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Biochemistry General

Liver microRNA-29b-3p positively correlates with relative enhancement values of magnetic resonance imaging and represses liver fi brosis

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This study aims to identify potential microRNAs (miRNAs) contribute to liver fibrosis progression and investigate how the miRNA is involved. We recruited totally 58 patients. Magnetic resonance imaging was employed to detect fibrosis. Classification of liver fibrosis was carried out by Ishak scoring system. Cell viability was tested using cell counting kit-8. Measurements of mRNA and protein expressions were conducted using real-time quantitative polymerase chain reaction and western blotting. Luciferase reporter assay was recruited for determination of miR-29b-3p targets. We found that relative enhancement (RE) values were reduced with the increases in fibrosis stages and was negatively associated with Ishak scores. In comparison with patients without liver fibrosis, miR-29b-3p level was remarkably reduced in those with liver fibrosis. Its level was found to be positively associated with RE values. Transforming growth factor beta 1 (TGF-β1)-induced hepatic stellate cell (HSC) activation significantly decreased miR-29b-3p expression. However, miR-29b-3p overexpression repressed TGF-β1-induced collagen I protein and alpha-smooth muscle actin (α-SMA) expression. As expected, its overexpression also reduced cell viability. We found that miR-29b-3p directly bind to signal transducer and activator of transcription 3 (STAT3) and suppressed its expression. Our study demonstrates that low expression of miR-29b-3p may contribute to the progression of liver fibrosis by suppressing STAT3.

Keywords; collagen I, liver fibrosis, microRNA (miR)-29b-3p, signal transducer and activator of transcription 3 (STAT3), transforming growth factor beta 1 (TGF-β1)

Protein Structure

Trp: a conserved aromatic residue crucial to the interaction of a scorpion peptide with sodium channels

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Anti-tumour-analgesic peptide (AGAP), one scorpion toxin purified from *Buthus martensii* Karsch, was known as its analgesic and anti-tumour activities. Trp^{38}, a conserved aromatic residue of AGAP, might play important roles in its interaction with sodium channels. In this study, a mutant W38F was generated and effects of W38F were examined on $hNa_v1.4$, $hNa_v1.5$ and $hNa_v1.7$ by using whole-cell patch-clamp, which were closely associated to the biotoxicity of skeletal and cardiac muscles and pain signalling. The data showed that W38F decreased the inhibition effects of peak currents of $hNa_v1.7$, $hNa_v1.4$ and $hNa_v1.5$ compared with AGAP, notably, W38F reduced the analgesic activity compared with AGAP. The results suggested that Trp^{38} be a crucial amino acid involved in the interaction with these three sodium channels. The decreased analgesic activity of W38F might result from its much less inhibition of $hNa_v1.7$. These findings provided more information about the relationship between structure and function of AGAP and may facilitate the modification of other scorpion toxins with pharmacological effects.

Keywords; AGAP, analgesic activity, Nav1.7, scorpion toxin, voltage-gated sodium channel

Protein Interaction and Recognition

Studies on the gene regulation involved in the lytic–lysogenic switch in Staphylococcus aureus temperate bacteriophage

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Antirepressor proteins of bacteriophages are chiefly involved in interfering with the function of the repressor protein and forcing the bacteriophage to adopt the lytic cycle. The genome of *Staphylococcus aureus* phage, Phi11 has already been sequenced; from the genome sequence, we amplified *gp07* gene and analysed its involvement in the developmental pathway of Phi11. Our results indicate that Gp07 functions as a novel antirepressor

and regulates the developmental pathway of Phi11 by enhancing the binding of the Cro repressor protein to its cognate operator. We also report our finding that the CI repressor protein of Phi11 binds to the putative operator of Gp07 and regulates its expression. We further report that *S.aureus* transcriptional repressor LexA and coprotease RecA play a crucial role in the lytic–lysogenic switching in Phi11. We also identified that the N-terminal domain (Bro-N) of Gp07 is actually responsible for enhancing the binding of Cro repressor to its cognate operator. Our results suggest that Phi11 prophage induction is different from other bacteriophages. This study furnishes a first-hand report regarding the regulation involved in the developmental pathway of Phi11.

