Journal of Biochemistry

Vol.168, No.4（2020 年 10 月 発 行）

ダイジェスト

Editorial

Kenji Kadomatsu

It has only been just over a year since I last published an editorial in the *Journal of Biochemistry*, but the world we currently find ourselves living in could not be more different. Autumn is the conference season in Japan and the schedule leading up to the end of the year is usually packed with business trips, presentations and welcome receptions, but this year has thrown up all sorts of new challenges as we navigate unchartered waters in both our professional and private lives, and grapple with the unfamiliar demands of online conferences, meetings and lectures....

JB Reviews

GTP metabolic reprogramming by IMPDH2: unlocking cancer cells' fuelling mechanism

Satoshi Kofuji[1] and Atsuo T Sasaki[2,3,4,5]

[1]Department of Developmental and Regenerative Biology, Medical Research Institute, Tokyo Medical and Dental University (TMDU), 1–5–45 Yushima, Bunkyo-ku, Tokyo 113–8510, Japan, [2]Division of Hematology and Oncology, Department of Internal Medicine, University of Cincinnati College of Medicine, 3125 Eden Ave., Cincinnati, OH 45267–0508, USA, [3]Department of Cancer Biology, University of Cincinnati College of Medicine, 3125 Eden Ave., OH 45267–0508, USA, [4]Department of Neurosurgery, Brain Tumor Center at UC Gardner Neuroscience Institute, 3113 Bellevue Ave, Cincinnati, OH 45267–0508, USA, [5]Institute for Advanced Biosciences, Keio University, Kakuganji 246–2, Mizukami, Tsuruoka City, Yamagata 997–0052, Japan

Growing cells increase multiple biosynthetic processes in response to the high metabolic demands needed to sustain proliferation. The even higher metabolic requirements in the setting of cancer provoke proportionately greater biosynthesis. Underappreciated key aspects of this increased metabolic demand are guanine nucleotides and adaptive mechanisms to regulate their concentration. Using the malignant brain tumour, glioblastoma, as a model, we have demonstrated that one of the rate-limiting enzymes for guanosine triphosphate (GTP) synthesis, inosine monophosphate dehydrogenase-2 (IMPDH2), is increased and IMPDH2 expression is necessary for the activation of *de novo* GTP biosynthesis. Moreover, increased IMPDH2 enhances RNA polymerase I and III transcription directly linking GTP metaboli sm to both anabolic capacity as well as nucleolar enlargement historically observed as associated with cancer. In this review, we will review in detail the basis of these new discoveries and, more generally, summarize the current knowledge on the role of GTP metabolism in cancer.

Keywords; glioblastoma, GTP, IMPDH, nucleolus, ribosome

Emerging roles of phosphatidylinositol 4-phosphate and phosphatidylinositol 4,5-bisphosphate as regulators of multiple steps in autophagy

Takashi Baba[1,2] and Tamas Balla[2]

[1]Department of Biological Informatics and Experimental Therapeutics, Graduate School of Medicine, Akita University, 1–1–1 Hondo, Akita 010–8543, Japan, [2]Section on Molecular Signal Transduction, Eunice Kennedy Shriver National Institutes of Child Health and Human Development, National Institutes of Health, 35A Convent Drive, Bethesda, MD 20892–3752, USA

Inositol phospholipids are low-abundance regulatory lipids that orchestrate diverse cellular functions in eukaryotic organisms. Recent studies have uncovered involvement of the lipids in multiple steps in autophagy. The late endosome–lysosome compartment plays critical roles in cellular nutrient sensing and in the control of both the initiation of autophagy and the late stage of eventual degradation of cytosolic materials destined for elimination. It is particularly notable that inositol lipids are involved in almost all steps of the autophagic process. In this review, we summarize how inositol lipids regulate and contribute to autophagy through the endomembrane compartments, primarily focusing on PI4P and PI(4,5)P_2.

Keywords; autophagy, late endosome/lysosome, phosphoinositide

BIOCHEMISTRY

Biochemistry General

Role of Apis cerana cerana N-terminal asparagine amidohydrolase (AccNtan1) in oxidative stress

Guangdong Zhao[1], Chen Wang[1], Ying Wang[2], Lijun Wang[1], Baohua Xu[2] and Xingqi Guo[1]

[1]State Key Laboratory of Crop Biology, College of Life Sciences, Shandong Agricultural University, Daizong Road No.61, Taian, Shandong 271018, PR China, [2]College of Animal Science and Technology, Shandong Agricultural University, Daizong Road No.61, Taian, Shandong 271018, PR China

N-Terminal asparagine amidohydrolase is a component of the ubiquitin-dependent N-end rule pathway of protein degradation that has been implicated in a variety of physiological functions, including the sensing of heme, oxygen, nitric oxide, selective elimination of misfolded proteins and the repair of DNA. We identified the *Apis cerana cerana* N-terminal asparagine amidoh ydrolase (*AccNtan1*) gene from *A. cerana cerana* and investiga ted its role in oxidation resistance. Multiple sequence alignmen

ts and phylogenetic analysis revealed that N-terminal asparagine amidohydrolase is highly conserved in insect species. Quantitative real-time polymerase chain reaction analysis indicated that the expression levels of *AccNtan1* were significantly lower in the wing, honey sac and abdomen than in other tissues and were sig nificantly higher in early stages of development, including the la rva, prepupa and pink-eyed pupa stages, than in later stages. We further observed that *AccNtan1* expression was induced by sever al environmental stressors, including aberrant temperature, H_2O_2, UV, heavy metals and pesticides. Moreover, a bacteriostatic assay suggested that overexpression of AccNtan1 enhances the resistance of bacteria to oxidative stress. In addition, knockdown of *AccNtan1* using RNA interference significantly affected the ex pression levels of most antioxidant genes and the activity levels of several antioxidant enzymes. Thus, we hypothesize that AccN tan1 plays important roles in environmental stress responses and antioxidative processes.

