、中尾亜希子）
編集協力者：小川　崇（木原生物学研究所）石川善弘（京都大学再生医科学研究所）小出隆規（早稲田大学生命化学科）住吉秀明（大分大学生体分子構造機能制御）中里浩一（日本体育大学運動生理学）二木杉子（大阪大学蛋白質研究所）藤崎ひとみ（ニッピバイオマトリックス研究所）

２．新評議員の推薦

以下の９名が新評議員として推薦され、メール理事会にて既に承認済であることが報告された。

宇田川　信之　（松本歯科大学生化学）
上條　竜太郎　（昭和大学歯学部口腔生化学）
須田　直人　（東京医科歯科大学大学院顔面矯正学）
槻木　恵一　（神奈川歯科大学大学院口腔病理学）
中邨　智之　（関西医科大学薬理学）
前野　正夫　（日本大学歯学部衛生学）
山根　明　（鶴見大学歯学部薬理学）
小栗　佳代子　（国立病院機構名古屋医療センター臨床研究センター）
平川　聡史　（愛媛大学皮膚科）

３．平成２０年度大高賞審査結果

６名の応募があり、選考委員（渡辺秀人、野水基義、宇谷厚志、雑賀司珠也、宿南知佐）による審査の結果、以下の２名が選出されたことが報告された。

- 基礎系：輪千浩史氏（星薬科大学・薬学部・臨床化学）

Sato, F., et al.: Distinct steps of cross-linking, self-association, and maturation of tropoelastin are necessary for elastic fiber formation., J Mol Biol., 369, 841-851, 2007.

- 臨床系：細野幸三氏（名古屋大学・医学部附属病院・整形外科）

  Hosono, K., et al.: Hyaluronan oligosaccharides inhibit tumorigenicity of osteosarcoma cell lines MG-63 and LM-8 in vitro and in vivo via perturbation of hyaluronan-rich pericellular matrix of the cells., Am J Pathol, 171, 274-286, 2007.

４． 第３９回学術大会優秀演題賞
優秀演題賞は下記とする旨報告があった。
Ａ１８（松本嘉寛）、Ａ２７（岡田愛子）、Ａ３０（鳥越清之）、Ａ３３（佐藤　隆）、Ａ３４（平川聡史）、Ｐ１０（高坂一貴）

５． その他の学会賞については、本年度は該当者無しとの報告がなされた。

II. 審議事項

１． 平成１９年度収支決算報告が上野事務局長よりあり、審議の後に承認された。

２． 平成２０年度予算案が上野事務局長より提示された。岡田理事長より総会補助金の増額、PPCTSS 大会への補助金の拠出が提案され審議した結果、総会補助金の増額は引き続き検討課題とすること、来年の PPCTSS 大会に学会予算から３０万円の補助をすることが承認された。

３． 岡田理事長より、第４２回日本結合組織学会学術大会会長として妹尾理事を推薦したい旨の提案があり、審議の上承認された。妹尾理事も応諾した。第５７回マトリックス研究会と合同開催の方向で検討することとなった。

４． 平成２１年度大高賞選考委員長について審議の上、稲垣理事を選出した。

５． 平成２１年度理事・監事選挙管理委員会の設置について藤原理事を選挙管理委員長とする提案があり、審議の上承認された。

６． 新理事の担当業務について審議がなされ、石川理事、澤井理事が総務、上野理事、鍋島理事が将来計画、西田理事が財務、野水理事が編集をそれぞれ担当することとなった。

７． 新評議員推薦・承認手続きに関して細則の改訂について事務局より提案があり、審議の上　承認された。これに伴い会則、細則を以下の通り改定する。

- 日本結合組織学会　会則　第２１条

  理事及び監事は評議員の中から評議員が選出し、理事会の承認を経て、総会で決定する。会長は理事会及び評議員会の議を経て推薦され、総会で決定する。評議員は評議員の推薦により理事会で承認し、評議員会および総会に報告する。