Keywords; antirepressor, Bro-N, KilA-C, lytic–lysogenic switch, Phi11

Linker DNA and histone contributions in nucleosome binding by p53

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The tumour suppressor protein p53 regulates various genes involved in cell-cycle arrest, apoptosis and DNA repair in response to cellular stress, and apparently functions as a pioneer transcription factor. The pioneer transcription factors can bind nucleosomal DNA, where many transcription factors are largely restricted. However, the mechanisms by which p53 recognizes the nucleosomal DNA are poorly understood. In the present study, we found that p53 requires linker DNAs for the efficient formation of p53-nucleosome complexes. p53 forms an additional specific complex with the nucleosome, when the p53 binding sequence is located around the entry/exit region of the nucleosomal DNA. We also showed that p53 directly binds to the histone H3-H4 complex via its N-terminal 1–93 amino acid region. These results shed light on the mechanism of nucleosome recognition by p53.

Keywords; chromatin, histone, nucleosome, p53, pioneer transcription factor

Glycobiology and Carbohydrate Biochemistry

Analysis of GPI-anchored proteins involved in germline stem cell proliferation in the Caenorhabditis elegans germline stem cell niche

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Stem cells divide and undergo self-renewal depending on the signals received from the stem cell niche. This phenomenon is indispensable to maintain tissues and organs in individuals. However, not all the molecular factors and mechanisms of self-renewal are known. In our previous study, we reported that glycosylphosphatidylinositol (GPI)-anchored proteins (GPI-APs) synthesized in the distal tip cells (DTCs; the stem cell niche) are essential for germline stem cell proliferation in *Caenorhabditis elegans*. Here, we characterized the GPI-APs required for proliferation. We selected and verified the candidate GPI-APs synthesized in DTCs by RNA interference screening and found that F57F4.3 (GFI-1), F57F4.4 and F54E2.1 are necessary for germline proliferation. These proteins are likely involved in the same pathway for proliferation and activated by the transcription factor PQM-1. We further provided evidence suggesting that these GPI-APs act through fatty acid remodelling of the GPI anchor, which is essential for association with lipid rafts. These findings demonstrated that GPI-APs, particularly F57F4.3/4 and F54E2.1, synthesized in the germline stem cell niche are located in lipid rafts and involved in promoting germline stem cell proliferation in *C. elegans*. The findings may thus shed light on the mechanisms by which GPI-APs regulate stem cell self-renewal.

Keywords; Caenorhabditis elegans, germline stem cell proliferation, lipid raft, roles of glycoconjugates, stem cell niche

Biochemistry in Cell Membranes

Functional characterization and tissue localization of the facilitative glucose transporter GLUT12

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Facilitative glucose transporters (GLUTs) play crucial roles in glucose utilization and homeostasis. GLUT12 was initially isolated as a novel GLUT4-like transporter involved in insulin-dependent glucose transport. However, tissue distribution and biochemical properties of GLUT12 are not well understood. In

this study, we investigated the basic kinetic properties and tissue distribution of GLUT12. Human GLUT12 and GLUT1 were overexpressed and purified using Ni-NTA column chromatography. Reconstituted proteoliposomes showed time-dependent D-glucose transport activity, which was inhibited by phloretin and dehydroascorbate. Dose dependence of glucose transport revealed a K_M and V_{max} values of 6.4 mM and 1.2 μmol/mg/min, respectively, indicating that GLUT12 is a high-affinity type GLUT. Glucose transport by GLUT12 was inhibited by ATP and glucose-1-phosphate, glucose-6-phosphate and disaccharides (properties similar to those of GLUT1). Indirect immunohistochemistry revealed the distribution of mouse GLUT12 in the apical region of distal tubules and collecting ducts in the kidney and epithelial cells of the jejunum. In addition to these cells, GLUT12 was present in chromaffin cells in the adrenal medulla, the anterior pituitary lobe, as well as the thyroid and pyloric glands. These tissue distributions suggest a unique function of GLUT12, besides that of an insulin-dependent glucose transport.
Keywords; glucose transporter, GLUT, GLUT12, kinetics, tissue distribution

