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ダイジェスト

JB Reviews

The nucleolus from a liquid droplet perspective

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The nucleolus is a membrane-less organelle sequestered from the nucleus by liquid droplet formation through a liquid–liquid phase separation (LLPS). It plays important roles in cell homoeostasis through its internal thermodynamic changes. Reversible nucleolar transitions between coalescence and dispersion are dependent on the concentrations, conformations and interactions of its molecular liquid droplet-forming components, including DNA, RNA and protein. The liquid droplet-like properties of the nucleolus enable its diverse dynamic roles. The liquid droplet formation mechanism, by which the nucleolus is sequestered from the nucleoplasm despite the absence of a membrane, explains a number of complex nucleolar functions.

Keywords: liquid droplet, nucleolus, ribosome, heterochromatin, Pol I

Emerging solvatochromic push–pull dyes for monitoring the lipid order of biomembranes in live cells

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Solvatochromic dyes have emerged as a new class of fluorescent probes in the field of lipid membranes due to their ability to identify the lipid organization of biomembranes in live cells by changing the colour of their fluorescence. This type of solvatochromic function is useful for studying the heterogeneous features of biomembranes caused by the uneven distribution of lipids and cholesterols in live cells and related cellular processes. Therefore, a variety of advanced solvatochromic dyes have been rapidly developed over the last decade. To provide an overview of the works recently developed solvatochromic dyes have enabled, we herein present some solvatochromic dyes, with a particular focus on those based on pyrene and Nile red. As these dyes exhibit preferable photophysical properties in terms of fluorescence microscopy applications and unique distribution/localization in cellular compartments, some have already found applications in cell biological and biophysical studies. The goal of this review is to provide information to researchers who have never used solvatochromic dyes or who have not discovered applications of such dyes in biological studies.

Keywords: biomembranes, fluorescence imaging, lipid order, polarity, solvatochromic dyes

BIOCHEMISTRY

Biochemistry General

The protein N-terminal acetyltransferase A complex contributes to yeast mitophagy via promoting expression and phosphorylation of Atg32

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Mitophagy is an evolutionarily conserved catabolic process that selectively degrades damaged or superfluous mitochondria via autophagy. Although mitophagy is considered to be critical to maintain cellular homeostasis, detailed mechanisms of mitophagy remain largely unknown. In the budding yeast *Saccharomyces cerevisiae*, the protein N-terminal acetyltransferase A (NatA) complex is important for transcriptional induction of the pro-mitophagic factor Atg32 and efficient degradation of mitochondria under prolonged respiratory conditions. Overexpression of Atg32 only partially recovers mitophagy in cells lacking NatA, raising the possibility that NatA may contribute to mitophagy via additional mechanisms. Here, we demonstrate that Atg32 phosphorylation, which is required for facilitating mitophagy, is altered in respiring NatA-deficient cells. Hyperphosphorylation of Atg32 partially rescues mitophagy in cells lacking NatA. Notably, mitophagy is mostly restored in NatA-null cells overexpressing hyperphosphorylated Atg32. Loss of NatA does not impair the interaction of phosphorylated Atg32 with Atg11, a scaffold protein critical for selective autophagy, suggesting that NatA-dependent Atg32 phosphorylation promotes mitophagy independently of Atg32–Atg11 interactions. We propose that NatA-mediated protein N-terminal acetylation acts in Atg32 expression and phosphorylation to drive mitophagy.

Keywords: Atg32, autophagy, mitochondria, NatA, Ppg1, yeast

MiR-142–5p promotes retinoblastoma cell proliferation, migration and invasion by targeting PTEN

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The study intends to probe the functions of miR-142-5p in retinoblastoma (RB) and the relationship between miR-142-5p and phosphatase and tensin homolog deleted on chromosome ten (PTEN). In our study, miR-142-5p and PTEN mRNA expression in RB tissue, serum of RB patients and RB cell lines were investigated by quantitative real-time polymerase chain reaction (qRT-PCR). The proliferation, migration, invasion and cell apoptosis were measured using MTT assay, BrdU assay, Transwell experiments and flow cytometry analysis, respectively. Binding sites between miR-142-5p and PTEN were predicted by the TargetScan database and were confirmed via qRT-PCR, western blot and dual-luciferase reporter gene assay. It was demonstrated that miR-142-5p expression was elevated in RB tissue, serum of RB patients and RB cell lines. MiR-142-5p overexpression remarkably promoted the proliferation, migration, invasion and inhibited the apoptosis of WERI-RB-1 cells while miR-142-5p knockdown induced opposite effects in Y79 cells. MiR-142-5p decreased PTEN expression in both mRNA and protein expression levels, and PTEN was identified as a target gene of miR-142-5p. Cotransfection of PTEN overexpression plasmids reversed the influences of miR-142-5p on RB cells. In conclusion, miR-142-5p enhances proliferation, migration and invasion of RB cell by targeting PTEN.

