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ダイジェスト

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Ferroptosis model system by the re-expression of BACH1

Riko Irikura, Hironari Nishizawa, Kazuma Nakajima, Mie Yamanaka, Guan Chen, Kozo Tanaka, Masafumi Onodera, Mitsuyo Matsumoto, Kazuhiko Igarashi (Volume 174, Issue 3, September 2023, Pages 239–252, https://doi.org/10.1093/jb/mvad036)

Jaw1/LRMP is associated with the maintenance of Golgi ribbon structure

Wataru Okumura, Kazuko Tadahira, Takuma Kozono, Miwa Tamura-Nakano, Hiroyuki Sato, Hitomi Matsui, Taeko Dohi, Jack Rohrer, Takashi Tonozuka, Atsushi Nishikawa (Volume 173, Issue 5, May 2023, Pages 383–392, https://doi.org/10.1093/jb/mvad004)

A lipid scramblase TMEM41B is involved in the processing and transport of GPI-anchored proteins

Shu-Ya Cao, Yi-Shi Liu, Xiao-Dong Gao, Taroh Kinoshita, Morihisa Fujita (Volume 174, Issue 2, August 2023, Pages 109–123, https://doi.org/10.1093/jb/mvad041)

Cooperative DNA-binding activities of Chp2 are critical for its function in heterochromatin assembly

Anisa Fitri Rahayu, Aki Hayashi, Yuriko Yoshimura, Reiko Nakagawa, Kyohei Arita, Jun-ichi Nakayama (Volume 174, Issue 4, October 2023, Pages 371–382, https://doi.org/10.1093/jb/mvad050)

γ-enolase (ENO2) is methylated at the Nτ position of His-190 among enolase isozymes

Fumiya Kasai, Koichiro Kako, Syunsuke Maruhashi, Toru Uetake, Yuan Yao, Hiroaki Daitoku, Akiyoshi Fukamizu (Volume 174, Issue 3, September 2023, Pages 279–289, https://doi.org/10.1093/jb/mvad042)

PPARα activation partially drives NAFLD development in liver-specific Hnf4a-null mice

Carlos Ichiro Kasano-Camones, Masayuki Takizawa, Noriyasu Ohshima, Chinatsu Saito, Wakana Iwasaki, Yuko Nakagawa, Yoshio Fujitani, Ryo Yoshida, Yoshifumi Saito, Takashi Izumi, Shin-Ichi Terawaki, Masakiyo Sakaguchi, Frank J Gonzalez, Yusuke Inoue (Volume 173, Issue 5, May 2023, Pages 393–411, https://doi.org/10.1093/jb/mvad005)

Inner nuclear membrane proteins Lem2 and Bqt4 interact with different lipid synthesis enzymes in fission yeast

Yasuhiro Hirano, Yasuha Kinugasa, Yoshino Kubota, Chikashi Obuse, Tokuko Haraguchi, Yasushi Hiraoka (Volume 174, Issue 1, July 2023, Pages 33–46, https://doi.org/10.1093/jb/mvad017)

Structural insights into a bacterial β-glucosidase capable of degrading sesaminol triglucoside to produce sesaminol: toward the understanding of the aglycone recognition mechanism by the C-terminal lid domain

Taro Yanai, Yukino Takahashi, Eri Katsumura, Naoki Sakai, Kohei Takeshita, Riki Imaizumi, Hiroaki Matsuura, Shuntaro Hongo, Toshiyuki Waki, Seiji Takahashi, Masaki Yamamoto, Kunishige Kataoka, Toru Nakayama, Satoshi Yamashita (Volume 174, Issue 4, October 2023, Pages 335–344, https://doi.org/10.1093/jb/mvad048)

Direct binding of calmodulin to the cytosolic C-terminal regions of sweet/umami taste receptors

Atsuki Yoshida, Ayumi Ito, Norihisa Yasui, Atsuko Yamashita (Volume 174, Issue 5, November 2023, Pages 451–459, https://doi.org/10.1093/jb/mvad060)