CELL

Cytoskeleton, Cell Motility, and Cell Shape

The PKGIα/VASP pathway is involved in insulin- and high glucosedependent regulation of albumin permeability in cultured rat podocytes

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Podocytes, the principal component of the glomerular filtration barrier, regulate glomerular permeability to albumin via their contractile properties. Both insulin- and high glucose (HG)-dependent activation of protein kinase G type Iα (PKGIα) cause reorganization of the actin cytoskeleton and podocyte disruption. Vasodilator-stimulated phosphoprotein (VASP) is a substrate for PKGIα and involved in the regulation of actin cytoskeleton dynamics. We investigated the role of the PKGIα/VASP pathway in the regulation of podocyte permeability to albumin. We evaluated changes in high insulin- and/or HG-induced transepithelial albumin flux in cultured rat podocyte monolayers. Expression of PKGIα and downstream proteins was confirmed by western blot and immunofluorescence. We demonstrate that insulin and HG induce changes in the podocyte contractile apparatus via PKGIα-dependent regulation of the VASP phosphorylation state, increase VASP colocalization with PKGIα, and alter the subcellular localization of these proteins in podocytes. Moreover, VASP was implicated in the insulin- and HG-dependent dynamic remodelling of the actin cytoskeleton and, consequently, increased podocyte permeability to albumin under hyperinsulinaemic and hyperglycaemic conditions. These results indicate that insulin- and HG-dependent regulation of albumin permeability is mediated by the PKGIα/VASP pathway in cultured rat podocytes. This molecular mechanism may explain podocytopathy and albuminuria in diabetes.
Keywords; high glucose, insulin, podocytes, protein kinase G type Iα;VASP

Long non-coding RNA PHACTR2-AS1 promotes tongue squamous cell carcinoma metastasis by regulating Snail

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Long non-coding RNA is an endogenous non-coding RNA that has currently been proved to be an important player in cancer cell biology. In the present study, we investigated the biological role of PHACTR2-AS1 in tongue squamous cell carcinoma (TSCC). PHACTR2-AS1 was preferentially localized in the cytoplasm, and was notably upregulated in TSCC tissues. High PHACTR2-AS1 was correlated with tumour differentiation, metastatic clinical features, relapse and shortened survival time. Depletion of PHACTR2-AS1 did not affect TSCC cell viability and colony formation ability, whereas substantially inhibited cell migration and invasion *in vitro* and lung metastasis *in vivo*. Mechanistically, PHACTR2-AS1 could sponge miR-137 to increase Snail expression, resulting in triggering epithelial–mesenchymal transition process, thereby promoting TSCC cell metastasis. Taken together, our data for the first time elucidate the metastasis-promoting role of PHACTR2-AS1 in TSCC, hinting a new therapeutic target for metastatic TSCC patients.
Keywords; epithelial–mesenchymal transition, metastasis, PHACTR2-AS1, prognosis, tongue squamous cell carcinoma

Receptors and Signal Transduction

Genetic dissection of Ragulator structure and function in amino acid-dependent regulation of mTORC1

