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ダイジェスト

JB Reviews

Featured article of the month

Signal amplifi cation in flow cytometry for cell surface antigen analysis

Takeshi Mori[1] and Yoshiki Katayama[1]

[1]Faculty of Engineering, Department of Chemistry and Biochemistry, Kyushu University, 744 Moto-oka, Nishi-ku, Fukuoka, Japan

Signal enhancing systems have been introduced to enable detection of cell surface antigens by flow cytometry. Cell surface antigens are important targets that describe the function and lineage of cells. Although flow cytometry is an effective tool for analysing cell surface antigens, this technique has poor sensitivity, which prohibits the detection of many important antigens on cell membranes. Thus, signal amplification is essential for developing practical tools for evaluating cell surface antigens by flow cytometry. Using a bright fluorophore or fluorescent polymer incorporated into antibodies is a straightforward strategy to improve flow cytometry sensitivity but may affect the functional characteristics of the labelled antibody. In contrast, enzymatic signal amplification is a more practical and efficient strategy to improve sensitivity that should not affect antibody activity. Although enzymatic signal amplification still has a number of drawbacks, this approach is a promising strategy to analyse cell surface antigens.

Keywords: cell surface antigen, flow cytometry, fluorimetry, immunofluorescence, signal amplification

Neurodegenerative disorders and sterile infl ammation: lessons from a Drosophila model

Firzan Nainu[1], Emil Salim[2,3], Rangga Meidianto Asril[1,2], Aki Hori[2] and Takayuki Kuraishi[2]

[1]Faculty of Pharmacy, Universitas Hasanuddin, Jl. Perintis Kemerdekaan Km.10 Tamalanrea, Makassar, Indonesia, [2]Faculty of Pharmacy, Universitas Sumatera Utara, Jl. Tri Dharma No. 5 Pintu 4 Kampus USU, Medan, Indonesia, [3]Faculty of Pharmacy, Institute of Medical, Pharmaceutical and Health Sciences, Kanazawa University, Shizenken, Kakuma-machi, Kanazawa, Ishikawa, Japan

Central nervous system (CNS)-related disorders, including neurodegenerative diseases, are common but difficult to treat. As effective medical interventions are limited, those diseases will likely continue adversely affecting people's health. There is evidence that the hyperactivation of innate immunity is a hallmark of most neurodegenerative diseases, including Alzheimer's disease, Parkinson's disease, amyotrophic lateral sclerosis and polyglutamine diseases. In mammalian and fly CNS, the presence of noninfectious ligands, including danger-associated molecular patterns, is recognized by (micro)glial cells, inducing the expression of proinflammatory cytokines. Such inflammation may contribute to the onset and progression of neurodegenerative states. Studies using fruit flies have shed light on the types of signals, receptors and cells responsible for inducing the inflammation that leads to neurodegeneration. Researchers are using fly models to assess the mechanisms of sterile inflammation in the brain and its link to progressive neurodegeneration. Given the similarity of its physiological system and biochemical function to those of mammals, especially in activating and regulating innate immune signalling, *Drosophila* can be a versatile model system for studying the mechanisms and biological significance of sterile inflammatory responses in the pathogenesis of neurodegenerative diseases. Such knowledge would greatly facilitate the quest for a novel effective treatment for neurodegenerative diseases.

Keywords: CNS, *Drosophila*, innate immunity, neurodegenerative diseases, sterile inflammation

BIOCHEMISTRY

Biochemistry General

A novel angiotensin-I converting enzyme inhibitory peptide derived from the glutelin of vinegar soaked black soybean and its antihypertensive effect in spontaneously hypertensive rats

Yueyuan Zhang[1], Yanling Zhang[2], Peiyao Chen[1], Fengjue Shu[3], Kai Li[1], Liansheng Qiao[2], Zujun Chen[4] and Lingzhi Wang[1]

[1]School of Life Science, Beijing University of Chinese Medicine, No. 11, Bei San Huan Dong Lu, Chaoyang District, Beijing, China, [2]School of Chinese Materia Medica, Beijing University of Chinese Medicine, No. 11, Bei San Huan Dong Lu, Chaoyang District, Beijing, China, [3]ICF International Inc., 3 Corporate Blvd, NE#100, Atlanta, GA, USA, [4]Department of Cardiovascular Surgery, Cardiovascular Institute and Fuwai Hospital, Peking Union Medical College and Chinese Academy of Medical Sciences, No. 167 North Lishi Road, Xicheng District, Beijing, China