Keywords: miR-142-5p, PTEN, retinoblastoma

Direct and specific binding of cholesterol to the mitochondrial translocator protein (TSPO) using PhotoClick cholesterol analogue

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The translocator protein (TSPO) is a five-helix transmembrane protein localized to the outer mitochondria membrane. Radioligand binding assays and chemical crosslinking showed TSPO to be a high affinity cholesterol-binding protein. In this report, we show that TSPO in mitochondrial fractions from MA-10 mouse tumour Leydig cells can interact directly and competitively with the clickable photoreactive cholesterol analogue. PhotoClick cholesterol showed saturable photoaffinity labelling of TSPO that could be specifically immunoprecipitated with anti-TSPO antibody, following the click reaction with the fluorescent-azide probe, tetramethylrhodamine (TAMRA)-azide. Moreover, excess cholesterol reduced the photolabelling of both total mitochondrial proteins and TSPO. Together, the results of this study demonstrated direct binding of PhotoClick cholesterol to TSPO and that this interaction occurs at physiologically relevant site(s).

Keywords: cholesterol, click chemistry, photoaffinity labelling, translocator protein (TSPO)

Development of biparatopic bispecific antibody possessing tetravalent scFv-Fc capable of binding to ROBO1 expressed in hepatocellular carcinoma cells

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There is no standard structural format of the biparatopic bispecific antibody (bbsAb) which is used against the target molecule because of the diversity of biophysical features of bispecific antibodies (bsAbs). It is therefore essential that the interaction between the antibody and antigen is quantitatively analyzed to design antibodies that possess the desired properties. Here, we generated bsAbs, namely, a tandem scFv-Fc, a diabody-Fc, and an immunofusion–scFv-Fc–scFv, that possessed four scFv arms at different positions and were capable of recognizing the extracellular domains of ROBO1. We examined the interactions between these bsAbs and ROBO1 at the biophysical and cellular levels. Of these, immunofusion–B2212A scFv-Fc–B5209B scFv was stably expressed with the highest relative yield. The kinetic and thermodynamic features of the interactions of each bsAb with soluble ROBO1 (sROBO1) were validated using surface plasmon resonance and isothermal titration calorimetry. In all bsAbs, the immunofusion–scFv-Fc–scFv format showed homogeneous interaction with the antigen with higher affinity compared with that of monospecific antibodies. In conclusion, our study presents constructive information to design druggable bbsAbs in drug applications.

Keywords: antibody engineering, biophysical chemistry, bispecific antibody, hepatocellular carcinoma, ROBO1

Protein Structure

Detailed structure of mouse interferon α2 and its interaction with Sortilin

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Interferon α (IFNα) is a type I interferon, an essential cytokine employed by the immune system to fight viruses. Although a number of the structures of type I interferons have been reported, most of the known structures of IFNα are in complex with its receptors. There are only two examples of structures of free IFNα: one is a dimeric X-ray structure without side-chain information; and another is an NMR structure of human IFNα. Although we have shown that Sortilin is involved in the secretion of IFNα, the details of the molecular interaction and the secretion mechanism remain unclear. Recently, we solved the X-ray structure of mouse Sortilin, but the structure of mouse IFNα remained unknown. In this study, we determined the crystal structure of mouse IFNα2 at 2.1 Å resolution and investigated its interaction with Sortilin. Docking simulations suggested that Arg22 of mouse IFNα2 is important for the interaction with mouse Sortilin. Mutation of Arg22 to alanine facilitated IFNα2 secretion, as determined by flow cytometry, highlighting the contribution of this residue to the interaction with Sortilin. These results suggest an important role for Arg22 in mouse IFNα for Sortilin-mediated IFNα trafficking.

Keywords: cytokine secretion, interaction, interferon-alpha, simulation, x-ray structure

Glycobiology and Carbohydrate Biochemistry

PCYT2 synthesizes CDP-glycerol in mammals and reduced PCYT2 enhances the expression of functionally glycosylated α-dystroglycan

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α-Dystroglycan (α-DG) is a highly glycosylated cell-surface protein. Defective *O*-mannosyl glycan on α-DG is associated with muscular dystrophies and cancer. In the biosynthetic pathway of the *O*-mannosyl glycan, fukutin (FKTN) and fukutin-related protein (FKRP) transfer ribitol phosphate (RboP). Previously, we reported that FKTN and FKRP can also transfer glycerol phosphate (GroP) from CDP-glycerol (CDP-Gro) and showed the inhibitory effects of CDP-Gro on functional glycan synthesis by preventing glycan elongation *in vitro*. However, whether mammalian cells have CDP-Gro or associated synthetic machinery has not been elucidated. Therefore, the function of CDP-Gro in mammals is largely unknown. Here, we reveal that cultured human cells and mouse tissues contain CDP-Gro using liquid chromatography tandem–mass spectrometry (LC–MS/MS). By performing the enzyme activity assay of candidate recombinant proteins, we found that ethanolamine-phosphate cytidylyltransferase (PCYT2), the key enzyme in *de novo* phosphatidylethanolamine biosynthesis, has CDP-Gro synthetic activity from glycerol-3-phosphate (Gro3P) and CTP. In addition, knockdown of PCYT2 dramatically reduced cellular CDP-Gro. These results indicate that PCYT2 is a CDP-Gro synthase in mammals. Furthermore, we found that the expression of functionally glycosylated α-DG is increased by reducing PCYT2 expression. Our results suggest an important role for CDP-Gro in the regulation of α-DG function in mammals.