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Ragulator is a heteropentameric protein complex consisting of two roadblock heterodimers wrapped by the membrane anchor p18/Lamtor1. The Ragulator complex functions as a lysosomal membrane scaffold for Rag GTPases to recruit and activate mechanistic target of rapamycin complex 1 (mTORC1). However, the roles of Ragulator structure in the regulation of mTORC1 function remain elusive. In this study, we disrupted Ragulator structure by directly anchoring RagC to lysosomes and monitored the effect on amino acid-dependent mTORC1 activation. Expression of lysosome-anchored RagC in p18-deficient cells resulted in constitutive lysosomal localization and amino acid-independent activation of mTORC1. Co-expression of Ragulator in this system restored the amino acid dependency of mTORC1 activation. Furthermore, ablation of Gator1, a suppressor of Rag GTPases, induced amino acid-independent activation of mTORC1 even in the presence of Ragulator. These results demonstrate that Ragulator structure is essential for amino acid-dependent regulation of Rag GTPases via Gator1. In addition, our genetic analyses revealed new roles of amino acids in the regulation of mTORC1 as follows: amino acids could activate a fraction of mTORC1 in a Rheb-independent manner, and could also drive negative-feedback regulation of mTORC1 signalling via protein phosphatases. These intriguing findings contribute to our overall understanding of the regulatory mechanisms of mTORC1 signalling.

Keywords; lysosome, mTORC1, p18, Rag, Ragulator, Rheb

Involvement of PP2A methylation in the adipogenic differentiation of bone marrow-derived mesenchymal stem cell

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Bone marrow-derived mesenchymal stem cells (BM-MSCs) are multipotent stem cells with ability to self-replicate and differentiate into mesodermal derivatives, such as adipocytes and osteoblasts. BM-MSCs are a critical component of the tumour microenvironment. They support tumour progression by recruiting additional BM-MSCs and by differentiating into myofibroblasts (also called cancer-associated fibroblasts). Protein phosphatase 2A (PP2A) is an essential serine/threonine protein phosphatase that regulates a broad range of cellular signalling. PP2A forms a heterotrimer to dephosphorylate specific substrates. The reversible methylesterification (methylation) of Leu309 in the catalytic subunit of PP2A (PP2Ac) regulates biogenesis of the PP2A holoenzyme. It is unknown whether the methylation of PP2Ac plays a role in BM-MSC differentiation. Our experiments determined that protein levels of PP2A subunits and PP2A methyltransferase (LCMT-1) are significantly altered during differentiation. PP2Ac methylation levels in BM-MSCs decrease over time in response to an adipogenic differentiation stimulus. However, blockage of PP2A demethylation using the PP2A dimethyl-esterase inhibitors enhanced adipocyte differentiation. This suggests that PP2Ac demethylation is involved in adipocyte differentiation resistance. The results of our study provide a greater understanding of the regulation of BM-MSCs differentiation by PP2A holoenzyme.

Keywords; BM-MSCs, differentiation, PP2A, protein methylation

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JB REVIEW

G-quadruplex binding protein Rif1, a key regulator of replication timing

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DNA replication is spatially and temporally regulated during S phase to execute efficient and coordinated duplication of entire genome. Various epigenomic mechanisms operate to regulate the timing and locations of replication. Among them, Rif1 plays a major role to shape the ‘replication domains’ that dictate which segments of the genome are replicated when and where in the nuclei. Rif1 achieves this task by generating higher-order chromatin architecture near nuclear membrane and by recruiting a protein phosphatase. Rif1 is a G4 binding protein, and G4 binding activity of Rif1 is essential for replication timing regulation in fission yeast. In this article, we first summarize strategies by which cells regulate their replication timing and then describe how Rif1 and its interaction with G4 contribute to regulation of chromatin architecture and replication timing.

Keywords; chromatin structure, DNA replication, G-quadruplexes, DNA–protein interaction, Rap1 interacting factor 1 (Rif1),

replication timing

BIOCHEMISTRY

Protein Structure

Identification of quasi-stable water molecules near the Thr73–Lys13 catalytic diad of Bacillus sp. TB-90 urate oxidase by X-ray crystallography with controlled humidity