Keywords; Apis cerana cerana, environmental stress, N-terminal asparagine amidohydrolase, oxidative stress, RNA inference

Mangiferin alleviates endoplasmic reticulum stress in acute liver injury by regulating the miR-20a/miR-101a-Nrf2 axis

Shaoxun Li[1,2], Shuanghong Jin[1,2], Weilai Chen[3], Jiake Yu[1,4], Peipei Fang[1,4], Guangyao Zhou[1], Jie Li[1], Lingxiang Jin[1], Yiping Chen[4], Ping Chen[5,6] and Chenwei Pan[1,4]

[1]Department of Infectious Disease, The Second Affiliated Hospital and Yuying Children's Hospital of Wenzhou Medical University, No. 109 West College Road, Wenzhou 325027, Zhejiang, China, [2]Department of Infectious Disease, The Second School of Medicine, Wenzhou Medical University, No. 109 West College Road, Wenzhou 325027, Zhejiang China, [3]Department of Neurology, Wenzhou People's Hospital, No. 57 Canghou Road, Wenzhou 325000, Zhejiang, China, [4]Department of Pediatric Hepatitis and Liver Disease, The Second Affiliated Hospital and Yuying Children's Hospital of Wenzhou Medical University, No. 109 West College Road, Wenzhou 325027, Zhejiang, China, [5]Department of Infectious Disease, Shulan Hospital, No. 848 Dongxin Road, Hangzhou 310006, Zhejiang, China, [6]Department of Infectious Disease, The Third People's Hospital of Zhengzhou, No. 136 Nanshuncheng Road, Zhengzhou 450000, Henan, China

This study aimed to investigate the mechanism of mangiferin on regulating endoplasmic reticulum (ER) stress in acute liver injury. The mouse model of acute liver injury was established by injection of LPS/D-GalN. The primary mouse hepatocytes were stimulated with LPS to induce the *in vitro* model. The effect of miR-20a/101a on the luciferase activity of Nrf2 3′-UTR was ass essed by luciferase reporter assay. Mangiferin improved the liver function, inhibited the oxidative stress and ER stress and down-regulated the expressions of miR-20a and miR-101a in LPS/D-GalN-induced mice and LPS-induced hepatocytes. The knock-down of miR-20a and miR-101a co-operatively alleviated ER stress of LPS-induced hepatocytes. miR-20a and miR-101a both targeted Nrf2 and the over-expression of miR-20a or miR-101a decreased Nrf2 protein level, while their silences increased Nrf2 protein level. The silence of miR-20a and miR-101a promoted Nrf2 expression and inhibited the ER stress in LPS-induced hepatocytes, while the knockdown of Nrf2 reversed these effects. The over-expression of miR-20a and miR-101a eliminated the effects of mangiferin on Nrf2 protein level and ER stress in LPS-induced hepatocytes and Nrf2 over-expression altered these trends. Our findings suggest that mangiferin alleviates ER stress in acute liver injury by regulating the miR-20a/miR-101a-Nrf2 axis.

Keywords; acute liver injury, endoplasmic reticulum stress, mangiferin, miR-20a, miR-101a

A novel soybean protein disulphide isomerase family protein possesses dithiol oxidation activity: identifi cation and characterization of GmPDIL6

Aya Okuda[1,2], Motonori Matsusaki[1], Taro Masuda[1], Ken Morishima[3], Nobuhiro Sato[3], Rintaro Inoue[3], Masaaki Sugiyama[3] and Reiko Urade[1,2]

[1]Division of Agronomy and Horticultural Science, Graduate School of Agriculture, Kyoto University, Uji, Kyoto 611–0011, Japan, [2]Reiko Urade, Institute for Integrated Radiation and Nuclear Science, Kyoto University, Kumatori, Sennan-gun, Osaka 590–0494, Japan, [3]Institute for Integrated Radiation and Nuclear Science, Kyoto University, Kumatori, Sennan-gun, Osaka 590–0494, Japan

Secretory and membrane proteins synthesized in the endoplasmic reticulum (ER) are folded with intramolecular disulphide bonds, *viz*. oxidative folding, catalysed by the protein disulphide isomerase (PDI) family proteins. Here, we identified a novel soybean PDI family protein, GmPDIL6. GmPDIL6 has a single thioredoxin-domain with a putative *N*-terminal signal peptide and an active centre (CKHC). Recombinant GmPDIL6 forms various oligomers binding iron. Oligomers with or without iron binding and monomers exhibited a dithiol oxidase activity level comparable to those of other soybean PDI family proteins. However, they displayed no disulphide reductase and extremely low oxidative refolding activity. Interestingly, GmPDIL6 was mainly expressed in the cotyledon during synthesis of seed storage proteins and *GmPDIL6* mRNA was up-regulated under ER stress. GmPDIL6 may play a role in the formation of disulphide bonds in nascent proteins for oxidative folding in the ER.

Keywords; endoplasmic reticulum, protein disulphide isomerase, protein folding, unfolded protein response, soybean

Nano-second protein dynamics of key residue at Position 38 in catechol-O-methyltransferase system: a time-resolved fl uorescence study

Fan Liu[1] and Jianyu Zhang[1]

[1]School of Pharmaceutical Science and Technology, Tianjin University, 92 Weijin Road, Nankai District, Tianjin 300072, China

Human catechol-*O*-methyltransferase, a key enzyme related to neurotransmitter metabolism, catalyses a methyl transfer from *S*-adenosylmethionine to catechol. Although extensive studies aim to understand the enzyme mechanisms, the connection of protein dynamics and enzyme catalysis is still not clear. Here, W38in (Trp143Phe) and W38in/Y68A (Trp143Phe with Tyr68Ala) mutants were carried out to study the relationship of dynamics and catalysis in nano-second timescale using time-resolved fluorescence lifetimes and Stokes shifts in various solvents. The comprehensive data implied the mutant W38in/Y68A with lower activity is more rigid than the 'WT'−W38in, suggesting the importance of flexibility at residue 38 to maintain the optimal catalysis.