Vinegar soaked black soybean is a traditional Chinese food widely used for the treatment of hypertension. While its pharmacodynamic substance was not fully unveiled. It contained abundant glutelin, thus the purpose of this study was to obtain potent antihypertensive peptides from vinegar soaked black soybean. Black soybean was soaked with vinegar and then glutelin was first catalyzed by alcalase. Ultrafiltration, ion exchange chromatography and reversed-phase high performance liquid chromatography were sequentially applied to separate and purify

the angiotensin-I converting enzyme (ACE) inhibitory peptides from glutelin hydrolysates. As a result, the fraction L1-4 with the highest ACE inhibitory activity (83.41%) at the final concentration of 0.01 mg/mL was obtained and five peptides were then identified. These peptides were further optimized by virtual screening combining with *in silico* proteolysis. Finally, a novel tetrapeptide Phe-Gly-Ser-Phe (FGSF) was obtained. FGSF exhibited high *in vitro* ACE inhibitory activity (IC_{50}=117.11 μM) and *in vivo* hypotensive effect which maximally reduced systolic blood pressure of 21.95 mmHg at 20 mg/kg body weight in spontaneously hypertensive rats. Our study demonstrated that FGSF derived from vinegar soaked black soybean might be used as a promising ingredient for pharmaceuticals against hypertension and its related diseases.

Keywords: angiotensin-I converting enzyme inhibitory peptides, antihypertensive effect, glutelin, molecular simulation, vinegar soaked black soybean

Protein Structure

Temperature-sensitive mutants of MscL mechanosensitive channel

Naoto Owada[1], Megumi Yoshida[1], Kohei Morita[1] and Kenjiro Yoshimura[1]

[1]Department of Machinery and Control Systems, College of Systems Engineering and Science, Shibaura Institute of Technology, 307 Fukasaku, Minuma-ku, Saitama-shi, Saitama, Japan

MscL is a mechanosensitive channel that undergoes a global conformational change upon application of membrane stretching. To elucidate how the structural stability and flexibility occur, we isolated temperature-sensitive (Ts) mutants of *Escherichia coli* MscL that allowed cell growth at 32°C but not at 42°C. Two Ts mutants, L86P and D127V, were identified. The L86P mutation occurred in the second transmembrane helix, TM2. Substitution of residues neighbouring L86 with proline also led to a Ts mutation, but the substitution of L86 with other amino acids did not result in a Ts phenotype, indicating that the Ts phenotype was due to a structural change of TM2 helix by the introduction of a proline residue. The D127V mutation was localized in the electrostatic belt of the bundle of cytoplasmic helices, indicating that stability of the pentameric bundle of the cytoplasmic helix affects MscL structure. Together, this study described a novel class of MscL mutations that were correlated with the thermodynamic stability of the MscL structure.

Keywords: αhelix, bacteria, gain-of-function, mechanosensitive channel, temperature-sensitive mutants

Glycobiology and Carbohydrate Biochemistry

Glycoengineering of HEK293 cells to produce high-mannose-type N-glycan structures

Wei-Wei Ren[1], Ze-Cheng Jin[1], Weijie Dong[2], Toshihiko Kitajima[1], Xiao-Dong Gao[1] and Morihisa Fujita[1]

[1]Key Laboratory of Carbohydrate Chemistry and Biotechnology, Ministry of Education, School of Biotechnology, Jiangnan University, Wuxi, Jiangsu 214122, China, [2]College of Basic Medical Sciences, Dalian Medical University, Dalian 116044, Liaoning, China