- 日本結合組織学会　細則　第３条　３項

  前記１及び２項の何れの場合も、評議員２名以上の推薦を付して、本人の履歴書及び主たる業績の目録を理事長宛に提出する。新評議員は評議員の推薦に基づき理事会（含メール理事会）で審議のうえ承認し、評議員会および総会に報告する。ただし評議員会において過半数の反対があれば取り消しすることができる。６月末までに理事会で決定した新評議員は当該年度から評議員会費を納め、次年度の理事選挙権を認める。７月以降１２月末までに決定した新評議員は当該年度は正会員費を納め次年度より評議員会費を納める。理事選挙権は翌々年度から発効する。

８． 大高賞応募資格の年齢に関して規定文が曖昧であったため､「翌年３月３１日時点で４５歳未満としてはどうか」との提案があり、審議の上承認された。「選考委員は、委員長に加えて基礎系及び臨床系選考委員各２名の合計５名がこれに当たる。」としてはどうかとの提案があり、審議の上承認された。応募に際して推薦書があったほうがよいのではないかとの提案があったが、畑理事より、この件については以前議論があり推薦書は不要とされた経緯の説明があった。「推薦書（A4 一枚程度、様式　自由）を添付してもよい。」とされた。なお、大高賞の選考基準や選考委員の任命は選考委員長に一任であること、受賞は原則として１名であることが再確認された。

９． 学会現状について事務局より報告があった。収入増加に向け、特に理事においてはホームページでのリンク広告を企業に紹介のうえ成約に向け働きかけて欲しいとの提案があった。また会員増加に向けて積極的

な推薦をすることが合意された。

１０．　CTR 誌に関して二宮理事より、出版社のミスで「JSCTR Page」が掲載されていないことが複数回あったとの報告がなされた。さらに JSCTR Page の活用法として、理事長就任や学会開催の挨拶などを掲載すること、また編集委員会の役割として、この JSCTR Page への掲載情報の収集と CTR への投稿を盛んにすることが報告された。岡田理事長より、出版社との初期の話し合いの時点で CTR 誌の日本側 Associate Editor として３名（林理事、二宮理事、岡田理事）が就任しているが、任期は決まっていない。今後、より活発に推進できる方向で人数も含めて任期や選出方法を検討して行きたいとの提案があり、林理事を委員長として委員会を設置し検討することが承認された。

１１．　藤原理事より、会員名簿作成の件について、会員情報はエクセルファイルとして事務局にある、このファイルをＣＤに焼いて配布するなら１枚あたり４００－５００円、印刷冊子の場合には簡易製本であれば２５０円程度で、６００部１５万円程度で作成可能であるとの報告があった（郵送費用は別途）。審議の結果、今年度中に冊子体として発行することが承認された。掲載情報など詳細については藤原理事を委員長とする委員会で原案を作成し理事会に諮ることとなった。

１２．　評議員から正会員への変更手続きについては今後も「メール理事会で承認」とすることが確認された。

１３．　伊東理事より、学術大会時の理事会に加えもう１回（合計年２回）実施してはどうかという提案がなされた。この件については審議の時間がなくなったため、後日メール理事会で審議するものとする。

平成 20 年度　メール理事会承認事項

（平成 20 年 4 月 1 日～平成 21 年 4 月 30 日現在）

平成 20 年 7 月 25 日

新評議員推薦；推薦者・畑 隆一郎、加藤靖正
磯川 桂太郎 先生(日本大学歯学部解剖学教室第２講座)

平成 20 年 12 月 19 日

新評議員推薦；推薦者・岡田保典、望月早月
榎本宏之 先生（慶應義塾大学医学部整形外科）

平成 21 年 3 月 3 日

木全弘治先生より HA2010 国際学会（第８回ヒアルロン酸国際カンファレンス）後援依頼があり、理事会にて審議。結合組織学会に関連する学会であり、経済的な負担もないため、後援を承認。