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Urate oxidases (UOs) catalyze the cofactor-independent oxidation of uric acid, and an extensive water network in the active site has been suggested to play an essential role in the catalysis. For our present analysis of the structure and function of the water network, the crystal qualities of *Bacillus* sp. TB-90 urate oxi dase were improved by controlled dehydration using the humid air and glue-coating method. After the dehydration, the $P2_12_12$ crystals were transformed into the $I222$ space group, leading to an extension of the maximum resolution to 1.42 Å. The dehydration of the crystals revealed a significant change in the five-water-molecules' binding mode in the vicinity of the catalytic diad, indicating that these molecules are quasi-stable. The pH profile analysis of $\log(k_{cat})$ gave two pK_a values: pK_{a1} at 6.07 ± 0.07 and pK_{a2} at 7.98 ± 0.13. The site-directed mutagenesis of K13, T73 and N276 involved in the formation of the active-site water netw ork revealed that the activities of these mutant variants were sig nificantly reduced. These structural and kinetic data suggest that the five quasi-stable water molecules play an essential role in the catalysis of the cofactor-independent urate oxidation by reducing the energy penalty for the substrate-binding or an on–off switchi ng for the proton-relay rectification.

Keywords; catalytic mechanism, conformational flexibility, enzyme kinetics, water structure, X-ray crystallography

Structural insight and stability of TNFR-Fc fusion protein (Etanercept) produced by using transgenic silkworms

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Therapeutic proteins expressed using transgenic animals have been of great interest for several years. Especially, transgenic silkworm has been studied intensively because of its ease in handling, low-cost, high-yield and unique glycosylation patterns. However, the physicochemical property of the therapeutic protein expressed in transgenic silkworm remains elusive. Here, we constructed an expression system for the TNFR-Fc fusion protein (Etanercept) using transgenic silkworm. The TNFR-Fc fusion protein was employed to N-glycan analysis, which revealed an increased amount of afucosylated protein. Evidence from surface plasmon resonance analysis showed that the TNFR-Fc fusion protein exhibit increased binding affinity for Fcγ receptor IIIa and FcRn compared to the commercial Etanercept, emphasizing the profit of expression system using transgenic silkworm. We have further discussed the comparison of higher order structure, thermal stability and aggregation of the TNFR-Fc fusion protein.

Keywords; Fc fusion protein, glycosylation, physicochemical property, silkworm, therapeutic protein

Interplay of isoform 1N4R tau protein and amyloid-β peptide fragment 25–35 in reducing and non-reducing conditions

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Amyloid-β (Aβ) peptide and tau protein are two hallmark proteins in Alzheimer's disease (AD); however, the parameters, which mediate the abnormal aggregation of Aβ and tau, have not been fully discovered. Here, we have provided an optimum method to purify tau protein isoform 1N4R by using nickel-nitrilotriacetic acid agarose chromatography under denaturing condition. The biochemical and biophysical properties of the purified protein were further characterized using *in vitro* tau filament ass embly, tubulin polymerization assay, circular dichroism (CD) sp ectroscopy and atomic force microscopy. Afterwards, we invest igated the effect of tau protein on aggregation of Aβ (25–35) pe ptide using microscopic imaging and cell viability assay. Incuba tion of tau at physiologic and supra-physiologic concentrations with $A\beta_{25-35}$ for 40 days under reducing and non-reducing cond itions revealed formation of two types of aggregates with distin ct morphologies and dimensions. In non-reducing condition, the co-incubated sample showed granular aggregates, while in reduc ing condition, they formed annular protofibrils. Results from cell viability assay revealed the increased cell viability for the co-incubated sample. Therefore, the disassembling action shown by tau protein on $A\beta_{25-35}$ suggests the possibility that tau may have a protective role in preventing Aβ peptide from acquiring the cyt otoxic, aggregated form against oxidative stress damages.