Therapeutic proteins are a developing part of the modern biopharmaceutical industry, providing novel therapies to intractable diseases including cancers and autoimmune diseases. The human embryonic kidney 293 (HEK293) cell line has been widely used to produce recombinant proteins in both basic science and industry. The heterogeneity of glycan structures is one of the most challenging issues in the production of therapeutic proteins. Previously, we knocked out genes encoding α1,2-mannosidase-Is, *MAN1A1*, *MAN1A2* and *MAN1B1*, in HEK293 cells, establishing a triple-knockout (T-KO) cell line, which produced recombinant protein with mainly high-mannose-type N-glycans. Here, we further knocked out *MAN1C1* and *MGAT1* encoding another Golgi α1,2-mannosidase-I and N-acetylglucosaminyltransferase-I, respectively, based on the T-KO cells. Two recombinant proteins, lysosomal acid lipase (LIPA) and immunoglobulin G1 (IgG_1), were expressed in the quadruple-KO (QD-KO) and quintuple-KO (QT-KO) cell lines. Glycan structural analysis revealed that all the hybrid-type and complex-type N-glycans were eliminated, and only the high-mannose-type N-glycans were detected among the recombinant proteins prepared from the QD-KO and QT-KO cells. Overexpression of the oncogenes *MYC* and *MYCN* recovered the slow growth in QD-KO and QT-KO without changing the glycan structures. Our results suggest that these cell lines could be suitable platforms to produce homogeneous therapeutic proteins.

Keywords: α1,2-mannosidase-I, genome editing, HEK293, homogeneous N-glycan, therapeutic protein

MOLECULAR BIOLOGY

DNA-Protein Interaction

Cross-talk between the three furA orthologs in Mycobacterium smegmatis and the contribution to isoniazid resistance

Chun-Hui Gao[1], Wen-Ping Wei[1], Hui-Ling Tao[1], Li-Kai Cai[1], Wan-Zhong Jia[2], Lihua Hu[1] and Min Yang[1]

[1]State Key Laboratory of Agricultural Microbiology, Huazhong Agricultural University, No. 1, Shizishan Street, Hongshan District, Wuhan, China, [2]The State Key Laboratory of Veterinary Etiological Biology, Lanzhou Veterinary Research Institute, No. 1, Xujiaping, Chengguan District, Chinese Academy of Agricultural Sciences, Lanzhou, China

The ferric uptake regulator A (FurA) plays an essential role in responding to oxidative stress in mycobacteria. The genome of *Mycobacterium smegmatis* harbours three FurA orthologs; however, the potential cross-talk and contribution to drug resistance of different *furA* operon remain underdetermined. In this

study, we characterized the cross-regulation and effect in drug resistance of these orthologs from *M. smegmatis*. Cross-binding of FurA protein to *furA* promoter was observed. The binding of FurA1 to *furA3p* and FurA2 to *furA1p* or *furA3p* is even more pronounced than their self-binding. The three FurA proteins are all functional at repressing the expression of the peroxidase enzyme *katG1/katG2 in vivo*. When overexpressing any of the *furA* orthologs in *M. smegmatis*, the bacteria become more resistant to isoniazid (INH). This pattern is consistent with that in *Mycobacterium bovis*. However, the knockdown of *furA* does not affect the INH sensitivity. This is the first report of cross-talk and contribution to drug resistance of all three *furA* orthologs in *M. smegmatis*.

Keywords: cross-talk, furA, isoniazid resistance, katG, mycobacteria

CELL

Tumor and Immunology

Toll-like receptor 7 regulates osteoclastogenesis in rheumatoid arthritis

Kyoung-Woon Kim[1], Bo-Mi Kim[1], Ji-Yeon Won[2], Kyung-Ann Lee[2], Hae-Rim Kim[2] and Sang-Heon Lee[2]

[1]Convergent Research Consortium in Immunologic Disease, Seoul St. Mary's Hospital College of Medicine, The Catholic University, 222 Banpo-daero, Seocho-gu, Seoul, Korea, [2]Department of Rheumatology, Research Institute of Medical Science, Konkuk University School of Medicine, 120–1 Neungdong-ro (Hwayang-dong), Gwangjin-gu, Seoul, Korea