Keywords; catechol-*O*-methyltransferase, mechanism, protein dynamics, Stokes shift, time-resolved fluorescence

Protein Structure

The N-terminal amino-latch region of Hlg2 component of staphylococcal bi-component *γ*-haemolysin is dispensable for prestem release to form *β*-barrel pores

Kein Takeda[1], Yoshikazu Tanaka[2] and Jun Kaneko[1]

[1]Department of Microbial Biotechnology, Graduate School of Agricultural Science, Tohoku University, 468−1 Aramaki Aza Aoba, Aoba-ku, Sendai 980−8572, Japan, [2]Laboratory of Applied Biological Molecular Science, Graduate School of Life Sciences, Tohoku University, 2−1−1 Katahira, Aoba-ku, Sendai 980−8577, Japan

The contribution of N-terminal regions of staphylococcal bi-component *γ*-haemolysin toxin components to haemolytic activity towards human erythrocyte cells was investigated in this study. A deletion construct of N-terminal amino acids 1−10 of Hlg2 (Hlg2 ΔN10), which is the S-component protein of *γ*-haemolysin, had little effect on its haemolytic activity, whereas N-terminal 1−11 amino acid deletion (Hlg2 ΔN11) significantly delayed haemolysis. Moreover, a deletion of N-terminal amino acids 1−17 of LukF, which is the F-component protein of *γ*-haemolysin, increased its haemolytic activity in combination with either the wild-type or Hlg2 ΔN10. Unlike the N-terminal amino-latch region of staphylococcal *α*-haemolysin, which is a single component *β*-barrel pore-forming toxin, the N-terminal regions present in *γ*-haemolysin components are dispensable for the haemolytic activity of the bi-component toxin. These results strengthen our recent proposal that staphylococcal bi-component *γ*-haemolysin toxin uses an N-terminal amino-latch independent molecular switch for prestem release during the formation of *β*-barrel pores.

Keywords; amino-latch, *β*-barrel pore-forming, bi-component toxin, *γ*-haemolysin, Staphylococcus aureus

Protein Interaction and Recognition

C-terminal aromatic residue of Plasmodium ferredoxin important for the interaction with ferredoxin: NADP(H) oxidoreductase: possible involvement for artemisinin resistance of human malaria parasites

Yoko Kimata-Ariga[1], Asako Sakamoto[1], Miho Kamatani[1], Takashi Saitoh[2] and Toshiharu Hase[3]

[1]Department of Biological Chemistry, College of Agriculture, Graduate School of Sciences and Technology for Innovation, Yamaguchi University, Yoshida, Yamaguchi 753−8515, Japan, [2]Department of Medicinal Chemistry, Faculty of Pharmaceutical Sciences, Hokkaido University of Science, Maeda, Teine, Sapporo, Hokkaido 006−8585, Japan, [3]Laboratory of Regulation of Biological Reactions, Division of Protein Chemistry, Institute for Protein Research, Osaka University, Suita, Osaka 565−0871, Japan

The malaria parasite (*Plasmodium* sp.) contains a plastid-derived organelle called the apicoplast, which is essential for the grow th of the parasite. In this organelle, a redox system comprising plant-type ferredoxin (Fd) and Fd: NADP(H) oxidoreductase (FNR) supplies reducing power for the crucial metabolic pathwa ys. Electron transfer between *Plasmodium falciparum* Fd (PfFd) and FNR (PfFNR) is performed with higher affinity and specifi city than those of plant Fd and FNR. We investigated the struct ural basis for such superior protein−protein interaction by focu ssing on the *Plasumodium*-specific regions of PfFd. Significant contribution of the C-terminal region of PfFd for the electron transfer with PfFNR was revealed by exchanging the C-terminal three residues between plant Fd and PfFd. Further site-directed mutagenesis of the PfFd C-terminal residues indicated that the presence of aromatic residue at Positions 96 and 97 contributes to the lower K_m for PfFNR. Physical binding analyses using fluo rescence and calorimetric measurements supported the results. A mutation from Asp to Tyr at position 97 of PfFd was recently re ported to be strongly associated with *P. falciparum* resistance to artemisinin, the front line anti-malarial drug. Thus, the enhanced interaction of PfFd D97Y protein with PfFNR could be involved in artemisinin resistance of human malaria parasites.

Keywords; artemisinin, ferredoxin, ferredoxin: NADP(H) oxidoreductase, malaria parasite, protein−protein interaction

Enzymology

The roles of histidine and tyrosine residues in the active site of collagenase in Grimontia hollisae

Kaichi Hayashi[1], Takeaki Ikeuchi[1], Ryo Morishita[1], Jun Qian[1],

Kenji Kojima[1], Teisuke Takita[1], Keisuke Tanaka[2], Shunji Hattori[2] and Kiyoshi Yasukawa[1]

[1]Division of Food Science and Biotechnology, Graduate School of Agriculture, Kyoto University, Sakyo-ku, Kyoto 606–8502, Japan, [2]Research Institute of Biomatrix, Nippi, Incorporated, 520–11, Kuwabara, Toride, Ibaraki 302–0017, Japan

Collagenase from the *Grimontia hollisae* strain 1706B (Ghcol) is a zinc metalloproteinase with the zinc-binding motif $H^{492}E$ XXH^{496}. It exhibits higher collagen-degrading activity than the collagenase from *Clostridium histolyticum*, which is widely used in industry. We previously examined the pH and temperature dependencies of Ghcol activity; Glu493 was thought to contribute acidic pK_a (pK_{e1}), while no residue was assigned to contribute alkaline pK_a (pK_{e2}). In this study, we introduced nine single mutations at the His or Tyr residues in and near the active site. Our results showed that H412A, H485A, Y497A, H578A and H737A retained the activities to hydrolyze collagen and gelatin, while H426A, H492A, H496A and Y568A lacked them. Purification of active variants H412A, H485A, H578A and H737A, along with inactive variants H492A and H496A, were successful. H412A preferred (7-methoxycoumarin-4-yl)acetyl-L-Lys-L-Pro-L-Leu-Gly-L-Leu-[N^3-(2,4-dinitrophenyl)-L-2,3-diaminopropionyl]-L-Ala-L-Arg-NH_2 to collagen, while H485A preferred collagen to the peptide, suggesting that His412 and His485 are importa nt for substrate specificity. Purification of the active variant Y4 97A and inactive variants H426A and Y568A were unsuccessf ul, suggesting that these three residues were important for stabili ty. Based on the reported crystal structure of clostridial collagen ase, Tyr568 of Ghcol is suggested to be involved in catalysis and may be the ionizable residue for pK_{e2}.