THE JB REVIEWER AWARD

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JB COMMENTARY

BACH to the ferroptosis

Fuminori Tokunaga
Department of Medical Biochemistry, Graduate School of Medicine, Osaka Metropolitan University, 1–4–3 Asahi-machi, Abeno-ku, Osaka 545–8585, Japan

Ferroptosis is a form of regulated cell death characterized by iron-dependent phospholipid peroxidation and is closely related to various diseases. System Xc^-, a cystine/glutamate antiporter, and glutathione peroxidase 4 (GPX4) are key molecules in ferroptosis. Erastin and RSL3, known as inhibitors of system Xc^-

and GPX4, respectively, are commonly used as ferroptosis inducers. Broad-Complex, Tramtrack and Bric a brac (BTB) and Cap'n'collar (CNC) homology 1 (BACH1), a heme-binding transcription repressor, promotes pro-ferroptotic signalling, and therefore, *Bach1*-deficient cells are resistant to ferroptosis. Irikura *et al.* **(**Ferroptosis model system by the re-expression of BACH1. *J. Biochem.* 2023; **174**: 239–52) constructed *Bach1*-re-expressing immortalized mouse embryonic fibroblasts (iMEFs) from *Bach1*$^{-/-}$ mice, which induce ferroptosis simply through the depletion of 2-mercaptoethanol from the culture medium. Transcriptional repression by re-expressed BACH1 induces suppressed glutathione synthesis and increases labile iron. Furthermore, ferroptosis initiated by BACH1-re-expressing iMEFs is propagated to surrounding cells. Thus, the BACH1-re-expression system is a novel and powerful tool to investigate the cellular basis of ferroptosis.

Keywords: *BACH1,* ferroptosis, glutathione, system Xc^-, transcriptional repression

REGULAR PAPERS

BIOCHEMISTRY

Biochemistry General

Transglutaminase mediates the hardening of fish egg envelope produced by duplication of factor XIIIA gene during the evolution of Teleostei

Shigeki Yasumasu[1], Miyuki Horie[2], Mayuko Horie[2], Kodai Sakuma[2], Chihiro Sato[1], Hikari Sato[1], Taiki Nakajima[1], Tatsuki Nagasawa[3], Mari Kawaguchi[1] and Ichiro Iuchi[1]

[1]Department of Materials and Life Sciences, Faculty of Science and Technology, Sophia University, 7–1 Kioi-cho, Chiyoda-ku, Tokyo 102–8554, Japan; [2]Graduate School of Science and Technology, Sophia University, 7–1 Kioi-cho, Chiyoda-ku, Tokyo 102–8554, Japan and [3]School of Life Science and Technology, Tokyo Institute of Technology, 2–12–1 Ookayama, Meguro-ku, Tokyo 152–8550, Japan

During the fertilization of fish eggs, the hardening of the egg envelope is mediated by transglutaminase (hTGase). After fertilization, TGase undergoes processing. We isolated hTGase from extracts of unfertilized and water-activated rainbow trout eggs. Rainbow trout hTGase (Rt-hTGase) appeared as an 80 kDa protein, and its processed form was 55 kDa. Their N-terminal amino acid sequences were nearly identical, suggesting processing in the C-terminal region. The specific activities were not significantly different, indicating that C-terminal processing does not activate the enzyme itself. We cloned the cDNA by reverse transcription polymerase chain reaction (RT-PCR) using degenerate primers followed by RACE-PCR. The deduced amino acid sequence of the cDNA was similar to that of factor XIII subunit A (FXIIIA). Molecular phylogenetic and gene syntenic analyses clearly showed that hTGase was produced by duplication of FXIIIA during the evolution to Teleostei. The 55 kDa processed form of Rt-hTGase is predominantly composed of an enzyme domain predicted from the amino acid sequence of the cDNA. It is hypothesized that the C-terminal domain of Rt-hTGase binds to egg envelope proteins, and that processing allows the enzyme to move freely within the egg envelope, increasing substrate–enzyme interaction and thereby accelerating hardening.

