Journal of Biochemistry

Vol.169, No.5（2021 年 5 月 発 行）

ダイジェスト

JB Commentary

Regulation of lysosomal positioning via TMEM55B phosphorylation

Makoto Araki[1] and Kenji Kontani[1]

[1]Department of Biochemistry, Meiji Pharmaceutical University, 2–522–1 Noshio, Kiyose, Tokyo 204–8588, Japan

Lysosomes are dynamic organelles that are transported along microtubules bidirectionally via kinesin and dynein motor proteins. Lysosomal positioning, which is determined by the balance of the bidirectional lysosomal movement, changes under various conditions and affects lysosomal functions such as autophagy and antigen presentation. A recent study by Takemasu et al. (Phosphorylation of TMEM55B by Erk/MAPK regulates lysosomal positioning. *J. Biochem.* 2019; 166:175–185) has shown that phosphorylation of the transmembrane protein TMEM55B is involved in the retrograde lysosomal trafficking towards the perinuclear region. They found that TMEM55B is phosphorylated upon stimulation with various ligands and that Erk/MAPK mediates the TMEM55B phosphorylation. They have also revealed that a phosphorylation mimic mutant of TMEM55B enhances perinuclear lysosomal clustering compared to the wild-type TMEM55B. These findings suggest that TMEM55B phosphorylation by Erk/MAPK is responsible for regulating lysosomal positioning in response to external stimuli.

Keywords; Erk, lysosomes, phagocytosis, phosphorylation, TMEM55B

JB Reviews

Regulation of Reelin functions by specifi c proteolytic processing in the brain

Mitsuharu Hattori[1] and Takao Kohno[1]

[1]Department of Biomedical Science, Graduate School of Pharmaceutical Sciences, Nagoya City University, Nagoya, Aichi 467–8603, Japan

The secreted glycoprotein Reelin plays important roles in both brain development and function. During development, Reelin regulates neuronal migration and dendrite development. In the mature brain, the glycoprotein is involved in synaptogenesis and synaptic plasticity. It has been suggested that Reelin loss or decreased function contributes to the onset and/or deterioration of neuropsychiatric diseases, including schizophrenia and Alzheimer's disease. While the molecular mechanisms underpinning Reelin function remain unclear, recent studies have suggested that the specific proteolytic cleavage of Reelin may play central roles in the embryonic and postnatal brain. In this review, we focus on Reelin proteolytic processing and review its potential physiological roles.

Keywords; ADAMTS, Alzheimer's disease, brain, metalloprotease, Reelin

Structural catalog of core Atg proteins opens new era of autophagy Research

Kazuaki Matoba[1] and Nobuo N Noda[1]

[1]Institute of Microbial Chemistry, 3–14–23 Kamiosaki, Shinagawa-ku, Tokyo 141–0021, Japan

Autophagy, which is an evolutionarily conserved intracellular degradation system, involves *de novo* generation of autophagosomes that sequester and deliver diverse cytoplasmic materials to the lysosome for degradation. Autophagosome formation is mediated by approximately 20 core autophagy-related (Atg) proteins, which collaborate to mediate complicated membrane dynamics during autophagy. To elucidate the molecular functions of these Atg proteins in autophagosome formation, many researchers have tried to determine the structures of Atg proteins by using various structural biological methods. Although not sufficient, the basic structural catalog of all core Atg proteins was established. In this review article, we summarize structural biological studies of core Atg proteins, with an emphasis on recently unveiled structures, and describe the mechanistic breakthroughs in autophagy research that have derived from new structural information.