Keywords; Clostridium histolyticum, collagen, collagenase, Grimontia hollisae, variant

Biochemistry in Diseases and Aging

Excessively activated plasminogen in human plasma cleaves VWF multimers and reduces collagen-binding activity

Kenshi Togashi[1], Satoshi Suzuki[2], Sae Morita[3], Yuki Ogasawara[4], Yasutada Imamura[1,2,3] and Yongchol Shin[1,2,3]

[1]Graduate School of Engineering, [2]Department of Applied Chemistry, Faculty of Engineering, [3]Department of Chemistry and Life Science, School of Advanced Engineering, Kogakuin University, 2665–1 Nakano, Hachioji, Tokyo 192–0015, [4]Department of Analytical Biochemistry, Meiji Pharmaceutical University, Tokyo, Japan

Plasmin (Pm) is a serine protease that can dissolve fibrin clots. Several possible functions of Pm in blood other than fibrinolysis have been proposed. To explore the effects of Pm on primary haemostasis, we evaluated the cleavage of von Willebrand factor multimers (VWFMs) in human plasma by streptokinase (SK)-activated plasminogen (Pg) and the binding ability of the digested VWFMs to collagen. SK-activated Pg and ADAMTS13 (a VWF-cleaving enzyme) in human plasma cleaved VWFMs in conformation-dependent manners through dialysis to the urea-containing buffer. However, VWFMs in human plasma under vortex-based shear stress were cleaved by SK-activated Pg but not by ADAMTS13. These results suggested that the VWFM-cleavage sites in human plasma are exposed to some extent by vortex-based shear stress for Pm but not for ADAMTS13. Additionally, we revealed that cleavage by SK-activated Pg reduced VWFMs' binding ability to collagen, and VWFMs in human plasma were cleaved by Pm at several sites. These results suggest that SK-activated Pg degrades VWFMs, reduces their binding abilities to collagen and affects primary haemostasis. Because excessive Pg activation can degrade fibrinogen/fibrin, we propose that SK-activated Pg in blood may cause impaired primary and secondary haemostasis.

Keywords; ADAMTS13, fibrinogen, plasmin, thrombotic thrombocytopenic purpura, von Willebrand factor

BIOTECHNOLOGY

Bioactive Substances

Non-electrophilic TRPA1 agonists, menthol, carvacrol and clotrimazole, open epithelial tight junctions via TRPA1 activation

Minagi Mukaiyama[1], Takeo Usui[2] and Yoko Nagumo[3,4]

[1]Graduate School, [2]Faculty of Life and Environmental Sciences Microbiology Research Center for Sustainability (MiCS), [3]Faculty of Life and Environmental Sciences Alliance for Research on the Mediterranean and North Africa (ARENA), University of Tsukuba, Tsukuba, Ibaraki 305–8572, Japan

Activation of the transient receptor potential A1 channel (TRPA1) by electrophilic agonists was reported to induce the opening of tight junctions (TJs). Because compounds that increase TJ permeability can be paracellular permeability enhancers, we investigated the effect of non-electrophilic TRPA1 activators, including food ingredients (menthol and carvacrol) and medication (clotrimazole), on epithelial permeability. We show that all three compounds induced increase of the permeability of fluorescein isothiocyanate-conjugated dextran (4 kDa) and decrease of transepithelial electrical resistance, accompanied by Ca^{2+} influx and cofilin activation in epithelial MDCK II monolayers. These phenotypes were attenuated by pretreatment of a TRPA1 antagonist, suggesting TRPA1-mediated opening of TJs. These results suggest that non-electrophilic TRPA1 activators with established safety can be utilized to regulate epithelial barriers.

Keywords; carvacrol, clotrimazole, menthol, tight junction, TRPA1

Immunological Engineering

Site-specifi c epitope insertion into recombinant proteins using the MAP tag system

Ayami Wakasa[1], Mika K Kaneko[2], Yukinari Kato[2,3], Junichi Takagi[1] and Takao Arimori[1]

[1]Laboratory of Protein Synthesis and Expression, Institute for Protein Research, Osaka University, 3–2 Yamadaoka, Suita, Osaka 565–0871, Japan, [2]Department of Antibody Drug Development, Tohoku University Graduate School of Medicine, [3]New Industry Creation Hatchery Center, Tohoku University, 2–1 Seiryo-machi, Aoba-ku, Sendai, Miyagi 980–8575, Japan

The MAP tag system comprises a 14-residue peptide derived from mouse podoplanin and its high-affinity monoclonal antibody PMab-1. We determined the crystal structure of PMab-1 complexed with the MAP tag peptide and found that the recognition required only the N-terminal 8 residues of MAP tag sequence, enabling the shortening of the tag length without losing the affinity for PMab-1. Furthermore, the structure illustrated that the MAP tag adopts a U-shaped conformation when bound by PMab-1, suggesting that loop-inserted MAP tag would assume conformation compatible with the PMab-1 binding. We inserted the 8-residue MAP tag into multiple loop regions in various proteins including fibronectin type III domain and G-protein-coupled receptors and tested if they maintain PMab-1 reactivity. Despite the conformational restraints forced by the insertion position, all MAP-inserted mutants were expressed well in mammalian cells at levels comparable to the non-tagged proteins. Furthermore, the binding by PMab-1 was fully maintained even for the mutant where MAP tag was inserted at a structurally restricted β-hairpin, indicating that the MAP tag system has unique feature that allows placement in the middle of protein domain at desired locations. Our results indicate the versatile utility of the MAP tag system in 'site-specific epitope insertion' application.