Keywords: fertilization, fish, FXIIIA, gene duplication, hardening of egg envelope

Activation of platelet-derived growth factor receptors regulate connective tissue growth factor protein levels via the AKT pathway in malignant mesothelioma cells

Tomoya Suehiro[1], Khoja Mouhand Ahmad[1], Nguyen Truong Duc Hoang[2], Bingwen Xu[1], Honoka Komatsu[1], Komei Kurachi[1], Hiroki Nikawa[3], Yuichi Mine[4] , Tohru Matsuki[5], Katsura Asano[6,7,8] and Makiko Fujii[1]

[1]Department of Genomic Oncology and Oral Medicine, Graduate School of Biomedical and Health Science, Hiroshima University, 1–2–3 Kasumi Minami-ku, Hiroshima 734–8553, Japan; [2]Oncology Department, Faculty of Medicine, University of Medicine and Pharmacy at Ho Chi Minh City, 217 Hong Bang, Ward 11, District 5, Ho Chi Minh 70000, Vietnam; [3]Department of Oral Biology and Engineering, Graduate School of Biomedical and Health Sciences, Hiroshima University, 1–2–3 Kasumi Minami-ku, Hiroshima 734–8553, Japan; [4]Department of Medical Systems Engineering, Graduate School of Biomedical and Health Sciences, Hiroshima University, 1–2–3 Kasumi Minami-ku, Hiroshima 734–8553, Japan; [5]Department of Cellular Pathology, Institute for Developmental Research, Aichi Developmental Disability Center, 713–8 Kamiya, Kasugai, Aichi, 480–0304, Japan; [6]Molecular Cellular and Developmental Biology Program, Division of Biology, Kansas State University, 116 Ackert Hall, Manhattan, KS 66506, USA; [7]Graduate School of Integrated Sciences for Life, Hiroshima University, 1–3–1 Kagamiyama, Higashi-Hiroshima, Hiroshima 739–8530, Japan. and [8]Hiroshima Research Center for Healthy Aging, Hiroshima University, 1–3–1 Kagamiyama, Higashi-Hiroshima, Hiroshima 739–8530, Japan

The incidence of malignant mesothelioma (MM), a disease linked to refractory asbestos exposure, continues to increase globally and remains largely resistant to various treatments. Our previous studies have identified a strong correlation between connective tissue growth factor (CTGF) protein expression and MM malignancy, underscoring the importance of understanding CTGF regulation in MM cells. In this study, we demonstrate for the first time that stimulation with platelet-derived growth factor receptor (PDGFR) ligand, PDGF-BB, increases CTGF protein expression levels without affecting CTGF mRNA levels. Inhibition of PDGFR resulted in a reduction of CTGF protein expres-

sion, indicating that PDGFR activation is essential in regulating CTGF protein expression in MM cells. PDGF-BB also activated the protein kinase B (AKT) pathway, and inhibition of AKT phosphorylation abolished the PDGFR-induced CTGF protein expression, suggesting that PDGFR acts upstream of CTGF via the AKT pathway. This reinforces the role of CTGF protein as a key regulator of MM malignancy. Additionally, PDGFR activation led to the phosphorylation of mTOR and 4E-BP1, critical regulators of protein synthesis downstream of AKT, suggesting that PDGFR controls CTGF protein expression through the regulation of CTGF mRNA translation.

Keywords: 4E-BP1, AKT–mTOR pathway, CTGF, malignant mesothelioma, PDGF

MOLECULAR BIOLOGY

Gene Expression

Absolute quantification of BACH1 and BACH2 transcription factors in B and plasma cells reveals their dynamic changes and unique roles

Takeshi Kurasawa[1], Akihiko Muto[1], Mitsuyo Matsumoto[1,2], Kyoko Ochiai[1], Kazutaka Murayama[3] and Kazuhiko Igarashi[1]

[1]Department of Biochemistry, Tohoku University Graduate School of Medicine, 2–1 Seiryo-machi, Aoba-ku, Sendai 980–8575, Japan; [2]Institute of Multidisciplinary Research for Advanced Materials, Tohoku University, 2–1–1 Katahira, Aoba-ku, Sendai 980–8577, Japan and [3]Division of Biomedical Measurements and Diagnostics, Tohoku University Graduate School of Biomedical Engineering, 2–1 Seiryo-machi, Aoba-ku, Sendai 980–8575, Japan