This study aimed to determine the regulatory role of toll-like receptor 7 (TLR7) in receptor activator of nuclear factor kappa-B ligand (RANKL) production and osteoclast differentiation in rheumatoid arthritis (RA). In confocal microscopy, the co-expression of TLR7, CD55 and RANKL was determined in RA synovial fibroblasts. After RA synovial fibroblasts were treated with imiquimod, the RANKL gene expression and protein production were determined by real-time polymerase chain reaction (PCR) and enzyme-linked immunosorbent assay (ELISA). Osteoclastogenesis from peripheral blood $CD14^+$ monocytes which were cultured with imiquimod was assessed by determining the numbers of tartrate-resistant acid phosphatase (TRAP)-positive multinucleated cells. The signal pathways mediating the TLR7-induced RANKL expression and osteoclastogenesis were analysed after inhibition of intracellular signal molecules and their phosphorylation. Imiquimod stimulated the expression of TLR7 and RANKL and production of RANKL in RA synovial fibroblasts, increasing the phosphorylation of TRAF6, IRF7, mitogen-activated protein kinases (MAPK), c-Jun and NFATc1. When $CD14^+$ monocytes were cultured with imiquimod or co-cultured with imiquimod-pre-treated RA synovial fibroblasts, they were differentiated into TRAP+ multinucleated osteoclasts in the absence of RANKL. TLR7 activation-induced osteoclastogenesis in RA through direct induction of osteoclast differentiation from its precursors and up-regulation of RANKL production in RA synovial fibroblasts. Thus, the blockage of TLR7 pathway could be a promising therapeutic strategy for preventing bone destruction in RA.

Keywords: osteoclastogenesis, RANKL, rheumatoid arthritis, synovial fibroblasts, toll-like receptor 7

Dysregulation of miR484-TUSC5 axis takes part in the progression of hepatocellular carcinoma

Shanzong Wang[1], Weijuan Wang[2], Xiaoguang Han[3], Youli Wang[1], Yunzhen Ge[1] and Zhen Tan[1]

[1]Department of Pathology, The Third People's Hospital of Linyi, Huaxia Road No. 117, Linyi Economic and Technological Development Zone, Linyi, Shandong, China, [2]Department of Gynaecology, The Third People's Hospital of Linyi, Huaxia Road No. 117, Linyi Economic and Technological Development Zone, Linyi, Shandong, China, [3]Department of Internal Medicine, The Third People's Hospital of Linyi, Huaxia Road No. 117, Linyi Economic and Technological Development Zone, Linyi, Shandong, China

Hepatocellular carcinoma (HCC) is one of the leading causes of cancer-related death worldwide. miR-484 is previously reported to be a crucial modulator during the process from precancerous lesion to cancer. Tumour suppressor candidate 5 (TUSC5) is a potential tumour suppressor, but its expression and function in HCC are obscure. In this study, we aimed to explore the roles of miR-484 and TUSC5 in HCC, and clarify the relationship between them. We demonstrated that miR-484 was significantly up-regulated in HCC, while TUSC5 was down-regulated. TUSC5 was validated as the target gene of miR-484 and both of them were associated with the prognosis of HCC patients. miR-484 mimics markedly promoted the malignant phenotypes while TUSC5 plasmid had the opposite effect. In conclusion, miR-484/TUSC5 is potential diagnostic biomarkers and therapy targets for HCC.

Keywords: hepatocellular carcinoma, miR-484, prognosis, TUSC5

BIOTECHNOLOGY

Biotechnology General

Stable mutants of restriction-defi cient/modifi cation-profi cient Bacillus subtilis 168: hub strains for giant DNA engineering

Mitsuhiro Itaya[1], Mitsuru Sato[1], Satoru Watanabe[2], Hirofumi Yoshikawa[2], Masaru Tomita[1] and Rintaro Sato[1]

[1]Institute for Advanced Biosciences, Keio University, Nipponkoku, Daihoji, Tsuruoka-shi, Yamagata, Japan, [2]Department of Bioscience, Tokyo University of Agriculture, Sakuragaoka, Setagaya-ku, Tokyo, Japan

Bacillus subtilis 168 has been explored as a platform for the

synthesis and transmission of large DNA. Two inherent DNA incorporation systems, natural transformation and pLS20-based conjugation transfer, enable rapid handling of target DNA. Both systems are affected by the *Bsu* restriction–modification system that recognizes and cleaves unmethylated *Xho*I sites, limiting the choice of target DNA. We constructed *B. subtilis* 168 with stable mutation for restriction-deficient and modification-proficient (r^-m^+). It was demonstrated that the r^-m^+ strains can incorporate and transfer synthesized DNA with multiple *Xho*I sites. These should be of value as hub strains to integrate and disseminate giant DNA between *B. subtilis* 168 derivatives.