Keywords; antibody, epitope tag system, flow cytometry, G-protein-coupled receptor, X-ray crystallography

Journal of Biochemistry

Vol.168, No.5（2020 年 11 月 発 行）

ダイジェスト

JB Review

Posttranscriptional modifi cations in mitochondrial tRNA and its implication in mitochondrial translation and disease

Tomizawa Kazuhito[1] and Fan-Yan Wei[1,2]

[1]Department of Molecular Physiology, Faculty of Life Sciences, Kumamoto University, Honjo 1–1–1, Chuo-ku, Kumamoto-shi, Kumamoto 860–8556, Japan, [2]Department of Modomics Biology and Medicine, Institute of Development, Aging and Cancer, Tohoku University, Seriyo-machi 4–1, Aoba-ku, Sendai-shi, Miyagi 980–8575, Japan

A fundamental aspect of mitochondria is that they possess DNA and protein translation machinery. Mitochondrial DNA encodes 22 tRNAs that translate mitochondrial mRNAs to 13 polypeptides of respiratory complexes. Various chemical modifications have been identified in mitochondrial tRNAs via complex enzymatic processes. A growing body of evidence has demonstrated that these modifications are essential for translation by regulating tRNA stability, structure and mRNA binding, and can be dynamically regulated by the metabolic environment. Importantly, the hypomodification of mitochondrial tRNA due to pathogenic mutations in mitochondrial tRNA genes or nuclear genes encoding modifying enzymes can result in life-threatening mitochondrial diseases in humans. Thus, the mitochondrial tRNA modification is a fundamental mechanism underlying the tight regulation of mitochondrial translation and is essential for life. In this review, we focus on recent findings on the physiological roles of 5-taurinomethyl modification (herein referred as taurine modification) in mitochondrial tRNAs. We summarize the findings in human patients and animal models with a deficiency of taurine modifications and provide pathogenic links to mitochondrial diseases. We anticipate that this review will help understand the complexity of mitochondrial biology and disease.

Keywords; Mitochondrial disease, MELAS, MERFF, taurine, tRNA modification

BIOCHEMISTRY

Biochemistry General

RNF8 induces autophagy and reduces infl ammation by promoting AKT degradation via ubiquitination in ulcerative colitis mice

Yu Zhu[1], Yan Shi[2], Xiquan Ke[1], Lanlan Xuan[3] and Zhenzeng Ma[1]

[1]Department of Gastroenterology, [2]Department of Ultrasonic Medicine, The First Affiliated Hospital of Bengbu Medical College, No.287 Changhuai Road, Longzihu District, Bengbu, Anhui 233004, China, [3]Department of Pathology, Anqing Hospital Affiliated to Anhui Medical University, Anqing Municipal Hospital, No.352 Renmin Road, Yingjiang District, Anqing, Anhui 246003, China

RING finger protein 8 (RNF8) is an E3 ligase that is pivotal for DNA repair. However, the role of RNF8 in ulcerative colitis (UC) remains unclear. The aim of this study is to investigate the effect and the mechanism of RNF8 on UC model induced by trinitrobenzene sulfonic acid (TNBS) in mice. Lentiviruses overexpressing RNF8 were injected into mice after the induction of UC. The histopathological changes in colon tissues were as-

sessed by haematoxylin and eosin staining. The mRNA level of RNF8 was detected by real-time quantitative polymerase chain reaction. The protein levels of RNF8, autophagy-related proteins (LC3 and P62) and AKT/mammalian target of rapamycin (mTOR) signalling-related proteins were measured by Western blot. The pro-inflammatory cytokines (tumour necrosis factor-α and interleukin-1β) were examined by immunohistochemical analysis. Immunoprecipitation was performed to analyse the interaction between RNF8 and AKT1. The TNBS-induced UC mice exhibited colonic damage and inflammation, accompanied by decreased RNF8 expression, impaired autophagy and increased phosphorylation levels of AKT and mTOR in the colon. However, these alterations were reversed by RNF8 overexpression. Furthermore, RNF8 bound to AKT1 and mediated its ubiquitination. Collectively, RNF8 overexpression protects against TNBS-induced UC, which might be due to its enhancement of autophagy by suppressing the AKT/mTOR signalling via AKT1 ubiquitination.

Keywords; AKT/mTOR, autophagy, RNF8, ubiquitination, ulcerative colitis

Protein Interaction and Recognition

A second hybrid-binding domain modulates the activity of Drosophila ribonuclease H1

Jose M González de Cózar[1], Maria Carretero-Junquera[1], Grzegorz L Ciesielski[1,2,3], Sini M Miettinen[4], Markku Varjosalo[4], Laurie S Kaguni[1,2], Eric Dufour[1] and Howard T Jacobs[1]

[1]Faculty of Medicine and Health Technology, FI-33014 Tampere University, Finland, [2]Department of Biochemistry and Molecular Biology, Michigan State University, East Lansing, MI 48824, USA, [3]Department of Chemistry, Auburn University at Montgomery, Montgomery, AL 36117, USA, [4]Institute of Biotechnology, FI-00014 University of Helsinki, Finland

In eukaryotes, ribonuclease H1 (RNase H1) is involved in the processing and removal of RNA/DNA hybrids in both nuclear and mitochondrial DNA. The enzyme comprises a C-terminal catalytic domain and an N-terminal hybrid-binding domain (HBD), separated by a linker of variable length, 115 amino acids in *Drosophila melanogaster* (*Dm*). Molecular modelling predicted this extended linker to fold into a structure similar to the conserved HBD. Based on a deletion series, both the catalytic domain and the conserved HBD were required for high-affinity binding to heteroduplex substrates, while loss of the novel HBD led to an ~90% drop in K_{cat} with a decreased K_M, and a large increase in the stability of the RNA/DNA hybrid-enzyme complex, supporting a bipartite-binding model in which the second HBD facilitates processivity. Shotgun proteomics following *in vivo* cross-linking identified single-stranded DNA-binding pro teins from both nuclear and mitochondrial compartments, resp ectively RpA-70 and mtSSB, as prominent interaction partners of *Dm* RNase H1. However, we were not able to document direct and stable interactions with mtSSB when the proteins were co-overexpressed in S2 cells, and functional interactions between them *in vitro* were minor.