平成 21 年 3 月 16 日

新評議員推薦；推薦者・藤原作平、吉岡秀克
松尾哲孝 先生（大分大学医学部マトリックス医学）

平成 21 年 4 月 13 日

学術賞推薦；推薦者・岡田保典、妹尾春樹
平成 21 年度学術賞 畑 隆一郎 先生（神奈川歯科大学生体機能学）

日本結合組織学会 評議員（平成 21 年 4 月現在）

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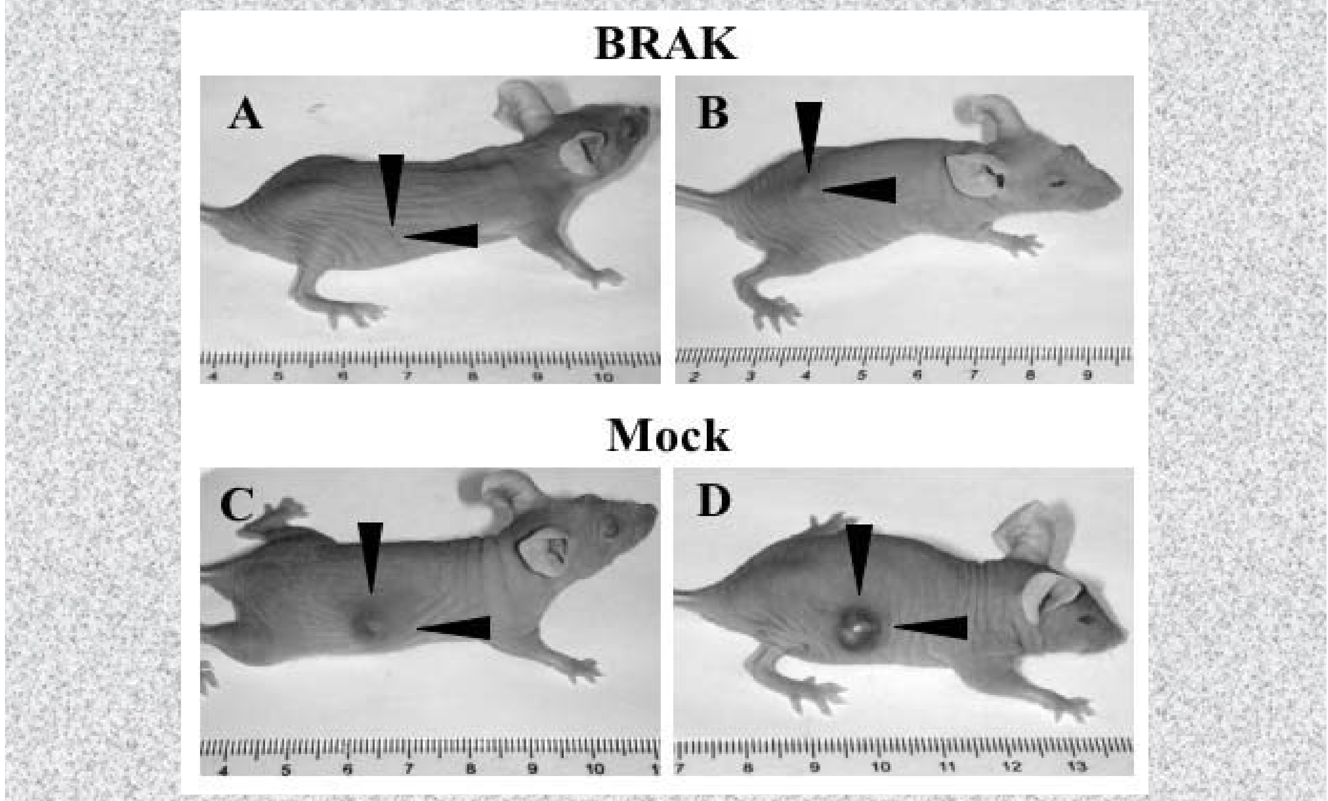
Molecular Targeting Therapy of Oral Cancer

BRAK

suppresses tumor growth



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