Keywords: α-dystroglycan, CDP-glycerol, cytidylyltransferase, O-mannosyl glycan, PCYT2

Enzymology

Sequential conformational changes in transmembrane domains of presenilin 1 in Aβ42 downregulation

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Alzheimer disease (AD) is the most common neurodegenerative disease worldwide. AD is pathologically characterized by the deposition of senile plaques in the brain, which are composed of an amyloid-β peptide (Aβ) that is produced through the multistep cleavage of amyloid precursor protein (APP) by γ-secretase. γ-Secretase is a membrane protein complex, which includes its catalytic subunit presenilin 1 (PS1). However, much about the structural dynamics of this enzyme remain unclear. We have previously demonstrated that movements of the transmembrane domain (TMD) 1 and TMD3 of PS1 are strongly associated with decreased production of the Aβ peptide ending at the 42nd residue (*i.e.* Aβ42), which is the aggregation-prone, toxic species. However, the association between these movements as well as the sequence of these TMDs remains unclear. In this study, we raised the possibility that the vertical movement of TMD1 is a prerequisite for expansion of the catalytic cavity around TMD3 of PS1, resulting in reduced Aβ42 production. Our results shed light on the association between the conformational changes of TMDs and the regulation of γ-secretase activity.

Keywords: γ-secretase, Alzheimer disease, conformational changes, presenilin, structural dynamics

Biochemistry in Diseases and Aging

SARS-CoV-2 spike protein binding selectively accelerates substrate-specifi c catalytic activity of ACE2

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Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is a novel coronavirus that has given rise to the devastating global pandemic. In most cases, SARS-CoV-2 infection results in the development of viral pneumonia and acute respiratory distress syndrome, known as 'coronavirus disease 2019' or COVID-19. Intriguingly, besides the respiratory tract, COVID-19 affects other organs and systems of the human body. COVID-19 patients with pre-existing cardiovascular disease have a higher risk of death, and SARS-CoV-2 infection itself may cause myocardial inflammation and injury. One possible explanation of such phenomena is the fact that SARS-CoV-2 utilizes angiotensin-converting enzyme 2 (ACE2) as the receptor required for viral entry. ACE2 is expressed in the cells of many organs, including the heart. ACE2 functions as a carboxypeptidase that can cleave several endogenous substrates, including angiotensin II, thus regulating blood pressure and vascular tone. It remains largely unknown if the SARS-CoV-2 infection alters the enzymatic properties of ACE2, thereby contributing to cardiovascular complications in patients with COVID-19. Here, we demonstrate that ACE2 cleavage of des-Arg9-bradykinin substrate analogue is markedly accelerated, while cleavage of angiotensin II analogue is minimally affected by the binding of spike protein. These findings may have implications for a better understanding of COVID-19 pathogenesis.

Keywords: ACE2, COVID-19, SARS-CoV-2, spike protein

MOLECULAR BIOLOGY

Gene Expression

The cap-specific m^{6}A methyltransferase, PCIF1/CAPAM, is dynamically recruited to the gene promoter in a transcription-dependent manner

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N^6-methyladenosine (m^{6}A), the most abundant modification in eukaryotic mRNAs, plays an important role in mRNA metabolism and functions. When adenosine is transcribed as the first cap-adjacent nucleotide, it is methylated at the ribose 2′-*O* and N^6 positions, thus generating N^6, 2′-*O*-dimethyladenosine (m^{6}Am). Phosphorylated C-terminal domain (CTD)-interacting factor 1 (PCIF1) is a novel cap-specific adenine N^6-methyltransferase responsible for m^{6}Am formation. As PCIF1 specifically interacts with the Ser5-phosphorylated CTD of RNA polymerase II (Pol II), which is a marker for the early phase of transcription, PCIF1 is speculated to be recruited to the early elongating Pol II. In this study, subcellular fractionation and immunofluorescence microscopy demonstrated that PCIF1 is mainly localized to the transcriptionally active chromatin regions in HeLa cells. Chromatin immunoprecipitation (ChIP) revealed that PCIF1 was predominantly localized to the promoter of a broad range of Pol II-transcribed genes, including several protein-coding genes and non-coding RNA genes. Moreover, PCIF1 accumulation on these promoters depended entirely on transcriptional activity and Ser5 phosphorylation of the CTD. These results suggest that PCIF1 dynamically localizes to the Pol II early in transcription and may efficiently catalyze N^6-methylation of the first adenosine residue of nascent mRNAs cotranscriptionally.

Keywords: CTD phosphorylation, mRNA capping, PCIF1/CAPAM, RNA m6A methylation, RNA polymerase II

Molecular Genetics

Serum INHB levels and ACE gene I/D polymorphism with increased risk for unexplained infertility

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Angiotensin converting enzyme (ACE) has a significant role in the angiogenesis of ovarian endothelium and the resumption of meiosis and folicular growth. However, there is no any study concerning ACE polymorphism and unexplained infertility (UI). The main aim of this study is that both identify ACE polymorphism and measure the serum ACE, anti-Mullerian hormone (AMH) and inhibin-B (INHB) levels in UI patients and controls in Turkish population. Forty-seven UI patients and 41 controls were involved in this study. To determine the ACE polymorphisms, DNA isolation and PCR were performed. Then, serum ACE, AMH and INHB levels were measured spectrophotometrically. Patients with UI had significantly higher serum INHB levels compared with controls ($P < 0.05$). Serum ACE levels were decreased, compared to controls; however, the decrease was not significant. Serum AMH levels did not significantly differ from controls. When the relationship was analysed between ACE insertion/deletion (I/D) polymorphism and infertility risk, and ID genotype was chosen as reference, it was found to be 2.33 times more risk of UI than the women have DD genotype [DD versus ID: odds ratio = 2.33, 95% confidence interval (0.88–6.19); $P = 0.086$]. This finding indicates that DD genotype may be high

risk for UI. Further studies are warranted to confirm this finding, especially with a larger population.