Keywords; biolayer interferometry, mitochondria, ribonuclease H, shotgun proteomics, single-stranded DNA-binding protein

LncRNA PCAT18/miR-301a/TP53INP1 axis is involved in gastric cancer cell viability, migration and invasion

Jin Dou[1], Daoyuan Tu[1], Haijian Zhao[1] and Xiaoyu Zhang[1]

[1]Department of General Surgery, Huai'an Second People's Hospital and the Affiliated Huai'an Hospital of Xuzhou Medical University, 62 Huaihai South Road, Huai'an, Jiangsu 223001, PR China

MiR-301a is as an oncogene involved in the regulation of gastric cancer (GC) progression, but the underlying mechanism is unclear. This study was to explore the lncRNA PCAT18/miR-301a/TP53INP1 axis in regulating the GC cell proliferation and metastasis. In the present study, GC tissues and cell lines were collected for the detection of PCAT18 expression. Herein, we found that PCAT18 is significantly decreases in human GC tissues and five GC cell lines. Overexpression of PCAT18 inhibits cell viability, invasion and migration of GC cells and tumour growth of GC xenograft tumours. PCAT18 negatively regulates the expression level of miR-301a. The interaction between PCAT18 and miR-301a is confirmed by RIP and RNA pull down. MiR-301a mimic increases cell viability and promotes cell migration and invasion and reverses the inhibitory action of PCAT18. TP53INP1 expression is negatively regulated by miR-301a and TP53INP1/miR-301a is involved in GC viability, migration and invasion. The promoting of PCAT18 on TP53INP1 expression is abolished by miR-301a overexpression. In conclusion, lncRNA PCAT18 acts as a tumour suppressor for GC and lncRNA PCAT18, miR-301a and TP53INP1 comprise a signal axis in regulating GC cell proliferation, migration and invasion.

Keywords; gastric cancer, lncRNA PCAT18, miR-301a, TP53INP1

Lipid Biochemistry

Arachidonate 12S-lipoxygenase of platelet-type in hepatic stellate cells of methionine and choline-defi cient diet-fed mice

Yoshiko Mori[1], Yuki Kawakami[1], Keita Kanzaki[1,2], Akemi Otsuki[1], Yuka Kimura[1], Hibiki Kanji[1], Ryoma Tanaka[1], Izumi Tsukayama[1], Nana Hojo[1], Toshiko Suzuki-Yamamoto[1], Takayo Kawakami[1] and Yoshitaka Takahashi[1]

[1]Department of Nutritional Science, Faculty of Health and Welfare Science, Okayama Prefectural University, 111 Kuboki, Soja, Okayama 719–1197, Japan, [2]Department of Clinical Nutrition, Faculty of Health Science and Technology, Kawasaki Univer-

sity of Medical Welfare, 288 Matsushima, Kurashiki, Okayama 701–0193, Japan

A role of 12-lipoxygenase in the progression of non-alcoholic steatohepatitis (NASH) is suggested, although the underlying mechanism is not entirely understood. The catalytic activity of 12*S*-lipoxygenase that was hardly observed in liver cytosol of normal chow-fed mice was clearly detectable in that of NASH model mice prepared by feeding a methionine and choline-deficient (MCD) diet. The product profile, substrate specificity and immunogenicity indicated that the enzyme was the platelet-type isoform. The expression levels of mRNA and protein of platelet-type 12*S*-lipoxygenase in the liver of MCD diet-fed mice were significantly increased compared with those of normal chow-fed mice. Immunohistochemical analysis showed that platelet-type 12*S*-lipoxygenase colocalized with *α*-smooth muscle actin as well as vitamin A in the cells distributing along liver sinusoids. These results indicate that the expression level of platelet-type 12*S*-lipoxygenase in hepatic stellate cells was increased during the cell activation in MCD diet-fed mice, suggesting a possible role of the enzyme in pathophysiology of liver fibrosis.

Keywords; *α*-smooth muscle actin, arachidonic acid, hepatic st ellate cells, non-alcoholic steatohepatitis (NASH), platelet-type 12S-lipoxygenase

Enzymology

Molecular characterization of a prolyl endopeptidase from a feather-degrading thermophile Meiothermus ruber H328

Fumi Yamamoto[1], Hironobu Morisaka[1], Mitsuyoshi Ueda[1] and Kunihiko Watanabe[1]

[1]Division of Applied Life Sciences, Graduate School of Life and Environmental Sciences, Kyoto Prefectural University, Shimogamo, Sakyo, Kyoto 606–8522, Japan

Prolyl endopeptidase from an aerobic and Gram-negative thermophile *Meiothermus ruber* H328 (MrPEP) was purified in native and recombinant forms, but both preparations had comparable characteristics. Production of the native MrPEP was increased 10-fold by adding intact chicken feathers. The gene for MrPEP (mrH_2860) was cloned from the genome of strain H328 and found to have no signal sequence at the N-terminus. MrPEP is composed of two major domains: the *β*-propeller domain and the peptidase domain with a typical active site motif and catalytic triad. Based on extensive investigations with different types of peptide substrates and FRETS-25Xaa libraries, MrPEP showed strict preferences for Pro residue at the P1 position but broader preferences at the P2 and P3 positions in substrate specificity with stronger affinity for residues at the P3 position of substrate peptides that are longer than four residues in length. In conclusion, the molecular characterization of MrPEP resembles its animal counterparts more closely than bacterial counterparts in function and structure.