Keywords; Aβ25–35, Alzheimer's disease, fibrillation, intrinsically disordered protein, tau purification

Protein Interaction and Recognition

RNA-recognition motifs and glycine and arginine-rich region cooperatively regulate the nucleolar localization of nucleolin

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Nucleolin (NCL) is a nucleolar protein *i.e.* involved in the regul ation of the nucleolar structure and functions, and consists of thr ee distinct regions: the N-terminal region; the middle region, whi ch contains four RNA-recognition motifs (RRMs); and the C-ter minal glycine- and arginine-rich (GAR) region. The primary fun ction of the RRMs and GAR is thought to be specific RNA bind ing. However, it is not well understood how these RNA-binding regions of NCL separately or cooperatively regulate its nucleolar localization and functions. To address this issue, we constructed mutant proteins carrying point mutations at the four RRMs indiv idually or deletion of the C-terminal GAR region. We found that the GAR deletion and the mutations in the fourth RRM (RRM4) decreased the nucleolar localization of NCL. Biochemical anal yses showed that NCL interacted directly with ribosomal RNAs (rRNAs) and G-rich oligonucleotides, and that this interaction was decreased by mutations at RRM1 and RRM4 and GAR dele tion. Although GAR deletion decreased the rRNA-binding activi ty of NCL, the mutant was efficiently coprecipitated with rRNAs and nucleolar proteins from cell extracts. These contradictory res ults suggest that NCL stably localizes to the nucleoli via the inter actions with rRNAs and nucleolar proteins via GAR, RRM1 and RRM4.

Keywords; G-quadruplex, nucleolus, ribosome biogenesis, RNA-recognition motif, rRNA

Glycobiology and Carbohydrate Biochemistry

Dermatan sulphate promotes neuronal differentiation in mouse and human stem cells

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Dermatan sulphate (DS), a glycosaminoglycan, is present in the extracellular matrix and on the cell surface. Previously, we showed that heparan sulphate plays a key role in the maintenance of the undifferentiated state in mouse embryonic stem cells (mESCs) and in the regulation of their differentiation. Chondroitin sulphate has also been to be important for pluripotency and differentiation of mESCs. Keratan sulphate is a marker of human pluripotent stem cells. To date, however, the function of DS in mESCs has not been clarified. Dermatan 4 sulfotransferase 1, which transfers sulphate to the C-4 hydroxyl group of *N*-acetylgalactosamine of DS, contributes to neuronal differentiation of mouse neural progenitor cells. Therefore, we anticipated that neuronal differentiation would be induced in mESCs in culture by the addition of DS. To test this expectation, we investigated neuronal differentiation in mESCs and human neural stem cells (hNSCs) cultures containing DS. In mESCs, DS promoted neuronal differentiation by activation of extracellular signal-regulated kinase 1/2 and also accelerated neurite outgrowth. In hNSCs, DS promoted neuronal differentiation and neuronal migration, but not neurite outgrowth. Thus, DS promotes neuronal differentiation in both mouse and human stem cells, suggesting that it offers a novel method for efficiently inducing neuronal differentiation.

Keywords; dermatan sulphate, human neural stem cells, mouse embryonic stem cells, neurite outgrowth, neuronal differentiation

AMPK differentially alters sulphated glycosaminoglycans under normal and high glucose milieu in proximal tubular cells

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Glycosaminoglycans (GAGs) and AMP-activated protein kinase (AMPK) are two critical molecular players involved in cellular homeostasis. Both of them are altered due to hyperglycaemia in the kidney, leading to the pathogenesis of diabetic nephropathy. Here, we have looked into the effect of AMPK modulation on sulphated GAG (sGAG) levels of tubular cells of proximal and distal origin to understand the mechanism of hyperglycaemia-mediated pathogenesis of the diabetic nephropathy. In MDCK cells (distal tubular cell) and NRK-52E (proximal tubular cell), AMPK inhibition resulted in increased sGAG levels under normal glucose conditions characteristically of heparan sulphate

class, whereas AMPK activation did not have any effect. High glucose (HG) condition did not alter sGAG levels in MDCK cell despite a decrease in AMPK phosphorylation. Subjecting NRK-52E cells to HG milieu significantly decreased sGAG levels more so of chondroitin/dermatan sulphate, which is significantly prevented when HG is co-treated with AMPK activator. Interestingly, knockdown of AMPK by AMPKα1/α2 siRNA showed increased sGAG levels in NRK-52E. Our results suggest that changes in sGAG level, in particular, as a result of AMPK modulation is differentially regulated and is dependent on cell type as well as its physiological status. Furthermore, activation of AMPK is beneficial in preventing the HG-mediated decrease in sGAGs in proximal tubular cells.