Keywords: angiotensin converting enzyme (ACE), anti-Mullerian hormone (AMH), inhibin-B (INHB), polymorphism, unexplained infertility (UI)

CELL

Stress Proteins and Molecular Chaperones

A cyclic lipopeptide surfactin is a species-selective Hsp90 inhibitor that suppresses cyanobacterial growth

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Heat shock protein 90 (Hsp90) is essential for eukaryotic cells, whereas bacterial homologs play a role under stresses and in pathogenesis. Identifying species-specific Hsp90 inhibitors is challenging because Hsp90 is evolutionarily conserved. We found that a cyclic lipopeptide surfactin inhibits the ATPase activity of Hsp90 from the cyanobacterium *Synechococcus elongatus* (*S.elongatus*) PCC 7942 but does not inhibit *Escherichia coli* (*E.coli*), yeast and human Hsp90s. Molecular docking simulations indicated that surfactin could bind to the N-terminal dimerization interface of the cyanobacterial Hsp90 in the ATP- and ADP-bound states, which provided molecular insights into the species-selective inhibition. The data suggest that surfactin inhibits a rate-limiting conformational change of *S.elongatus* Hsp90 in the ATP hydrolysis. Surfactin also inhibited the interaction of the cyanobacterial Hsp90 with a model substrate, and suppressed *S.elongatus* growth under heat stress, but not that of *E.coli*. Surfactin did not show significant cellular toxicity towards mammalian cells. These results indicate that surfactin inhibits the cellular function of Hsp90 specifically in the cyanobacterium. The present study shows that a cyclic peptide has a great specificity to interact with a specific homolog of a highly conserved protein family.

Keywords: cyanobacterium, cyclic peptide, Hsp90, molecular chaperone, surfactin

Cell Death

The disulfiram/copper complex induces apoptosis and inhibits tumour growth in human osteosarcoma by activating the ROS/JNK signalling pathway

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Given the huge cost, long research and development (R&D) time and uncertain side effects of discovering new drugs, drug repositioning of those approved to treat diseases clinically as new drugs for other pathological conditions, especially cancers, is a potential alternative strategy. Disulfiram (DSF), an old drug used to treat alcoholism, has been found to exhibit anticancer activity and improve chemotherapeutic efficacy in cancers by an increasing number of studies. In addition, the combination of DSF and copper may be a more effective therapeutic strategy. In this study, we report the toxicity of the disulfiram/copper (DSF/Cu) complex to human osteosarcoma (OS) both *in vitro* and *in vivo*. DSF/Cu significantly inhibited the proliferation and clonogenicity of OS cell lines. Furthermore, the generation of reactive oxygen species (ROS) was triggered by DSF/Cu, and cell arrest, autophagy and apoptosis were induced in an ROS-dependent manner. The underlying mechanism of this process was explored, and DSF/Cu may mainly inhibit OS by inducing apoptosis by activating the ROS/JNK pathway. DSF/Cu also inhibited OS growth in a xenograft model with low levels of organ-related toxicities. These results suggest that the DSF/Cu complex could be an efficient and safe option for the treatment of OS in the clinic.

Keywords: apoptosis, autophagy, disulfiram/copper, osteosarcoma, ROS/JNK signalling pathway

BIOTECHNOLOGY

Biotechnology General

Novel photochromic inhibitor for mitotic kinesin Eg5 which forms multiple isomerization states

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The mitotic kinesin Eg5 is a plus-end directed homotetrameric molecular motor essential for the formation of bipolar spindles during cell division. Kinesin Eg5 is overexpressed in cancer cells and hence considered as a target for cancer therapy; the inhibitors specific for Eg5 have been developed as anticancer drugs. In this study, we synthesized a novel functional photoresponsive inhibitor composed of spiropyran and azobenzene derivatives to control Eg5 function with multistage inhibitory activity accompanied by the formation of different isomerization states. The photochromic inhibitor spiropyran-sulfo-azobenzene (SPSAB) exhibited three isomerization states: spiro (SP)-*trans*,

merocyanine (MC)-*cis* and MC-*trans*, upon exposure to visible light, ultraviolet and in the dark, respectively. SPSAB-induced reversible changes in the inhibitory activity of ATPase and motor activities correlating with photoisomerization among the three states. Among the three isomerization states of SPSAB, the SP-*trans* isomer showed potent inhibitory activity at an IC_{50} value of 30 μM in the basal ATPase assay. MC-*trans* and MC-*cis* exhibited less inhibitory activity at IC_{50} values of 38 and 86 μM, respectively. The results demonstrated that the novel photochromic inhibitor enabled precise control of Eg5 function at three different levels using light irradiation.