Changes in the absolute protein amounts of transcription factors are important for regulating gene expression during cell differentiation and in responses to changes in the cellular and extracellular environment. However, few studies have focused on the absolute quantification of mammalian transcription factors. In this study, we established an absolute quantification method for the transcription factors BACH1 and BACH2, which are expressed in B cells and regulated by direct heme binding. The method used purified recombinant proteins as controls in western blotting and was applied to mouse naïve B cells in the spleen, as well as activated B cells and plasma cells. BACH1 was present in naïve B cells at approximately half the levels of BACH2. In activated B cells, BACH1 decreased compared to naïve B cells, whilst BACH2 increased. In plasma cells, BACH1 increased back to the same extent as in naïve B cells, whilst BACH2 was not detected. Their target genes, *Prdm1* and *Hmox1*, were highly induced in plasma cells. BACH1 was found to undergo degradation with lower concentrations of heme than BACH2. Therefore, BACH1 and BACH2 are similarly abundant in B cells but differ in heme sensitivity, potentially regulating gene expression differently depending on their heme responsiveness.

Keywords: absolute quantification, B cell, BACH1, BACH2, BTB and CNChomology 1, BTB and CNC homology 2, heme metabolism, iron metabolism, plasma cell, transcription factor

RNA Processing

Cancer-associated SF3B1 mutations inhibit mRNA nuclear export by disrupting SF3B1–THOC5 interactions

Gang Liu, Bo Zhao, Yueru Shi and Youzhong Wan

China-Japan Union Hospital of Jilin University, No. 126, Xiantai Street, Changchun, Jilin 130033, China

Mutations in SF3B1 are common in many types of cancer, promoting cancer progression through aberrant RNA splicing. Recently, mRNA nuclear export has been reported to be defective in cells with the SF3B1 K700E mutation. However, the mechanism remains unclear. Our study reveals that the K700E mutation in SF3B1 attenuates its interaction with THOC5, an essential component of the mRNA nuclear export complex THO. Furthermore, the SF3B1 mutation caused reduced binding of THOC5 with some mRNA and inhibited the nuclear export of these mRNAs. Interestingly, overexpression of THOC5 restores the nuclear export of these mRNAs in cells with the SF3B1 K700E mutation. Importantly, other types of cancer-associated SF3B1 mutations also inhibited mRNA nuclear export similarly, suggesting that it is common for cancer-associated SF3B1 mutations to inhibit mRNA nuclear export. Our research highlights the critical role of the THOC5–SF3B1 interaction in the regulation of mRNA nuclear export and provides valuable insights into the impact of SF3B1 mutations on mRNA nuclear export.

Keywords: mRNA nuclear export, SF3B1, THO, THOC5, U2 snRNP

CELL

Cell Death

Intracellular biliverdin dynamics during ferroptosis

Kazuma Nakajima[1], Hironari Nishizawa[1], Guan Chen[2], Shunichi Tsuge[1], Mie Yamanaka[1,3], Machi Kiyohara[1], Riko Irikura[1], Mitsuyo Matsumoto[1,4], Kozo Tanaka[2], Rei Narikawa[5] and Kazuhiko Igarashi[1]

[1]Department of Biochemistry, Tohoku University Graduate School of Medicine, 2–1 Seiryo-machi, Aoba-ku, Sendai, Miyagi 980–8575, Japan; [2]Department of Molecular Oncology, Institute of Development, Aging and Cancer, Tohoku University, 4–1 Seiryo-machi, Aoba-ku, Sendai, Miyagi 980–8575, Japan; [3]Gladstone Institute of Neurological Disease, Gladstone Institutes, 1650 Owens street, San Francisco, CA 94158, USA; [4]Center for Regulatory Epigenome and Diseases, Tohoku University Graduate School of Medicine, 2–1 Seiryo-machi, Aoba-ku, Sendai, Miyagi 980–8575, Japan and [5]Department of Biological Sciences, Graduate School of Science, Tokyo Metropolitan University, 1–1 Minami-Ohsawa, Hachioji, Tokyo 192–0397, Japan