Keywords; Atg proteins, autophagosome, autophagy, phase separation, structural biology

Potential roles of G-quadruplex structures in RNA granules for physiological and pathological phase separation

Sefan Asamitsu[1] and Norifumi Shioda[1]

[1]Department of Genomic Neurology, Institute of Molecular Embryology and Genetics (IMEG); Kumamoto University, 2–2–1 Honjo, Chuo-ku, Kumamoto 860–0811, Japan

Cellular liquid–liquid phase separation is a physiologically inevitable phenomenon in molecularly crowded environments inside cells and serves to compartmentalize biomolecules to facilitate several functions, forming cytoplasmic and nuclear RNA granules. Abnormalities in the phase separation process in RNA granules are implicated in the onset of several neurodegenerative diseases; the initial liquid-like phase-separated droplets containing pathogenic proteins are prone to aberrantly mature into solid-like droplets. RNAs are involved in the maturation of physiological and pathological RNA granules and are essential for governing the fate of phase-transition processes. Notably, RNA G-quadruplex (G4RNA), which is the secondary structure

of nucleic acids that are formed in guanine-rich sequences, appears to be an advantageous scaffold for RNA-derived phase separation because of its multivalent interactions with RNAs and RNA-binding proteins. Here, we summarize the properties of RNA granules in physiological and pathological phase separation and discuss the potential roles of G4RNA in granules.

Keywords; compartmentalization, neurodegenerative diseases, phase separation, RNA G-quadruplex, RNA granules

BIOCHEMISTRY

Biochemistry General

Silent information regulator type-1 mediates amelioration of inflamatory response and oxidative stress in lipopolysaccaride-induced acute respiratory distress syndrome

Yuan Zhan[1], Chunjian Yang[2], Qunhui Zhang[1] and Li Yao[1]

[1]Department of Intensive Care Unit, [2]Department of General Surgery, The Second People's Hospital of Hefei, No. 246 Heping Road, Yaohai District, Hefei 230011, Anhui, China

Silent information regulator type-1 (SIRT1) is crucial during the development of acute respiratory distress syndrome (ARDS). We aimed to explore whether SIRT1 activation could protect against ARDS. SIRT1 was activated by its agonist SRT1720. ARDS was induced by intraperitoneal injection of 5 mg/kg lipopolysaccharide (LPS). Lung injuries were determined by the lung wet/dry ratio, inflammatory cells in the broncho-alveolar lavage fluid (BALF) and histological analysis. Inflammatory cytokine release was detected by enzyme-linked immunosorbent assay. The accumulation of neutrophils was detected by myeloperoxidase activity. Oxidative stress was evaluated by malondialdehyde, reduced glutathione, superoxide dismutase and catalase activities. The protein expression levels were detected using western blot. SIRT1 activation, either by SRT1720 administration or recombinant SIRT1, expression eliminated high-dose LPS-induced mortality in mice, attenuated lung injury, influenced cytokine release in BALF and decreased oxidative stress in the lung tissues of ARDS mice. Mechanically, SRT1720 administration inhibited p65 phosphorylation in the lung tissues of ARDS mice. SIRT1 ameliorates inflammatory response and oxidative stress in LPS-induced ARDS.

Keywords; ARDS, inflammatory response, oxidative stress, SIRT1

Protein Structure

Structural studies of reelin N-terminal region provides insights into a unique structural arrangement and functional multimerization

Masamichi Nagae[1,2], Kei Suzuki[3], Norihisa Yasui[4], Terukazu Nogi[5], Takao Kohno[6], Mitsuharu Hattori[6] and Junichi Takagi[2]

[1]Department of Molecular Immunology, Research Institute for Microbial Diseases, [2]Laboratory of Molecular Immunology, Immunology Frontier Research Center (iFReC), Osaka University, 3–1 Yamada-Oka, Suita, Osaka 565–0871, Japan, [3]Institute for Protein Research, Osaka University, 3–2 Yamada-Oka, Suita, Osaka 565–0871, Japan, [4]Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Okayama University, 1–1–1, Tsushima-naka, Kita-ku, Okayama 700–8530, Japan, [5]Graduate School of Medical Life Science, Yokohama City University, Tsurumi-ku, Yokohama 230–0045, Japan, [6]Department of Biomedical Science, Graduate School of Pharmaceutical Sciences, Nagoya City University, Nagoya, Aichi 467–8603, Japan