Keywords: inhibitor, isomerization, mitotic kinesin, multiple states, photochromic compound

Gene and Protein Engineering

High-level expression of human CH2 domain from the Fc region in Pichia pastoris and preparation of anti-CH2 antibodies

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Pichia pastoris is a popular eukaryotic system employed for the fast, simple and inexpensive production of recombinant protein including biotherapeutics such as human albumin. The CH_2 domain of human Immunoglobulin G (IgG) is a promising scaffold for developing novel therapeutics. To accelerate the research of CH_2 domain, we have established a procedure to highly express human CH_2 domain (~150 mg/l) as well as human Fc (~30 mg/l) in yeast *P. pastoris*. The procedure yields, simultaneously, a major glycosylated (~70%) and non-glycosylated (~30%) fractions. They can be easily separated with high purity. Although both forms of CH_2 domain have essentially the same secondary structure, the presence of the glycan increased the thermal stability of the CH_2 domain by about 5°C as determined from calorimetry. The purified glycosylated CH_2 domain elicited polyclonal antibodies in mouse, recognizing not only the CH_2 domain, but also recombinant human Fc and the commercial IgG1 antibody Rituxan. Protein A and Protein G binding to the kink region between CH_2 domain and CH_3 domain of human Fc are used to purify therapeutic proteins. Therefore, these antibodies are candidates to develop a novel affinity material to purify human antibodies using their CH_2 domain.

Keywords: antibody, CH2 domain, N-glycan, Pichia pastoris, protein expression

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BIOCHEMISTRY

Biochemistry General

Effects of genetic polymorphisms on the sulfation of doxorubicin by human SULT1C4 allozymes

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Doxorubicin is a chemotherapeutic drug widely utilized in cancer treatment. An enzyme critical to doxorubicin metabolism is the cytosolic sulfotransferase (SULT) SULT1C4. This study investigated the functional impact of SULT1C4 single nucleotide polymorphisms (SNPs) on the sulfation of doxorubicin by SULT1C4 allozymes. A comprehensive database search was performed to identify various *SULT1C4* SNPs. Ten nonsynonymous *SULT1C4* SNPs were selected, and the corresponding cDNAs, packaged in pGEX-2TK expression vector, were generated via site-directed mutagenesis. Respective SULT1C4 allozymes were bacterially expressed and purified by affinity chromatography. Purified SULT1C4 allozymes, in comparison with the wild-type enzyme, were analysed for sulphating activities towards doxorubicin and 4-nitrophenol, a prototype substrate. Results obtained showed clearly differential doxorubicin-sulphating activity of SULT1C4 allozymes, implying differential metabolism of doxorubicin through sulfation in individuals with distinct *SULT1C4* genotypes.

Keywords: doxorubicin polymorphism cytosol single nucleotide polymorphism sulfotransferase enzymes sulfation

Unusual aggregation property of recombinantly expressed cancer-testis antigens in mammalian cells

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Transient expression of human intracellular proteins in human embryonic kidney (HEK) 293 cells is a reliable system for obtaining soluble proteins with biologically active conformations. Contrary to conventional concepts, we found that recombinantly expressed intracellular cancer-testis antigens (CTAs) showed frequent aggregation in HEK293 cells. Although experimental subcellular localization of recombinant CTAs displayed proper cytosolic or nuclear localization, some proteins showed aggregated particles in the cell. This aggregative property was not observed in recombinant housekeeping proteins. No significant correlation was found between the aggregative and biophysical properties, such as hydrophobicity, contents of intrinsically disordered regions and expression levels, of CTAs. These results can be explained in terms of structural instability of CTAs, which are specifically expressed in the testis and aberrantly expressed in cancer cells and function as a hub in the protein–protein network using intrinsically disordered regions. Hence, we speculate that recombinantly expressed CTAs failed to form this protein complex. Thus, unfolded CTAs formed aggregated particles in the cell.
Keywords: aggregation, CTAs, IDPs, immunotherapy, hydrophobicity

Protein Interaction and Recognition

Study on novel PtNP–sorafenib and its interaction with VEGFR2
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With the developments of nanodrugs, some drugs have combined with nanoparticles (NPs) to reduce their side-effects and increase their therapeutic activities. Here, a novel nanodrug platinum nanoparticle–sorafenib (PtNP–SOR) was proposed for the first time. By means of molecular dynamics simulation, the stability and biocompatibility of PtNP–SOR were investigated. Then, the interaction mechanism between PtNP–SOR and vascular endothelial growth factor receptor 2 (VEGFR2) was explored and compared with that of the peptide 2a coated PtNPs. The results showed that PtNP–SOR could bind to VEGFR2 more stably, which was driven by the Coulombic and strong dispersion interaction between PtNP–SOR and VEGFR2. According to their contributions obtained from the decomposition of binding free energies, the key residues in VEGFR2 were identified to form the specific space, which increased the affinity with PtNP–SOR. This study provided useful insights to the design of PtNP-drugs as well as important theoretical proofs to the interaction between PtNP–SOR and VEGFR2 at a molecular level, which can be of large help during the development and optimization of novel nanodrugs.
Keywords: biocompatibility, interaction, molecular mechanism, PtNP–sorafenib, VEGFR2