Ferroptosis is a cell death mechanism mediated by iron-dependent lipid peroxidation. Although ferroptosis has garnered attention as a cancer-suppressing mechanism, there are still limited markers available for identifying ferroptotic cells or assessing their sensitivity to ferroptosis. The study focused on biliverdin, an endogenous reducing substance in cells, and examined the dynamics of intracellular biliverdin during ferroptosis using a biliverdin-binding cyanobacteriochrome. It was found that intracellular biliverdin decreases during ferroptosis and that this decrease is specific to ferroptosis amongst different forms of cell death. Furthermore, the feasibility of predicting sensitivity to ferroptosis by measuring intracellular biliverdin was demonstrated using a ferroptosis model induced by the re-expression of the transcription factor BACH1. These findings provide further insight into ferroptosis research and are expected to contribute to the development of cancer therapies that exploit ferroptosis.
Keywords: BACH1, biliverdin, cyanobacteriochrome, ferroptosis, heme

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ダイジェスト

JB COMMENTARY

Exploring the roles of Lem2 and Bqt4 in lipid metabolism for nuclear envelope maintenance: a novel perspective

Kei-ichiro Ishiguro
Department of Chromosome Biology, Institute of Molecular Embryology and Genetics (IMEG), Kumamoto University, 2–2–1 Honjo, Chuo-ku, Kumamoto 860–0811, Japan

The nuclear envelope (NE) is a double-membrane structure critical for genome maintenance and cellular function, composed of the inner and outer nuclear membranes. In fission yeast, the inner nuclear membrane (INM) proteins Lem2 and Bqt4 are essential for maintaining NE integrity. The study published by Hiraoka group explores the interactions between Lem2 and Bqt4 with lipid synthesis enzymes, addressing their roles in NE maintenance. The authors identified Lem2- and Bqt4-binding proteins using immunoprecipitation and mass spectrometry, revealing that Lem2 interacts with lipid synthesis enzymes, whilst Bqt4 binds to an enzyme that involves in glucosylceramide synthesis. These findings suggest that Lem2 and Bqt4 independently contribute to NE structure and its integrity through distinct lipid metabolic pathways, highlighting their complementary roles in nuclear membrane homeostasis. This study represents a significant step forward in the field of NE biology to unravel the complexities of nuclear membrane dynamics.
Keywords: inner nuclear membrane (INM), lipid metabolism, lipid synthesis, nuclear envelope (NE)

RAPID COMMUNICATION

Bcl2l12, a novel protein interacting with Arf6, triggers Schwann cell differentiation programme

Wakana Miyata[1], Naoko Sakaibara[1], Kentaro Yoshinaga[1], Asahi Honjo[1], Mikito Takahashi[1], Tatsuya Ooki[1], Hideji Yako[1,2], Kazunori Sango[2], Yuki Miyamoto[1,3] and Junji Yamauchi[1,2,3] [1]Laboratory of Molecular Neurology, Department of Life Sciences, 1432–1 Horinouchi, Hachioji, Tokyo University of Pharmacy and Life Sciences, Tokyo 192–0392, Japan [2]Diabetic Neuropathy Project, Tokyo Metropolitan Institute of Medical Science, 2–1 Kamikitazawa, Setagaya, Tokyo 156–8506, Japan [3]Laboratory of Molecular Pharmacology, Department of Pharmacy, National Research Institute for Child Health and Development, 2–10–1 Okura, Setagaya, Tokyo 157–8535, Japan