Keywords; Meiothermus ruber, peptidase, prolyl endopeptidase, prolyl oligopeptidase, serine protease

Methylated derivatives of L-tyrosine in reaction catalyzed by L-amino acid oxidase: isotope and inhibitory effects

Małgorzata Pająk[1]

[1]Faculty of Chemistry, University of Warsaw, Pasteur 1 Str, 02–093 Warsaw, Poland

L-Amino acid oxidase (LAAO) is widely distributed in nature and shows important biological activity. It induces cell apoptosis and has antibacterial properties. This study was designed to investigate the effect of methyl substituent on its activity as methylated derivatives of L-tyrosine, labelled with short-lived B^+ em itters, have been used in oncological diagnostics. To study isoto pe effects in the oxidative deamination of *O*-methyl-L-tyrosine, the deuterated isotopomer, *i.e. O*-methyl-[2-^{2}H]-L-tyrosine, was synthesized by isotope exchange, catalyzed enzymatically by tryptophanase. Isotope effects were determined using the spectrophotometric non-competitive method. The values of isotope effects indicate that the *α*-C–H bond cleavage occurs in the rate determining step of the investigated reaction and *α*-hydrogen plays a role in the substrate binding process at the enzyme active site. The inhibitory effect on LAAO activity was studied with *α* -methyl-L-tyrosine and *N*-methyl-L-tyrosine. The mode of inhibition was determined based on Lineweavear–Burk plots intersections. *α*-Methyl-L-tyrosine has been found a mixed type inhibitor of the investigated enzyme, whereas *N*-methyl-L-tyrosine is a non-competitive inhibitor of LAAO.

Keywords; *α*-methyl-L-tyrosine, isotope effects, L-amino acid ox idase inhibition, *N*-methyl-L-tyrosine, *O*-methyl-L-tyrosine

P219L substitution in human D-amino acid oxidase impacts the ligand binding and catalytic effi ciency

Wanitcha Rachadech[1,2], Yusuke Kato[1], Rabab M Abou El-Magd[1], Yuji Shishido[1], Soo Hyeon Kim[1], Hirofumi Sogabe[1], Nobuo Maita[3], Kazuko Yorita[1] and Kiyoshi Fukui[1]

[1]Division of Enzyme Pathophysiology, Institute for Enzyme Research, Tokushima University, 3–18–15 Kuramoto, Tokushima 770–8503, Japan, [2]Division of Chemistry, Faculty of Science, Udon Thani Rajabhat University, 64 Thahan Road, Muang, Udon Thani 41000, Thailand, [3]Division of Disease Proteomics, Institute for Enzyme Research, Tokushima University, 3–18–15 Kuramoto, Tokushima 770–8503, Japan

Human D-amino acid oxidase (DAO) is a flavoenzyme that is implicated in neurodegenerative diseases. We investigated the impact of replacement of proline with leucine at Position 219 (P219L) in the active site lid of human DAO on the structural and enzymatic properties, because porcine DAO contains leucine at the corresponding position. The turnover numbers (k_{cat}) of P219L were unchanged, but its K_m values decreased compar

ed with wild-type, leading to an increase in the catalytic efficien cy (k_{cat}/K_m). Moreover, benzoate inhibits P219L with lower K_i va lue (0.7–0.9 μM) compared with wild-type (1.2–2.0 μM). Crystal structure of P219L in complex with flavin adenine dinucleotide (FAD) and benzoate at 2.25 Å resolution displayed conformatio nal changes of the active site and lid. The distances between the H-bond-forming atoms of arginine 283 and benzoate and the rel ative position between the aromatic rings of tyrosine 224 and be nzoate were changed in the P219L complex. Taken together, the P219L substitution leads to an increase in the catalytic efficien cy and binding affinity for substrates/inhibitors due to these stru ctural changes. Furthermore, an acetic acid was located near the adenine ring of FAD in the P219L complex. This study provides new insights into the structure–function relationship of human DAO.

Keywords; human D-amino acid oxidase, point-mutation, active site lid, structure–function relationship, X-ray crystallography

MOLECULAR BIOLOGY

Molecular Biology General

Long non-coding RNA ABHD11-AS1 boosts gastric cancer development by regulating miR-361-3p/PDPK1 signalling

Hairong Xin[1], Zhifeng Yan[1] and Jie Cao[2]

[1]Department of General Surgery, Shanxi Provincial Cancer Hospital, Taiyuan, Shanxi 030013, China, [2]Department of Cancer, Jiangsu Cancer Hospital & Jiangsu Institute of Cancer Research & The Affiliated Cancer Hospital of Nanjing Medical University, No. 42 Baiziting, Nanjing, Jiangsu 210009, China

Gastric cancer (GC) is one of the most common cancers in gastrointestinal malignant tumours. Long non-coding RNAs were widely reported to play a significant role in the regulation of occurrence or development of tumours. Bioinformatics analysis and a wide range of experiments were conducted to explore the expression status, specific function and molecular mechanism of long non-coding RNA ABHD11 antisense RNA 1 (ABHD11-AS1). ABHD11-AS1 knockdown repressed cell proliferation but enhanced cell apoptosis in function. We proved that miR-361-3p directly combines with the 3′wUTR of PDPK2 and ABHD11-AS1 cooperated with miR-361-3p to modulate PDPK2 mRNA and protein levels. Rescue assays confirmed that the miR-361-3p silence reversed the suppressive effect of ABHD11-AS1 deficiency. In summary, ABHD11-AS1 boosts GC development by regulating miR-361-3p/PDPK1 signalling.