Molecular recognition of a single-chain Fv antibody specific for GA-pyridine, an advanced glycation end-product (AGE), elucidated using biophysical techniques and synthetic antigen analogues
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Advanced glycation end-products (AGEs) are a heterogeneous group of compounds formed by non-enzymatic reaction between reducing-sugar and Arg/Lys in proteins and are involved in various diabetic complications. GA-pyridine is derived from glycolaldehyde and is one of the most cytotoxic AGEs. Here, we established a single-chain Fv (scFv) antibody against GA-pyridine, 73MuL9-scFv, and examined the details of its specificity and antigen recognition by using various techniques involving biophysics, chemical biology and structural biology. We also synthesized several compounds that differ slightly in regard to the position and number of GA-pyridine substituent groups, and revealed that GA-pyridine was specifically bound to 73MuL9-scFv. Thermodynamic analysis revealed that the association of GA-pyridine to 73MuL9-scFv was an exothermic and enthalpy driven reaction, and thus that the antigen recognition involved multiple specific interactions. Crystallographic analysis of the Fv fragment of 73MuL9-scFv revealed that several CH-π and

hydrogen bond interactions took place between the Fv-fragment and GA-pyridine, which was consistent with the results of thermodynamic analysis. Further studies using 73MuL9-scFv as a tool to clarify the relevance of GA-pyridine to diabetic complications are warranted.

Keywords: biophysical assays, crystallographic analysis, GA-pyridine, scFv antibody, synthetic antigen analogues

Glycobiology and Carbohydrate Biochemistry

Bisecting-GlcNAc on Asn388 is characteristic to ERC/mesothelin expressed on epithelioid mesothelioma cells

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Mesothelioma is a highly aggressive tumour associated with asbestos exposure and is histologically classified into three types: epithelioid-type, sarcomatoid-type and biphasic-type. The prognosis of mesothelioma patients is poor and there is no effective molecular-targeting therapy as yet. ERC/mesothelin is a glycoprotein that is highly expressed on several types of cancers including epithelioid mesothelioma, but also expressed on normal mesothelial cells. This is a predicted reason why there is no clinically approved therapeutic antibody targeting ERC/mesothelin. In the present study, we focussed on the differential glycosylation between ERC/mesothelin present on epithelioid mesothelioma and that on normal mesothelial cells and aimed to reveal a distinct feature of epithelioid mesothelioma cells. Lectin microarray analysis of ERC/mesothelin using cells and patient specimens showed significantly stronger binding of PHA-E_4 lectin, which recognizes complex-type *N*-glycans having a so-called bisecting-GlcNAc structure, to ERC/mesothelin from epithelioid mesothelioma cells than that from normal mesothelial cells. Further, liquid chromatography/mass spectrometry analysis on ERC/mesothelin from epithelioid mesothelioma cells confirmed the presence of a bisecting-GlcNAc attached to Asn388 of ERC/mesothelin. These results suggest that this glycoproteome could serve as a potential target for the generation of a highly selective and safe therapeutic antibody for epithelioid mesothelioma.

Keywords: bisecting-GlcNAc, epithelioid mesothelioma, ERC/mesothelin, glycosylation

Lipid Biochemistry

Erylysin A inhibits cytokinesis in Escherichia coli by binding with cardiolipin

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Cardiolipin (CL) localizes to curved membranes such as cristae in mitochondria as well as cell poles and division sites in rod-shaped bacteria. CL is believed to stabilize the membrane curvature by localizing to sites of negative curvature. However, this hypothesis has not been tested because of a lack of appropriate tools to distinguish CL inside and outside lipid bilayers. In this study, we provided the first evidence that CL localized to regions of negative curvature in *Escherichia coli* using the novel CL probe erylysin A-EGFP (EryA-EGFP). Staining in *E.coli* illustrated that CL localized to the inner leaflets at cell poles and the outer leaflets at division sites. Furthermore, we revealed that EryA-EGFP inhibited cytokinesis. We propose that cytokinesis completes after CL in the outer leaflets transfers to the inner leaflets at division sites by inspecting the mechanism of inhibition of cytokinesis. Moreover, the cytoskeletal protein RodZ was abnormally distributed when cytokinesis was inhibited by EryA-EGFP, suggesting that RodZ participates in cytokinesis. In summary, we revealed the detailed distribution of CL and proposed a new model of cytokinesis.