Schwann cells are glial cells in the peripheral nervous system (PNS); they wrap neuronal axons with their differentiated plasma membranes called myelin sheaths. Although the physiological functions, such as generating saltatory conduction, have been well studied in the PNS, the molecular mechanisms by which Schwann cells undergo their differentiation programme without apparent morphological changes before dynamic myelin sheath formation remain unclear. Here, for the first time, we report that Arf6, a small GTP/GDP-binding protein controlling morphological differentiation, and the guanine–nucleotide exchange factors cytohesin proteins are involved in the regulation of Schwann cell differentiation marker expression in primary Schwann cells. Specific inhibition of Arf6 and cytohesins by NAV-2729 and SecinH3, respectively, decreased expression of marker proteins 2′,3′-cyclic nucleotide 3′-phosphodiesterase (CNPase) and glial fibrillary acidic protein (GFAP). Similar results using promoter assays were observed using the IMS32 Schwann cell line. Furthermore, using an affinity-precipitation technique, we identified Bcl2-like 12 (Bcl2l12) as a novel GTP-bound Arf6-interacting protein. Knockdown of Bcl2l12 using a specific artificial miRNA decreased expression of marker proteins. The knockdown also led to decreased filamentous actin extents. These results suggest that Arf6 and Bcl2l12 can trigger Schwann cell differentiation, providing evidence for a molecular relay that underlies how Schwann cells differentiate.
Keywords: Arf6, Bcl2l12, cytohesin, differentiation before myelination, Schwann cell

REGULAR PAPER

BIOCHEMISTRY

Protein Structure

Open and closed structures of L-arginine oxidase by cryo-

electron microscopy and X-ray crystallography

Hiroki Yamaguchi[1,2] , Kazutoshi Takahashi[1,] , Nobutaka Numoto[3], Hiroshi Suzuki[2], Moemi Tatsumi[1], Akiko Kamegawa[2,4,5], Kouki Nishikawa[4,5], Yasuhisa Asano[6], Toshimi Mizukoshi[1], Hiroshi Miyano[1], Yoshinori Fujiyoshi[2,4,5] and Masayuki Sugiki[1]

[1]Research Institute for Bioscience Products & Fine Chemicals, Ajinomoto Co. Inc., 1–1 Suzuki-cho, Kawasaki-ku, Kawasaki 210–8681, Japan; [2]Advanced Research Institute, Tokyo Medical and Dental University, 1–5–45 Yushima, Bunkyo-ku, Tokyo 113–8501, Japan; [3]Medical Research Institute, Tokyo Medical and Dental University, 1–5–45 Yushima, Bunkyo-ku, Tokyo 113–8501, Japan; [4]CeSPIA Inc., 2–1–1 Otemachi, Chiyoda-ku, Tokyo 100–0004, Japan; [5]Joint Research Course for Advanced Biomolecular Characterization, Faculty of Agriculture, Tokyo University of Agriculture and Technology, 3–5–8 Saiwai-cho, Fuchu, Tokyo 183–8509, Japan and [6]Biotechnology Research Center and Department of Biotechnology, Toyama Prefectural University, 5180 Kurokawa, Imizu, Toyama 939–0398, Japan

L-arginine oxidase (AROD, EC 1.4.3.25) is an oxidoreductase that catalyses the deamination of L-arginine, with flavin adenine dinucleotide (FAD) as a cofactor. Recently identified AROD from *Pseudomonas* sp. TPU 7192 (PT-AROD) demonstrates high selectivity for L-arginine. This enzyme is useful for accurate assays of L-arginine in biological samples. The structural characteristics of the FAD-dependent AROD, however, remain unknown. Here, we report the structure of PT-AROD at a resolution of 2.3 Å by cryo-electron microscopy. PT-AROD adopts an octameric structure with D4 symmetry, which is consistent with its molecular weight in solution, estimated by mass photometry. Comparative analysis of this structure with that determined using X-ray crystallography reveals open and closed forms of the lid-like loop at the entrance to the substrate pocket. Furthermore, mutation of Glu493, located at the substrate binding site, diminishes substrate selectivity, suggesting that this residue contributes significantly to the high selectivity of PT-AROD.