Keywords: *Bacillus subtilis*, conjugation transfer, DNA synthesis, genome engineering, natural genetic transformation, restriction–modification

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ダイジェスト

JB Review

Featured article of the month

Deciphering the mechanism for induction of senescence-associated secretory phenotype (SASP) and its role in ageing and cancer development

Naoko Ohtani[1]

[1]Department of Pathophysiology, Graduate School of Medicine, Osaka City University, 1–4–3 Asahi-machi, Abeno-ku, Osaka, Japan

Cellular senescence is an irreversible form of cell cycle arrest that can be induced by persistent DNA damage, and is well known to function as an important tumour suppression mechanism. Cellular senescence is detected in aged organisms; thus, it is also recognized as a hallmark of organismal ageing. Unlike apoptotic cells, senescent cells can survive for long periods of time. Recently, it has been shown that the late stage of senescent cells are capable of expressing a variety of secreted proteins such as cytokines, chemokines and proteases, and this condition is now known as senescence-associated secretory phenotype (SASP). These secreted factors are involved in myriad of physiological functions including tissue repair and clearance of damaged cells. Alternatively, these factors may promote detrimental effects, such as chronic inflammation or cancer progression, should the SASP persist. Recent scientific advances have indicated that innate immune responses, particularly involving the cGAS–STING pathway, trigger SASP induction. Therefore, developing a strategy to regulate SASP may provide scientific insights for the management of age-associated diseases and the implementation of healthy ageing in the future.

Keywords: cellular senescence, cGAS–STING, deoxycholic acid (DCA), innate immunity, lipoteichoic acid (LTA), senescence-associated secretory phenotype (SASP)

BIOCHEMISTRY

Biochemistry General

Phosphorylation by protein kinase C stabilizes ABCG1 and increases cholesterol efflux

Taro Watanabe[1], Noriyuki Kioka[1], Kazumitsu Ueda[1,2] and Michinori Matsuo[1,3]

[1]Laboratory of Cellular Biochemistry, Division of Applied Life Sciences, Graduate School of Agriculture, Kyoto University, Kyoto, Japan, [2]Institute for Integrated Cell-Material Sciences (WPI-iCeMS), Kyoto University, Kyoto, Japan, [3]Department of Food and Nutrition, Faculty of Home Economics, Kyoto Women's University, Kyoto, Japan

ATP-binding cassette protein G1 (ABCG1) plays an important role in eliminating excess cholesterol from macrophages and in the formation of high-density lipoprotein (HDL), which contributes to the prevention and regression of atherosclerosis. The post-translational regulation of ABCG1 remains elusive, although phosphorylation by protein kinase A destabilizes ABCG1 proteins. We examined the phosphorylation of ABCG1 using HEK293 and Raw264.7 cells. ABCG1 phosphorylation was enhanced by treatment with 12-O-tetradecanoylphorbol-13-acetate (TPA), a protein kinase C (PKC) activator. PKC activation by TPA increased ABCG1 protein levels and promoted ABCG1-dependent cholesterol efflux to HDL. This activity was suppressed by Go6976, a PKCα/βI inhibitor, suggesting that PKC activation stabilizes ABCG1. To confirm this, the degradation rate of ABCG1 was analysed; ABCG1 degradation was suppressed upon PKC activation, suggesting that PKC phosphorylation regulates ABCG1 levels. To confirm this involvement, we co-expressed ABCG1 and a constitutively active form of PKCα in HEK cells. ABCG1 was increased upon co-expression. These results suggest that PKC-mediated phosphorylation, probably PKCα, stabilizes ABCG1, consequently increasing ABCG1-mediated cholesterol efflux, by suppressing ABCG1 degradation. PKC activation could thus be a therapeutic target to suppress the development of atherosclerosis.