Keywords; ABHD11-AS1, gastric cancer, miR-361-3p, PDPK1

Exosomes derived from chronic lymphocytic leukaemia cells transfer miR-146a to induce the transition of mesenchymal stromal cells into cancer-associated fi broblasts

Yanli Yang[1], Jun Li[1] and Yinghua Geng[1]

[1]Department of Hematology, The First Affiliated Hospital of Bengbu Medical College, No. 287 Changhuai Road, Bengbu 233004, Anhui Province, China

Chronic lymphocytic leukaemia (CLL) is the most prevalent leukaemia and remains incurable. Mesenchymal stem cells (MSCs) can promote tumour progression by differentiating into cancer-associated fibroblasts (CAFs). However, the mechanisms by which tumour cells induce the transition of MSCs to CAFs are still largely undefined. Exosomes can regulate recipient cellular function by mediating intracellular communication. This study aimed to investigate whether CLL cells regulate the transition of bone marrow-derived MSCs (BM-MSCs) to CAFs via exosomal miR-146a delivery. The exosomes were isolated from CLL cell line MEC-1 (CLL-Exo) and then co-cultured with BM-MSCs. The expression of α-smooth muscle actin (α-SMA) and fibroblast-activated protein (FAP) were determined by immunofluorescence, quantitative real-time polymerase chain reaction and western blot. A luciferase reporter assay was performed to verify whether ubiquitin-specific peptidase 16 (USP16) was a target of miR-146a. CLL-Exo treatment up-regulated miR-146a and down-regulated expression of CAF markers (α-SMA and FAP) and USP16. The inducing effect of CLL-Exo on CAF marker expression was compromised when miR-146a expression was inhibited in CLL-Exo. USP16 was confirmed as a direct target of miR-146a and USP16 overexpression in BM-MSCs abrogated the CLL-Exo-mediated up-regulation of CAF markers. Collectively, CLL-Exo delivered miR-146a into BM-MSCs where miR-146a mediated transition of BM-MSCs into CAFs by targeting USP16.

Keywords; cancer-associated fibroblasts, chronic lymphocytic leukaemia, mesenchymal stem cells, miR-146a, ubiquitin-specific peptidase 16

LncRNA HCG18 contributes to the progression of hepatocellular carcinoma via miR-214-3p/CENPM axis

Yuepei Zou[1], Zhonghua Sun[2] and Shuangming Sun[3]

[1]Zhengzhou University, No. 100 Science Ave, Gaoxin District, Zhengzhou 450001, China, [2]Medical Department, Affiliated Hospital of Shandong University of Traditional Chinese Medicine, No. 16369 Jingshi Road, Jinan 250011, China, [3]Security and Anti-Terrorism Department, People's Public Security University of China, No. 1 Muxidinanli, Xicheng District, Beijing 100038, China

Long non-coding RNA (lnc) HCG18 has been reported to contribute progression of a variety of tumours. However, its roles in hepatocellular carcinoma (HCC) remains unknown. In the current study, we intended to uncover the biological functions of HCG18 in HCC. Quantitative real-time polymerase chain reaction (qRT-PCR) was conducted to detect the expression of HCG18, microRNA-214-3p (miR-214-3p) and centromere protein M (CENPM) messenger RNA (mRNA). The role of HCG18

in the growth and migration were assessed by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay, colony formation assay, wound healing assay and flow cytometry *in vitro* and animal experiments *in vivo*. The results showed that HCG18 was highly expressed in HCC tissues. HCG18 silencing inhibited the proliferation and migration while induced the apoptosis of HCC cells. Besides, miR-214-3p was down-regulated in HCC cells. Further experiments revealed that miR-214-3p could directly bind to HCG18 and exerted an anti-tumour role to counteracted siHCG18-1-mediated influence in HCC cells. Moreover, miR-214-3p could directly interact with CENPM mRNA and down-regulating the expression of CENPM. While HCG18 could up-regulate the expression of CENPM through acting as a sponge of miR-214-3p. Therefore, those results suggested HCG18 functioned as an oncogene to promote the proliferation and migration of HCC cells via miR-214-3p/CENPM axis.

Keywords; hepatocellular carcinoma, HCG18, miR-214-3p, CENPM, progression

CELL

Cell Cycle

Nuclear accumulation of ZFP36L1 is cell cycle-dependent and determined by a C-terminal serine-rich cluster

Yuki Matsuura[1], Aya Noguchi[1], Shunsuke Sakai[1], Naoto Yokota[1] and Hiroyuki Kawahara[1]

[1]Laboratory of Cell Biology and Biochemistry, Department of Biological Sciences, Tokyo Metropolitan University, Tokyo 192–0397, Japan

ZFP36L1 is an RNA-binding protein responsible for mRNA decay in the cytoplasm. ZFP36L1 has also been suggested as a nuclear-cytoplasmic shuttling protein because it contains a potential nuclear localization signal and a nuclear export signal. However, it remains unclear how the nuclear localization of ZFP36L1 is controlled. In this study, we provide evidence that the nuclear accumulation of ZFP36L1 protein is modulated in a cell cycle-dependent manner. ZFP36L1 protein accumulation in fractionated nuclei was particularly prominent in cells arrested at G1-/S-phase boundary, while it was downregulated in S-phase cells, and eventually disappeared in G2-phase nuclei. Moreover, forced nuclear targeting of ZFP36L1 revealed marked down-regulation of this protein in S- and G2-phase cells, suggesting that ZFP36L1 can be eliminated in the nucleus. The C-terminal serine-rich cluster of ZFP36L1 is critical for the regulation of its nuclear accumulation because truncation of this probable disordered region enhanced the nuclear localization of ZFP36L1, increased its stability and abolished its cell cycle-dependent fluctuations. These findings provide the first hints to the question of how ZFP36L1 nuclear accumulation is controlled during the course of the cell cycle.

Keywords; CCCH-zinc finger domain, cell cycle, intronic ARE, nuclear RNA-binding protein, ZFP36