Keywords: amino acid oxidase, cryo-EM, L-arginine oxidase, single particle analysis, X-ray crystallography

Biochemical Pharmacology

Long-term estrogen-deprived estrogen receptor *α*-positive breast cancer cell migration assisted by fatty acid 2-hydroxylase

Koki Kanamaeda[1], Masayo Hirao-Suzuki[2], Takanobu Kobayashi[3], Yuhki Sato[4], Masahiro Ohara[5] and Shuso Takeda[1]

[1]Laboratory of Molecular Life Sciences, Faculty of Pharmacy and Pharmaceutical Sciences, Fukuyama University, Sanzou 1, Gakuen-cho, Fukuyama, Hiroshima 729–0292, Japan; [2]Laboratory of Xenobiotic Metabolism and Environmental Toxicology, Faculty of Pharmaceutical Sciences, Hiroshima International University, 5–1–1 Hiro-koshingai, Kure, Hiroshima 737–0112, Japan; [3]Kagawa School of Pharmaceutical Sciences, Tokushima Bunri University, 1314–1, Shido, Sanuki, Kagawa 769–2193, Japan; [4]Laboratory of Clinical Evaluation of Drug Efficacy, Faculty of Pharmacy and Pharmaceutical Sciences, Fukuyama University, Sanzou 1, Gakuen-cho, Fukuyama, Hiroshima 729–0292, Japan and [5] Department of Breast Oncology, Saitama Medical University International Medical Center, 1397–1 Yamane, Hidaka, Saitama 350–1298, Japan

The risk of breast cancer (BC) recurrence is high in postmenopausal women, though the underlying molecular mechanisms are not yet fully understood. We developed a long-term estrogen-deprived (LTED) cell line from MCF-7 cells, which we used as an *in vitro* model for aromatase inhibitor (AI)-resistant estrogen receptor *α* (ER*α*)-positive postmenopausal BC. We also describe the involvement of fatty acid 2-hydroxylase (FA2H) in the modulation of LTED cell migration. Small interfering RNA specific to *FA2H* (siFA2H) could reduce cell migration, whereas the introduction of plasmid expressing FA2H, but not its inactive mutant, resulted in enhanced migration. Moreover, proliferation of the LTED cells was not affected by modulation of FA2H expression. Fulvestrant (FUL), a selective estrogen receptor degrader used to treat AI-resistant ER*α*-positive postmenopausal BC, was found to induce degradation of ER*α* together with a decrease in ER-mediated transcription; however, FA2H protein expression and migration remained unchanged. Overall, the findings of this study suggest that FA2H is one of the drivers of LTED cell migration, and that LTED cells resistant to FUL therapy may be involved in malignancy and metastatic mechanisms.

Keywords: cell migration, fatty acid 2-hydroxylase, fulvestrant, long-term estrogen-deprived cells, postmenopausal breast cancer

Analytical Biochemistry

isoAsp-Quest: workflow development for isoAsp identification using database searches

Hiroaki Sakaue and Atsushi Kuno

Molecular and Cellular Glycoproteomics Research Group, Cellular and Molecular Biotechnology Research Institute, National Institute of Advanced Industrial Science and Technology (AIST), Central 5, 1–1–1 Higashi, Tsukuba, Ibaraki 305–8565, Japan

A recent study reported that isomerization of aspartyl residues (Asp) occurs in various tissues and proteins *in vivo*. For a comprehensive analysis of post-translational modifications, the mass spectrometry (MS)-based proteomic approach is a straightforward method; however, the isomerization of Asp does not alter its molecular weight. Therefore, a unique method is required to analyse Asp isomers using MS. Herein, we present a novel strategy, isoAsp-Quest, which is a database search-oriented isoAsp identification method. isoAsp is specifically converted to ^{18}O-labelled L*α*-Asp by the enzymatic reaction of protein L-isoaspartyl-*O*-methyltransferase (PIMT) in ^{18}O water with a mass shift of 2 Da, which, in principle, enables us to distinguish Asp

isomers. However, in practise, a labelled Lα-Asp signal overlaps with that of endogenous Lα-Asp, making detection challenging. Therefore, degradation of the endogenous Lα-Asp peptide by AspN and subsequent removal of AspN were performed prior to the PIMT reaction. This strategy was applied to bovine lens α-crystallin. Consequently, several Asp isomerization sites, consistent with human αA-crystallin, were identified in bovine αA-crystallin, indicating that this strategy is also effective for biological proteins. Therefore, isoAsp-Quest enables the analysis of Lβ-Asp in a straightforward and rapid workflow, which may be useful for the quality control of protein products and biomarker discovery.