Keywords: ABC protein, atherosclerosis, cholesterol, high-density lipoprotein (HDL), transporter

Protein Structure

Effect of sodium ions on conformations of the cytoplasmic loop of the PomA stator protein of Vibrio alginolyticus

Taira Mino[1], Tatsuro Nishikino[1], Hiroto Iwatsuki[1], Seiji Kojima[1] and Michio Homma[1]

[1]Division of Biological Science, Graduate School of Science,

Nagoya University, Chikusa-ku, Furo-cyo, Nagoya, Japan

The sodium driven flagellar stator of *Vibrio alginolyticus* is a hetero-hexamer membrane complex composed of PomA and PomB, and acts as a sodium ion channel. The conformational change in the cytoplasmic region of PomA for the flagellar torque generation, which interacts directly with a rotor protein, FliG, remains a mystery. In this study, we introduced cysteine mutations into cytoplasmic charged residues of PomA, which are highly conserved and interact with FliG, to detect the conformational change by the reactivity of biotin maleimide. *In vivo* labelling experiments of the PomA mutants revealed that the accessibility of biotin maleimide at position of E96 was reduced with sodium ions. Such a reduction was also seen in the D24N and the plug deletion mutants of PomB, and the phenomenon was independent in the presence of FliG. This sodium ions specific reduction was also detected in *Escherichia coli* that produced PomA and PomB from a plasmid, but not in the purified stator complex. These results demonstrated that sodium ions cause a conformational change around the E96 residue of $loop_{2-3}$ in the biological membrane.

Keywords: cytoplasmic region, flagellar stator, PomA, rotor–stator interaction, *Vibrio*

Biochemistry in Diseases and Aging

Impaired plasmalogen synthesis dysregulates liver X receptor-dependent transcription in cerebellum

Masanori Honsho[1], Fabian Dorninger[2], Yuichi Abe[1], Daiki Setoyama[3], Ryohei Ohgi[4], Takeshi Uchiumi[3], Dongchon Kang[3], Johannes Berger[2] and Yukio Fujiki[1]

[1]Medical Institute of Bioregulation, Kyushu University, 3–1–1 Maidashi, Higashi-ku, Fukuoka, Japan, [2]Department of Pathobiology of the Nervous System, Center for Brain Research, Medical University of Vienna, Spitalgasse 4, Vienna, Austria, [3]Department of Clinical Chemistry and Laboratory Medicine, Graduate School of Medical Sciences, Kyushu University, 3–1–1 Maidashi, Higashi-ku, Fukuoka, Japan, [4]Graduate School of Systems Life Sciences, Kyushu University, 744 Motooka, Nishi-ku, Fukuoka, Japan

Synthesis of ethanolamine plasmalogen (PlsEtn) is regulated by modulating the stability of fatty acyl-CoA reductase 1 (Far1) on peroxisomal membrane, a rate-limiting enzyme in plasmalogen synthesis. Dysregulation of plasmalogen homeostasis impairs cholesterol biosynthesis in cultured cells by altering the stability of squalene epoxidase (SQLE). However, regulation of PlsEtn synthesis and physiological consequences of plasmalogen homeostasis in tissues remain unknown. In the present study, we found that the protein but not the transcription level of Far1 in the cerebellum of the *Pex14* mutant mouse expressing Pex14p lacking its C-terminal region ($Pex14^{\Delta C/\Delta C}$) is higher than that from wild-type mouse, suggesting that Far1 is stabilized by the lowered level of PlsEtn. The protein level of SQLE was increased, whereas the transcriptional activity of the liver X receptors (LXRs), ligand-activated transcription factors of the nuclear receptor superfamily, is lowered in the cerebellum of $Pex14^{\Delta C/\Delta C}$ and the mice deficient in dihydroxyacetonephosphate acyltransferase, the initial enzyme for the synthesis of PlsEtn. These results suggest that the reduction of plasmalogens in the cerebellum more likely compromises the cholesterol homeostasis, thereby reducing the transcriptional activities of LXRs, master regulators of cholesterol homeostasis.