The large, secreted glycoprotein reelin regulates embryonic brain development as well as adult brain functions. Although reelin binds to its receptors via its central part, the N-terminal region directs multimer formation and is critical for efficient signal transduction. In fact, the inhibitory antibody CR-50 interacts with the N-terminal region and prevents higher-order multimerization and signalling. Reelin is a multidomain protein in which the central part is composed of eight characteristic repeats, named reelin repeats, each of which is further divided by insertion of a epidermal growth factor (EGF) module into two subrepeats. In contrast, the N-terminal region shows unique 'irregular' domain architecture since it comprises three consecutive subrepeats without the intervening EGF module. Here, we determined the crystal structure of the murine reelin fragment named RX-R1 including the irregular region and the first reelin repeat at 2.0-Å resolution. The overall structure of RX-R1 has a branched Y-shaped form. Interestingly, two incomplete subrepeats cooperatively form one entire subrepeat structure, though an additional subrepeat is inserted between them. We further reveal that Arg335 of RX-R1 is crucial for binding CR-50. A possible self-association mechanism via the N-terminal region is proposed based on our results.

Keywords; epitope mapping, inhibitory antibody CR-50, irregular region, protein crystallography, reelin

Protein Interaction and Recognition

The N2N3 domains of ClfA, FnbpA and FnbpB in Staphylococcus aureus bind to human complement factor H, and their antibodies enhance the bactericidal capability of human blood

Xinrui Mao[1], Junghyun Kim[1], QingFeng Zhang[1], TingTing Jiang[1], Dong Ho Ahn[2], Yunjin Jung[1], Misao Matsushita[3], Taeok Bae[4] and Bok Luel Lee[1]

[1]Host Defense Protein Laboratory, College of Pharmacy, Pusan National University, Busan 46241, Republic of Korea, [2]New Drug Development Section of Clips Company, Mapo-gu, Seoul 04168, Republic of Korea, [3]Department of Applied Biochemistry, Tokai University, Hiratsuka 259–1292, Japan, [4]Department of Microbiology and Immunology, Indiana University School of Medicine-Northwest, Gary, IN 46408, USA

In the complement system, the opsonin C3b binds to the bacterial cell surface and mediates the opsonophagocytosis. However, the cell-wall protein SdrE of *Staphylococcus aureus* inhibits the C3b activity by recruiting the complement regulatory protein factor H (fH). SdrE binds to fH via its N-terminal N2N3 domain, which are also found in six other staphylococcal cell-wall proteins. In this study, we report that not only the N2N3 domain of SdrE but also those of ClfA, FnbpA and FnbpB can bind to fH. When immobilized on a microplate, the N2N3 domains recruited fH and enhanced the factor I (fI)-mediated cleavage of C3b. When mixed with fH and *S. aureus* cells, the N2N3 domains inhibited the fH binding to *S. aureus* cells and reduced the fI-mediated C3b cleavage on the bacterial cell surface. The $F(ab)'_2$ fragments of the rabbit N2N3 antibodies also inhibited the fH binding to the *S. aureus* cell surface. When added to human blood, the N2N3 antibodies or the N2N3 domain proteins significantly increased the bactericidal activity. Based on these results, we conclude that, in *S. aureus*, not only SdrE but also ClfA, FnbpA and FnbpB can contribute to the inhibition of C3b-mediated opsonophagocytosis.

Keywords; adhesion, cell surface proteins, complement, factor H, *Staphylococcus aureus*

Binding of collagen gene products with titanium oxide

Qin Song[1], Shouhei Iku[2,3], Rachel Sammons[4], Kimitoshi Yagami[5], Toshitake Furusawa[6], Koichi Morimoto[7], Md Shiblur Rahaman[8], Masaaki Kurasaki[8], Seiichi Tokura[8] and Yoshinori Kubok[8]