Keywords: aspartic acid, isoAsp-Quest, isomerization, protein L-isoaspartyl-*O*-methyltransferase, proteomics

CELL

Biomembranes/Organelles and Protein Sorting

Mitochondria–giant lipid droplet proximity and autophagy suppression in nitrogen-depleted oleaginous yeast *Lipomyces starkeyi* cells

Lan Duan[1], Akinobu Togou[2], Keisuke Ohta[2,3] and Koji Okamoto[1]
[1]Laboratory of Mitochondrial Dynamics, Graduate School of Frontier Biosciences, Osaka University, 1–3 Yamadaoka, Suita, Osaka 565–0871, Japan; [2]Advanced Imaging Research Center, Kurume University School of Medicine, Kurume, Fukuoka 830–0011, Japan and [3]Division of Microscopic and Developmental Anatomy, Department of Anatomy, Kurume University School of Medicine, Kurume, Fukuoka 830–0011, Japan

Balancing energy production and storage is a fundamental process critical for cellular homeostasis in most eukaryotes that relies on the intimate interplay between mitochondria and lipid droplets (LDs). In the oleaginous yeast *Lipomyces starkeyi* under nitrogen starvation, LD forms a single giant spherical structure that is easily visible under a light microscope. Currently, how mitochondria behave in *L. starkeyi* cells undergoing giant LD formation remains unknown. Here we show that mitochondria transition from fragments to elongated tubules and sheet-like structures that are in close proximity to a giant LD in nitrogen-depleted *L. starkeyi* cells. Under the same conditions, mitochondrial degradation and autophagy are strongly suppressed, suggesting that these catabolic events are not required for giant LD formation. Conversely, carbon-depleted cells suppress mitochondrial elongation and LD expansion, whereas they promote mitochondrial degradation and autophagy. We propose a potential link of mitochondrial proximity and autophagic suppression to giant LD formation.

Keywords: autophagy, lipid droplet, mitochondria, oleaginous yeast

Extracellular Matrices and Cell Adhesion Molecules

Collagen isolated from human adipose tissue and its cellular affinity

Hisayo Yamaoka[1], Keiko Yamaoka[2], Hidenori Ishii[1], Hideyuki Tanaka[3], Makoto Yasuda[2], Shigekazu Watanabe[2], Makoto Hosoyamada[2] and Yuzo Komuro[1]
[1]Department of Plastic Surgery, School of Medicine, Teikyo University, 2–11–1 Kaga, Itabashi-ku, Tokyo 173–8605, Japan; [2]Faculty of Pharma-Science, Teikyo University, 2–11–1 Kaga, Itabashi-ku, Tokyo 173–8605, Japan and [3]Department of Anatomy, School of Medicine, Teikyo University, 2–11–1 Kaga, Itabashi-ku, Tokyo 173–8605, Japan

The use of collagen in cell cultures promotes cell proliferation and differentiation, and it has been commercialized. In this study, we separated and purified collagen from adipose tissue discarded during liposuction and prepared collagen-coated dishes. After collagen was identified from human adipose tissue, type identification and quantification were performed using SDS-PAGE and FPLC. Collagen type I was used to coat culture dishes. Human skin fibroblasts and human adipose tissue-derived stem cells were seeded at a density of 2.5×10^5 cells/ml on prepared dishes at a collagen concentration of 3 mg/ml and cultured for 7 days. Cell viability was then measured and analyzed. The WST-1 assay was used to evaluate the results. The amount of collagen in 300 g of adipose tissue was 25.5 mg for type I, 41.4 mg for type III, 10.6 mg for type IV, 6.5 mg for type V and 15 mg for type VI. The highest rates were observed for adipose stem cells cultured on human adipose tissue-derived collagen-coated dishes. In cell cultures, cell affinity was higher when cells and the substrate used were of the same origin, and affinity was stronger when the tissue of origin was the same.

Keywords: adipose, amino acid analysis, cell affinity, Collagen I, III～VI, SDS-PAGE method