Keywords; AMPK, diabetic nephropathy, high glucose, sulphated glycosaminoglycans, tubular kidney cells

Enzymology

Kinetics and thermodynamics of thermal inactivation for recombinant Escherichia coli cellulases, cel12B, cel8C, and polygalacturonase, peh28; biocatalysts for biofuel precursor production

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Lignocellulosic biomass conversion using cellulases/polygalacturonases is a process that can be progressively influenced by several determinants involved in cellulose microfibril degradation. This article focuses on the kinetics and thermodynamics of thermal inactivation of recombinant *Escherichia coli* cellulas es, cel12B, cel8C and a polygalacturonase, peh 28, derived from *Pectobacterium carotovorum* sub sp. *carotovorum*. Several consensus *motifs* conferring the enzymes' thermal stability in both cel12B and peh28 model structures have been detailed earlier, which were confirmed for the three enzymes through the curre nt study of their thermal inactivation profiles over the 20–80°C range using the respective activities on carboxymethylcellulo se and polygalacturonic acid. Kinetic constants and half-lives of thermal inactivation, inactivation energy, plus inactivation entropies, enthalpies and Gibbs free energies, revealed high stability, less conformational change and protein unfolding for cel12B and peh28 due to thermal denaturation compared to cel8C. The apparent thermal stability of peh28 and cel12B, along with their hydrolytic efficiency on a lignocellulosic biomass conversion as reported previously, makes these enzymes candidates for various industrial applications. Analysis of the Gibbs free energy values suggests that the thermal stabilities of cel12B and peh28 are entropy-controlled over the tested temperature range.

Keywords; catalysis, cellulase, kinetics, polygalacturonase, thermodynamics

Immunochemistry

A novel site-specifi c chemical conjugation of IgG antibodies by affi nity peptide for immunoassays

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Recently, there has been an increasing interest in site-specific modifications of antibodies used in immunoassays for disease diagnosis and as antibody therapeutics, such as antibody−drug conjugates. Previously, we established a site-specific chemical conjugation system using an IgG-Fc binding chemical conjugation affinity peptide (CCAP). CCAP could be used only for the modification of human IgG owing to the lack of affinity of CCAP to rodent IgG molecules. In this study, novel CCAP reagents are proposed, which can be used for both human and mouse IgG, based on the *Staphylococcus aureus* protein A domain-derived af finity peptides Z34C and Z33. Compared with the activity of a conventional randomly modified antibody, mouse IgG modified using this method had favourable features in two immunoassays, demonstrating the advantages of the proposed CCAP method in preserving antibody functionality during conjugation.

Keywords; antibody, conjugation, immunoassay, peptide, protein A, RPLA

Biochemical Pharmacology

BaeR participates in cephalosporins susceptibility by regulating the expression level of outer membrane proteins in Escherichia coli

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The two-component system BaeSR participates in antibiotics resistance of *Escherichia coli*. To know whether the outer membrane proteins involve in the antibiotics resistance mediated by BaeSR, deletion of *acrB* was constructed and the recombined pl

asmid p-*baeR* was introduced into *E. coli* K12 and K12Δ*acrB*. Minimum inhibitory concentrations (MICs) of antibacterial agents were determined by 2-fold broth micro-dilution method. Gene expressions related with major outer membrane proteins and multidrug efflux pump-related genes were determined by real-time quantitative reverse transcription polymerase chain reaction. The results revealed that the MICs of K12Δ*acrB* to the tested drugs except for gentamycin and amikacin decreased 2- to 16.75-folds compared with those of K12. When BaeR was overexpressed, the MICs of K12Δ*acrB*/p-*baeR* to ceftiofur and cefotaxime increased 2.5- and 2-fold, respectively, compared with their corresponding that of K12Δ*acrB*. At the same time, the expression levels of *ompC*, *ompF*, *ompW*, *ompA* and *ompX* showed significant reduction in K12Δ*acrB*/p-*baeR* as compared with K12Δ*acrB*. Moreover, the expression levels of *ompR*, *marA*, *rob* and *tolC* also significantly 'decreased' in K12Δ*acrB*/p-*baeR*. These findings indicated that BaeR overproduction can decrease cephalosporins susceptibility in *acrB*-free *E. coli* by decreasing the expression level of outer membrane proteins.