[1]College of Pharmacy and Bioengineering, Chengdu University, Chengdu, Sichuan 610106, China, [2]Suqian Yimei Technology Co., Biotechnology Industrial Park No. 1, Suqian, Jiangsu 223800, China, [3]Jiangsu Alphay Biological Technology Co., Ltd, 226009 Nantong, China, [4]Department of Biomaterials, School of Dentistry, Birmingham University, Birmingham, UK, [5]Department of Oral Health Promotion, Graduate School of Oral Medicine, Matsumoto Dental University, Shiojiri 399–0781, Japan, [6]Department of Bioengineering, Graduate School of Science and Engineering, Yamagata University, Tsuruoka 982–0001, Japan, [7]Department of Genetic Engineering, Kindai University, Kinokawa, Wakayama, Japan, [8]Faculty of Environmental Earth Science, Hokkaido University, Sapporo 060–0810, Japan

Titanium is the only metal to which osteoblasts can adhere and on which they can grow and form bone tissue *in vivo*, resulting in a strong bond between the implant and living bone. This discovery provides the basis for the universal medical application of Ti. However, the biochemical mechanism of bond formation is still unknown. We aimed to elucidate the mechanism of bond formation between collagen, which constitutes the main organic component of bone, and TiO_2, of which the entire surface of pure Ti is composed. We analysed the binding between the soluble collagen and TiO_2 by chromatography with a column packed with Ti beads of 45 μm, and we explored the association between collagen fibrils and TiO_2 (anatase) powders of 0.2 μm. We ran the column of chromatography under various elution conditions. We demonstrated that there is a unique binding affinity between Ti and collagen. This binding capacity was not changed even in the presence of the dissociative solvent 2M urea, but it decreased after heat denaturation of collagen, suggesting the contribution of the triple-helical structure. We propose a possible role of periodically occurring polar amino acids and the collagen molecules in the binding with TiO_2.

Keywords; anatase, chromatography, collagen, titanium beads, 2M urea

Biochemical Pharmacology

circGFRA1 affects the sensitivity of triple-negative breast cancer cells to paclitaxel via the miR-361–5p/TLR4 pathway

Shu-rong Zheng[1,2], Qi-di Huang[2], Zhi-hai Zheng[1,3], Zhong-tao Zhang[1] and Gui-long Guo[3]

[1]Department of General Surgery, Beijing Key Laboratory of Cancer Invasion and Metastasis Research & National Clinical Research Center for Digestive Diseases, Beijing Friendship Hospital, Capital Medical University, 95 Yong-an Road, Xi-Cheng District, Beijing 100050, China, [2]Department of Breast and Thyroid Surgery, The First Affiliated Hospital of Wenzhou Medical University, Ouhai District, Wenzhou, Zhejiang 325000, China, [3]Department of Colorectal and Anal Surgery, The First Affiliated Hospital of Wenzhou Medical University, Ouhai District, Wenzhou, Zhejiang 325000, China

In recent years, the role of circular RNAs (circRNAs) in tumours has attracted widespread attention. Some circRNAs have been reported to play a role in triple-negative breast cancer (TNBC). However, circRNAs have rarely been reported in terms of TNBC resistance. This study aimed to clarify that circGFRA1 affects the sensitivity of TNBC cells to paclitaxel (PTX) by the miR-361-5p/TLR4 pathway. Compared with the non-PTX-resistant TNBC cell line MDA-MB-231, the expression of circGFRA1 in the PTX-resistant TNBC cell line MDA-MB-231.PR was significantly increased. The small hairpin RNA-mediated circGFRA1 knockdown inhibited the resistance of TNBC cells to PTX. RNA pull-down assay and luciferase reporter gene assay confirmed the binding between circGFRA1 and miR-361-5p and between miR-361-5p and TLR4. It has been proven that circGFRA1 knockdown can inhibit the resistance of TNBC cells to PTX by promoting the expression of miR-361-5p, and subsequently reduce the expression of TLR4.