Keywords: cerebellum, cholesterol, liver X receptor, peroxisome, plasmalogens

Immunochemistry

Characterization of monoclonal antibodies recognizing each extracellular loop domain of occluding

Yoshimi Shimizu[1,2], Yoshitaka Shirasago[1], Takeru Suzuki[1,3], Tomoyuki Hata[4], Masuo Kondoh[4], Kentaro Hanada[1], Kiyohito Yagi[4] and Masayoshi Fukasawa[1]

[1]Department of Biochemistry and Cell Biology, National Institute of Infectious Diseases, Shinjuku-ku, Tokyo, Japan, [2]Department of Pharmaceutical Sciences, Teikyo Heisei University, Nakano-ku, Tokyo, Japan, [3]Department of Biological Science and Technology, Tokyo University of Science, Katsushika-ku, Tokyo, Japan, [4]Graduate School of Pharmaceutical Sciences, Osaka University, Suita, Osaka, Japan

The tight junction protein occludin (OCLN) is a four-pass transmembrane protein with two extracellular loops (ELs), and also functions as a co-receptor for hepatitis C virus (HCV). Recently, we reported the establishment of monoclonal antibodies (mAbs) recognizing each intact EL domain of OCLN that can strongly prevent HCV infection *in vitro* and *in vivo*, and these mAbs were applicable for flow cytometric (FCM) analysis, immunocytochemistry (ICC) and cell-based enzyme-linked immunosorbent assay. In the present study, we further examined the application of these anti-OCLN mAbs and characterized their binding properties. All four mAbs were available for immunoprecipitation. The three first EL (EL1)-recognizing mAbs were applicable for immunoblotting, but the second EL (EL2)-recognizing one was not. Using site-directed mutagenesis, we also determined residues of OCLN critical for recognition by each mAb. Our findings showed that the small loop between two cysteines of the EL2 domain is essential for the binding to one EL2-recognizing mAb and that the recognition regions by three EL1-recognizing mAbs overlap, but are not the same sites of EL1. To obtain a deeper understanding of OCLN biology and its potential as a therapeutic target, specific mAbs to detect or target OCLN in intact cells should be powerful tools for future studies.

Keywords: monoclonal antibody, occludin, tight junction

MOLECULAR BIOLOGY

Replication and Recombination

Matrin3 promotes homologous recombinational repair by regulation of RAD51

Lin Shi[1], Jiying Sun[1], Aiko Kinomura[1], Atsuhiko Fukuto[1,2], Yasunori Horikoshi[1] and Satoshi Tashiro[1]

[1]Department of Cellular Biology, Research Institute for Radiation Biology and Medicine, [2]Department of Ophthalmology and Visual Science, Graduate School of Biomedical and Health Sciences, Hiroshima University, Hiroshima, Japan

Matrin3 is a highly conserved inner nuclear matrix protein involved in multiple stages of RNA metabolism. Although Matrin3 may also play a role in DNA repair, its precise roles have remained unclear. In this study, we showed that the depletion of Matrin3 led to decreased homologous recombination (HR) efficiency and increased radiation sensitivity of cells. Matrin3-depleted cells showed impaired DNA damage-dependent focus formation of RAD51, a key protein in HR. These findings suggest that Matrin3 promotes HR by regulating RAD51.

Keywords: DNA double-strand breaks, DNA repair, homologous recombination, Matrin3, RAD51

Gene Expression

miR-146a and miR-196a-2 genes polymorphisms and its circulating levels in lung cancer patients

Randa H Mohamed[1], Heba F Pasha[1], Doaa M Gad[2] and Mostafa M Toam[3]

[1]Medical Biochemistry Department, Faculty of Medicine, Zagazig University, Zagazig, Egypt, [2]Chest Department, Faculty of Medicine, Zagazig University, Zagazig, Egypt, [3]Clinical Oncology and Nuclear Medicine Department, Faculty of Medicine, Zagazig University, Zagazig, Egypt

Recently, MicroRNAs polymorphisms and their serum expression have been linked to increase risk of various cancers. The aim of this study was to elucidate the association between single nucleotide polymorphisms of *miR-146a* and *miR-196a-2* and their serum expression and lung cancer risk. One hundred and twenty lung cancer patients and 120 health controls were included in this study. Genotyping and expression for miR-146a and miR-196a-2 were performed using polymerase chain reaction (PCR)-restriction fragment length polymorphism and quantitative real-time PCR. Individuals carrying *miR-146a* CG and CC genotypes had significantly increased risk for lung cancer than those carrying *miR-146a* GG genotype. MiR-146a expression significantly decreased in *miR-146a* CG and CC genotypes carriers as compared with GG genotype carriers. *MiR-196a-2* CT and TT genotypes were significantly associated with increased lung cancer while the highest expression of MiR-196a-2 was detected in *miR-196a-2* CC genotype carriers. Serum miR-146a was significantly decreased in lung cancer patients while serum miR-196a-2 expression was significantly increased in lung cancer patients. In conclusion, *miR-146a* and *miR-196a-2* genes polymorphisms and their circulating levels were associated with lung cancer risk in Egyptians and may be helpful in early detection of lung cancer.