Keywords; BaeSR, cephalosporins susceptibility, Escherichia coli, outer membrane proteins, two-component system

MOLECULAR BIOLOGY

Gene Expression

Cd(II)-binding transcriptional regulator interacts with isoniazid and regulates drug susceptibility in mycobacteria

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It is urgent to understand the regulatory mechanism of drug resistance in widespread bacterial pathogens. In *Mycobacterium tuberculosis*, several transcriptional regulators have been found to play essential roles in regulating its drug resistance. In this study, we found that an ArsR family transcription regulator encoded by Rv2642 (CdiR) responds to isoniazid (INH), a widely used anti-tuberculosis (TB) drug. CdiR negatively regulates self and adjacent genes, including *arsC* (arsenic-transport integral membrane protein ArsC). CdiR directly interacts with INH and Cd(II). The binding of INH and Cd(II) both reduce its DNA-binding activity. Disrupting *cdiR* increased the drug susceptibility to INH, whereas overexpressing *cdiR* decreased the susceptibility. Strikingly, overexpressing *arsC* increased the drug susceptibility as well as *cdiR*. Additionally, both changes in *cdiR* and *arsC* expression caused sensitivity to other drugs such as rifamycin and ethambutol, where the minimal inhibitory concentrations in the *cdiR* deletion strain were equal to those of the *arsC*-overexpressing strain, suggesting that the function of CdiR in regulating drug resistance primarily depends on *arsC*. Furthermore, we found that Cd(II) enhances bacterial resistance to INH in a CdiR-dependent manner. As a conclusion, CdiR has a critical role in directing the interplay between Cd(II) metal ions and drug susceptibility in mycobacteria.

Keywords; ArsR, arsC, cdiR, drug susceptibility, transcriptional regulator

CELL

Tumor and Immunology

Circular RNA hsa_circ_0102231 sponges miR-145 to promote non-small cell lung cancer cell proliferation by up-regulating the expression of RBBP4

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Circular RNAs (circRNAs) are important regulators in various cancers. Previous studies have found that hsa_circ_0102231 is an oncogene in lung adenocarcinoma. Here, we investigated its mechanism in the development of non-small cell lung cancer (NSCLC). We detected the levels of hsa_circ_0102231 in five NSCLC cell lines and one normal bronchial epithelium cell line. The interaction between hsa_circ_0102231 and miR-145 was predicted and confirmed by pull-down and luciferase assays. The nuclear mass separation assay and fluorescence *in situ* hybridization were used to detect the distribution of hsa_circ_0102231. Cell Counting Kit-8 and Transwell assays were used to assess the cell proliferative and invasive ability. Western blot and RT-qPCR, respectively, detected the protein and mRNA levels of RBBP4. The RBBP4 promoter activity was detected with a luciferase assay. We found that hsa_circ_0102231 level was higher in NSCLC cells. hsa_circ_0102231 is mainly localized to the cytoplasm. hsa_circ_0102231 promotes NSCLC cell proliferation and invasion by sponge for miR-145. miR-145 significantly decreases the RBBP4 promoter activity, and its mRNA and protein levels. RBBP4 is an oncogene to promote proliferation and invasion ability. Our findings suggest that hsa_circ_0102231 promotes proliferation and invasion by mediating the miR-145/RBBP4 axis in NSCLC, indicating that it might be a potential target for NSCLC treatment.

Keywords; hsa_circ_0102231, invasion, miR-145, non-small cell lung cancer (NSCLC), proliferation, RBBP4