Keywords; circGFRA1, miR-361-5p, PTX, TLR4, triple-negative breast cancer, Natural Science Foundation of Zhejiang Province of China [LY19H160026], Zhejiang Provincial Health Department [2019KY454], Wenzhou Bureau of Science and Technology [Y20170038, Y20170257]

MOLECULAR BIOLOGY

Molecular Biology General

Molecular interaction of cytotoxic anticancer analogues as inhibitors of *β*-tubulin protein against UACC-62 melanoma cell

Kuan-Chung Chen[1], Chi-Rei Wu[2] and Jin-Cherng Lien[3,4]

[1]School of Pharmacy, [2]Department of Chinese Pharmaceutical Sciences and Chinese Medicine Resources, [3]Master Program of Pharmaceutical Manufacture, China Medical University, Taichung 40402, Taiwan, [4]Department of Medical Research, Hospital of China Medical University, Taichung 40402, Taiwan

In previous research, a series of cytotoxic anticancer analogues related to 2-acylamino-1,4-naphthoquinone derivatives has been demonstrated. As microtubule plays an important role in many essential cellular processes such as mitosis, tubulin is an important target of anticancer drug. This study performed molecular docking simulation, pharmacophore model, comparative force field analysis model and comparative similarity indices analysis model to investigate the relationship between inhibitory activities and the properties of compounds, in order to further progress the development of cytotoxic anticancer analogues. These compounds have common H-bond interactions with key residues Lys254 and Lys352, but compounds with large R^2 substituent have different docking poses than compounds with small R^2 substituent. Some of derivatives such as compound **18** formed the H-bonds with residue Lys254 using the oxygen atoms in R^1 substituent and formed π-cation interactions with residue Lys352 using phenyl moiety of 1,4-naphthoquinone. The R^1 substituent of these compounds preferred to have disfavoured hydrophobic fields and favourable space towards the direction of residue Asn258, while the R^2 substituent of these compounds preferred to have about 2–3 carbon chain length hydrophobic substituent towards the direction of residues Ala316 and Lys352. These results offer some beneficial advices for further study in anticancer drug development process.

Keywords; *β*-tubulin, molecular docking simulation, pharmacophore features, quantitative structure–activity relationship (QSAR) models

Down-regulation of lncRNA SNHG5 relieves sepsis-induced acute kidney injury by regulating the miR-374a-3p/TLR4/NF-*κ*B pathway

Min Wang[1], Jilou Wei[1], Futai Shang[1], Kui Zang[1] and Peng Zhang[1]

[1]Department of Intensive Care Unit, The Affiliated Huai'an No. 1 People's Hospital of Nanjing Medical University, No. 6 Beijing West Road, Huai'an 223300, China

Sepsis is an acute systemic infectious disease engendered by infectious factors, which can cause the dysfunction of multiple organs, including acute kidney injury (AKI). Recently, more and more researchers are focussing on long noncoding RNA (lncRNA) that is closely associated with the development and progression of various diseases; however, the role and mechanism of lncRNA in sepsis-induced AKI are not fully understood. Here, we found a significant increase in the expression of lncRNA small nuclear RNA host gene 5 (SNHG5) in the serum of patients with sepsis than healthy controls. Similar results were obtained from mouse model of sepsis. Further investigations revealed that knockdown of SNHG5 improves the viability and reduces the rate of apoptosis and the generation of inflammatory cytokines in HK-2 and TCMK-1 cells treated with lipopolysaccharide. Mechanistically, we showed that SNHG5 can combine with microRNA-374a-3p (miR-374a-3p), which inhibits nuclear factor-*κ*B (NF-*κ*B) activity by targeting TLR4. In conclusion, our results demonstrate that SNHG5 may regulate sepsis-induced AKI via the miR-374a-3p/TLR4/NF-*κ*B pathway, therefore providing a new insight into the treatment of this disease.