Keywords: expression, lung cancer, polymorphisms, miR-146a, miR-196a-2

CELL

Receptors and Signal Transduction

GPR31 and GPR151 are activated under acidic conditions

Misaki Mashiko[1], Aya Kurosawa[1], Yuki Tani[1], Takashi Tsuji[1] and Shigeki Takeda[1]

[1]Division of Molecular Science, Department of Chemical Biology, Faculty of Science and Technology, Gunma University, 1–5–1 Tenjin-cho, Kiryu, Gunma, Japan

Recent studies have revealed that not only proton-sensing channels, but also one family of G protein-coupled receptors (GPCRs) comprising OGR1, GPR4, G2A and TDAG8 are responsible for the sensing of extracellular protons, or pH. Here, we report that two other GPCRs, GPR31 and GPR151, were also activated in acidic condition. Elevated pH of assay mixtures resulted in a remarkable increase in [^{35}S]GTPγS binding by GPR31–Giα and GPR151–Giα fusion proteins in a narrow range between pH 6 and 5. Our reporter gene assays with CHO cells expressing recombinant GPR31 or GPR151 also showed that activation was maximal at pH ～5.8. Although these results from *in vitro* and cellular assays revealed slightly different pH sensitivities, all of our results indicated that GPR31 and GPR151 sensed extracellular protons equally well as other proton-sensing GPCRs.

Keywords: G protein-coupled receptor, proton-sensing, orphan receptor

Differentiation/Development and Aging

Characterization of the human E2F4 promoter region and its response to 12-O-tetradecanoylphorbol-13-acetate

Hiroshi Hamada[1], Yuta Goto[1], Jun Arakawa[1], Erisa Murayama[1], Yui Ogawa[1], Midori Konno[1], Takahiro Oyama[2,3], Masashi Asai[1], Akira Sato[3], Sei-ichi Tanuma[3,4] and Fumiaki Uchiumi[1]

[1]Department of Gene Regulation, Faculty of Pharmaceutical Sciences, Tokyo University of Science, Noda-shi, Chiba-ken, Japan, [2]Hinoki Shinyaku Co., Ltd., 9–6 Nibancho, Chiyoda-ku, [3]Tokyo, Japan, Department of Biochemistry, Faculty of Pharmaceutical Sciences, Tokyo University of Science, Noda-shi, Chiba-ken, Japan, [4]Genomic Medical Science, Research Institute of Science and Technology, Tokyo University of Science, Noda-shi, Chiba-ken, Japan

The E2F transcription factors (TFs), which control the progression of the cell cycle in response to DNA-damage and various

stresses, are known to interact with a tumour suppressor, Retinoblastoma 1 (RB1). We previously showed that the response of the human *RB1* promoter to a 12-*O*-tetradecanoylphorbol-13-acetate (TPA) in HL-60 cells is mediated by a duplicated GGAA motif, which is also present in the 5′-upstream of the *E2F* family genes. The motifs are especially rich in the 5′-upstream of the *E2F4* gene. In the present study, we constructed luciferase (Luc) expression vectors containing a 466 bp of the 5′-upstream of the human *E2F4* gene. The transfection of this plasmid and deletion/mutation-introduced derivatives into HL-60 cells and a Luc reporter assay showed that duplicated and triplicated GGAA (TTCC) motifs in the *E2F4* promoter respond to TPA. As expected, electrophoretic mobility shift assay indicated that SPI1 (PU.1) binds to the GGAA motif-containing element. A quantitative RT-PCR and western blotting showed that the *E2F4* transcripts and its encoding proteins accumulate during the differentiation of HL-60 into macrophage-like cells. In contrast, the expression of the *E2F1* gene and the protein, which possibly acts as a cell cycle accelerator, was greatly diminished.
Keywords: differentiation, E2F, ETS, HL-60, TPA