Keywords: cardiolipin, cytokinesis, Esherichia coli, membrane structure, RodZ

Biochemistry in Diseases and Aging

MiR-20a-5p overexpression prevented diabetic cardiomyopathy via inhibition of cardiomyocyte apoptosis, hypertrophy, fibrosis and JNK/NF-κB signalling pathway

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Diabetic cardiomyopathy (DCM) is a common cardiovascular

disease. A declined miR-20a-5p was observed in hearts of diabetic mice, while its effect on DCM remains unknown. Herein, we established streptozotocin-induced DCM rat model and high glucose-stimulated H9C2 model of DCM. Then they were treated with adenovirus expressing miR-20a-5p to explore the function of miR-20a-5p. Insulin tolerance test and intraperitoneal glucose tolerance test assay revealed that miR-20a-5p reduced blood glucose level. Besides, miR-20a-5p improved cardiac dysfunction reflected by reduced heart weight/body weight and left ventricular diastolic pressure, and increased left ventricular systolic pressure and ± LV d*p*/d*t* max. MiR-20a-5p prevented cardiomyocyte apoptosis, along with the upregulated c-caspase-3, bax and downregulated bcl-2. Moreover, miR-20a-5p alleviated cardiac hypertrophy as the parameters of atrial natriuretic peptide, B-type natriuretic peptide and MyHC-β decreased. Also, miR-20a-5p attenuated the cardiac fibrosis demonstrated by decreased transforming growth factor-β1, collagen I levels and the inflammatory response manifested by reduced interleukin-6, tumour necrosis factor-α and IL-1β production. Furthermore, miR-20a-5p prevented Jun NH_2-terminal kinase (JNK) phosphorylation and nuclear factor-κB (NF-κB) p65nuclear translocation. Similarly, the effects of miR-20a-5p on DCM were confirmed in our *in vitro* experiments. Additionally, ROCK2 is a possible target gene of miR-20a-5p. ROCK2 overexpression reversed the protective effect of miR-20a-5p on DCM. Overall, miR-20a-5p may effectively ameliorate DCM through improving cardiac metabolism, and subsequently inhibiting inflammation, apoptosis, hypertrophy, fibrosis and JNK/NF-κB pathway via modulating ROCK2.

Keywords: apoptosis, diabetic cardiomyopathy, JNK/NF-κB signalling pathway, miR-20a-5p

Analytical Biochemistry

Development of a liquid chromatography–electrospray ionization tandem mass spectrometric method for the simultaneous analysis of free fatty acids

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Fatty acids (FAs) play important roles in several physiological and pathophysiological processes, functioning as both nonesterified free FAs (FFAs) and components of other lipid classes. Although many lipid classes are readily measured using liquid chromatography–electrospray ionization tandem mass spectrometry (LC-ESI-MS/MS), the measurement of FFAs by this method is not straightforward because of inconsistent fragmentation behaviours. In this study, we describe a strategy to measure FFAs using conventional reverse-phase LC-ESI-MS/MS, without derivatization. The strategy combines three key methods: (i) an isocratic LC separation with a high organic solvent ratio, (ii) postcolumn base addition, and (iii) pseudo-multiple reaction monitoring. The method facilitates the measurement of ultra-long-chain FAs, the accumulation of which is a common biochemical abnormality in peroxisomal disorders. This study delivers a broad strategy that measures a wide spectrum of FFA species in complex biological samples.

Keywords: ammonium hydroxide, free fatty acid, isocratic LC, LC-MS/MS, pseudo-MRM

MOLECULAR BIOLOGY

Gene Expression

Retinoic acid stimulates transcription of the rat SHARP-2 gene via multiple pathways

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Members of the enhancer of split- and hairy-related protein (SHARP) family, SHARP-1 and SHARP-2, are basic helix–loop–helix transcriptional repressors and belong to the clock genes. In this study, an effect of retinoic acid (RA) on the *SHARP family* gene expression in the differentiated cells was examined. RA rapidly and temporarily induced the SHARP-2 mRNA expression in hepatic H4IIE cells. Then, whether the SHARP-2 mRNA expression is altered by dexamethasone (Dex), insulin, and the combination of RA and Dex or RA and insulin was examined. Dex had different effects on the expression of SHARP-2 mRNA in the presence or absence of RA. Then, the molecular mechanisms were investigated using inhibitors of various signaling molecules. The RA-induction of SHARP-2 mRNA level was mainly inhibited by LY294002, staurosporine, and actinomycin D, respectively. Finally, whether RA acts on the transcriptional regulatory region of the *SHARP-2* gene was analysed using luciferase reporter gene assay. At least two RA-responsive regions were mapped at the nucleotide sequences between −3,700 and −1,600 of the *SHARP-2* gene. In addition, this effect was dependent on the RA receptor and retinoid X receptor. Thus, we conclude that RA stimulated transcription of the *SHARP-2* gene via multiple pathways.

Keywords: gene transcription, hepatic H4IIE cells, retinoic acid, SHARP-2, signaling pathway

Molecular Genetics

Japonica Array NEO with increased genome-wide coverage and abundant disease risk SNPs

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Ethnic-specific SNP arrays are becoming more important to increase the power of genome-wide association studies in diverse population. In the Tohoku Medical Megabank Project, we have been developing a series of Japonica Arrays (JPA) for genotyping participants based on reference panels constructed from whole-genome sequence data of the Japanese population. Here, we designed a novel version of the SNP array for the Japanese population, called Japonica Array NEO (JPA NEO), comprising a total of 666,883 markers. Among them, 654,246 tag SNPs of autosomes and X chromosome were selected from an expanded reference panel of 3,552 Japanese, 3.5KJPNv2, using pairwise r^2 of linkage disequilibrium measures. Additionally, 28,298 markers were included for the evaluation of previously identified disease risk markers from the literature and databases, and those present in the Japanese population were extracted using the reference panel. Through genotyping 286 Japanese samples, we found that the imputation quality r^2 and INFO score in the minor allele frequency bin >2.5–5% were >0.9 and >0.8, respectively, and >12 million markers were imputed with an INFO score >0.8. From these results, JPA NEO is a promising tool for genotyping the Japanese population with genome-wide coverage, contributing to the development of genetic risk scores.