Keywords; acute kidney injury, miR-374a-3p, NF-*κ*B pathway, sepsis, SNHG5

Gene Expression

LncRNA HEIH promotes cell proliferation, migration and invasion by suppressing miR-214-3p in gastric carcinoma

Lei Jiang[1], Luyao Zhang[2], Qian Chen[2], Shigang Qiao[3], Feng Zhou[4] and Min Han[5]

[1]Department of Orthopedics, Taizhou People's Hospital, 366 Taihu Road, Taizhou, Jiangsu, China, [2]Department of Oncology, The Affiliated Suzhou Hospital of Nanjing Medical University, 242 Guangji Road, Suzhou 215008, Jiangsu, China, [3]Department of Anesthesiology, The Affiliated Suzhou Science and Technology Town Hospital of Nanjing Medical University, No. 1 Lijiang Road, Suzhou 215000, China, [4]Department of Ultrasonography, The Affiliated Suzhou Science and Technology Town Hospital of Nanjing Medical University, No. 1 Lijiang Road, Suzhou 215000, China, [5]Department of Gastroenterology, The Affiliated Suzhou Hospital of Nanjing Medical University, 16 Baita Road, Suzhou 215000, Jiangsu, China

The present study aimed to investigate the function of long noncoding RNA HEIH in gastric carcinoma (GC). Adjacent normal tissues and GC tissues were obtained from 72 patients. Real-time quantitative polymerase chain reaction (RT-qPCR) was utilized to measure the expression of HEIH in cancer tissues and cells. Cell Counting Kit-8 and transwell assays were employed to evaluate cell proliferation, migration and invasion. An Annexin V-fluorescein-isothiocyanate (FITC)/propidium iodide (PI) Apoptosis Detection Kit was used to evaluate the apoptosis ratio. RT-qPCR was used to detect the expression level of miR-214-3p. The expression of HEIH in GC tissues was higher than in adjacent normal tissues. The expression of HEIH was up-

regulated in MKN-45, NCL-N87, KATO III cell lines compared within normal gastric epithelial cells. Knockdown of lncRNA HEIH significantly decreased the number of migrated and invaded cells. Additionally, downregulation of HEIH could increase GC cell apoptosis compared with the non-specific control (NC) group. We also proved that miR-214-3p was the direct target of lncRNA HEIH, and that overexpression of miR-214-3p could reverse the effects of HEIH. Silencing of HEIH could suppress Gastric Carcinoma cell proliferation, migration and invasion by inhibiting miR-214-3p. Thus, HEIH might represent a novel biomarker and therapeutic target.

Keywords; gastric cancer, HEIH, long non-coding RNA, miR-214-3p

BIOTECHNOLOGY

Gene and Protein Engineering

A sweet protein monellin as a non-antibody scaffold for synthetic binding proteins

Norihisa Yasui[1], Kazuaki Nakamura[1] and Atsuko Yamashita[1]

[1]Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Okayama University, 1–1–1 Tsushima-naka, Kita-ku, Okayama 700–8530, Japan

Synthetic binding proteins that have the ability to bind with molecules can be generated using various protein domains as non-antibody scaffolds. These designer proteins have been used widely in research studies, as their properties overcome the disadvantages of using antibodies. Here, we describe the first application of a phage display to generate synthetic binding proteins using a sweet protein, monellin, as a non-antibody scaffold. Single-chain monellin (scMonellin), in which two polypeptide chains of natural monellin are connected by a short linker, has two loops on one side of the molecule. We constructed phage display libraries of scMonellin, in which the amino acid sequence of the two loops is diversified. To validate the performance of these libraries, we sorted them against the folding mutant of the green fluorescent protein variant (GFPuv) and yeast small ubiquitin-related modifier. We successfully obtained scMonellin variants exhibiting moderate but significant affinities for these target proteins. Crystal structures of one of the GFPuv-binding variants in complex with GFPuv revealed that the two diversified loops were involved in target recognition. scMonellin, therefore, represents a promising non-antibody scaffold in the design and generation of synthetic binding proteins. We termed the scMonellin-derived synthetic binding proteins ‘SWEEPins’.

Keywords; combinatorial library, non-antibody scaffold, phage display, single-chain monellin, synthetic binding proteins

Vol.169 Issue 6（2021年6月発行号）以降はCovid-19の影響を受け2021年8月8日現在発行が発行されていません。ご迷惑をおかけいたし誠に申し訳ございません。