Keywords: disease risk alleles, ethnic-specific SNP array, genome-wide coverage, genotype imputation, Tohoku Medical Megabank Project

CELL

Neurobiology

Abundant oleoyl-lysophosphatidylethanolamine in brain stimulates neurite outgrowth and protects against glutamate toxicity in cultured cortical neurons

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Lysophosphatidylethanolamines (LPEs) are bioactive lysophospholipids that have been suggested to play important roles in several biological processes. We performed a quantitative analysis of LPE species and showed their composition in mouse brain. We examined the roles of oleoyl-LPE (18 : 1 LPE), which is one of the abundant LPE species in brain. In cultured cortical neurons, application of 18 : 1 LPE-stimulated neurite outgrowth. The effect of 18 : 1 LPE on neurite outgrowth was inhibited by Gq/11 inhibitor YM-254890, phospholipase C (PLC) inhibitor U73122, protein kinase C (PKC) inhibitor Go6983 or mitogen-activated protein kinase (MAPK) inhibitor U0126. Additionally, 18 : 1 LPE increased the phosphorylation of MAPK/extracellular signal-regulated kinase 1/2. These results suggest that the action of 18 : 1 LPE on neurite outgrowth is mediated by the Gq/11/PLC/PKC/MAPK pathway. Moreover, we found that application of 18 : 1 LPE protects neurons from glutamate-induced excitotoxicity. This effect of 18 : 1 LPE was suppressed by PKC inhibitor Go6983. These results suggest that 18 : 1 LPE protects neurons from glutamate toxicity via PKC inhibitor Go6983-sensitive PKC subtype. Collectively, our results demonstrated that 18 : 1 LPE stimulates neurite outgrowth and protects against glutamate toxicity in cultured cortical neurons. Our findings provide insights into the physiological or pathological roles of 18 : 1 LPE in the brain.

Keywords: cultured cortical neuron, glutamate toxicity, mass spectrometry, neurite outgrowth, phospholipid

Tumor and Immunology

LINC01207 is up-regulated in gastric cancer tissues and promotes disease progression by regulating miR-671–5p/DDX5 axis

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LINC01207 is involved in the progression of some cancers. This study was designed to delve into the biological function and mechanism of LINC01207 in gastric cancer. Quantitative polymerase chain reaction (qPCR) was adopted to examine the expression levels of LINC01207, miR-671–5p, dead-box polypeptide 5 (DDX5) mRNA in gastric cancer tissues and cells. After LINC01207 was overexpressed or depleted, MTT and bromodeoxyuridine (BrdU) assays were conducted to detect cell proliferation. Transwell assay was employed to detect cell migration and invasion. Western blot was used to detect the expression of DDX5 protein in cells. Bioinformatics analysis, luciferase reporter assay and RNA pull-down assay were performed to predict and validate the binding site between miR-671–5p and LINC01207 or DDX5. LINC01207 and DDX5 mRNA were upregulated in gastric cancer, while miR-671–5p was downregulated; high expression of LINC01207 and transfection of miR-671–5p inhibitors facilitated the proliferation of gastric cancer cells; however, knocking down LINC01207 and the overexpression of miR-671–5p mimics had opposite biological effects. LINC01207 and miR-671–5p were interacted and miR-671–5p was negatively regulated by LINC01207. MiR-671–5p could reverse the function of LINC01207. DDX5 was a downstream target of miR-671–5p and was positively modulated by LINC01207. LINC01207 promotes the proliferation and metastasis of gastric cancer cells by regulating miR-671–5p/DDX5 axis.

Keywords: DDX5, gastric cancer, LINC01207, miR-671–5p

BIOTECHNOLOGY

New Devices in Biotechnology

A mixing microfl uidic chip for real-time NMR monitoring of macromolecular reactions

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NMR spectroscopy permits real-time monitoring of reactions that involve changes in the spectra of reactants. MICCS (MIcro Channelled Cell for Synthesis monitoring) is a microfluidic chip for such purposes, which is used to rapidly activate reactions by mixing the reactant solutions in the chip inserted into the typical NMR tube. Although it allows monitoring of chemical reactions of small compounds, its simple mixing system dependent on diffusion in the microchannel was not suitable for macromolecules such as proteins with low diffusion rates. Here, we developed a new microfluidic chip based on MICCS by incorporating a mixer of split-and-recombination type within the microchannel. We applied it to monitoring of the protein-folding reaction in a stopped-flow mode. A solution of denaturant-unfolded RNase A was injected from a syringe pump into the microchip set inside the NMR magnet and mixed with a buffer for dilution to reach the folding condition. Immediately after dilution, the reaction was initiated and detected by a series of NMR measurements that were synchronized with activation and inactivation of the pump. The process was repeated for accumulation of the data. By analysing the change of the spectra by factor analysis, a kinetic constant of $0.57\,\mathrm{min}^{-1}$ was obtained.

Keywords: Folding, Microfluidics, NMR, Reaction, Structure