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ダイジェスト

JB Review

Featured article of the month.

Factors regulating axon regeneration via JNK MAP kinase in Caenorhabditis elegans

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Axon regeneration following nerve injury is a highly conserved process in animals. The nematode *Caenorhabditis elegans* is an excellent model for investigating the molecular mechanisms of axon regeneration. Recent studies using *C. elegans* have shown that the c-Jun N-terminal kinase (JNK) plays the important role in axon regeneration. Furthermore, many factors have been identified that act upstream of the JNK cascade after axotomy. This review introduces these factors and describes their roles during the regulation of axon regeneration.

Keywords: axon regeneration, c-Jun N-terminal kinase, Caenorhabditis elegans, signalling pathway, svh gene

BIOCHEMISTRY

Biochemistry General

Quercetin attenuates high glucose-induced injury in human retinal pigment epithelial cell line ARPE-19 by up-regulation of miR-29b

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Quercetin is a kind of distinctive bioactive flavonoid that has potent anti-oxidant, anti-inflammatory and anti-diabetic properties. The present article was designed to check the effect of quercetin on diabetic retinopathy. Adult retinal pigment epithelial cell line (ARPE)-19 cells were pre-treated with quercetin and then stimulated by high glucose. Cell damage was evaluated by CCK-8 assay, flow cytometer, quantitative reverse transcriptase polymerase chain reaction (qRT-PCR), enzyme-linked immunosorbent assay, 2,7-dichlorofluorescein diacetate probe and western blot. The association between quercetin and miR-29b expression as well as the downstream pathways was studied by qRT-PCR and western blot. Pre-treating ARPE-19 cells with quercetin clearly attenuated high glucose-induced viability loss, apoptosis, MCP-1 and IL-6 overproduction and reactive oxygen species (ROS) generation. Quercetin down-regulated p53, Bax and cleaved-caspase-3 expression, while up-regulated CyclinD1, CDK4 and Bcl-2. miR-29b was low expressed in high glucose-treated cell, but quercetin elevated its expression. Moreover, the protective action of quercetin towards ARPE-19 cells was attenuated when miR-29b was suppressed. Also, quercetin promoted PTEN/AKT pathway, while inhibited NF-κB pathway via a miR-29b-dependent way. These data illustrated quercetin possibly possess the anti-diabetic retinopathy potential, as quercetin clearly attenuated high glucose-evoked damage in ARPE-19 cells. The protective action of quercetin may due to its regulation on miR-29b expression as well as PTEN/AKT and NF-κB pathways.

Keywords: apoptosis, diabetic retinopathy, inflammation, NF-κB pathway, PTEN/AKT pathway

Downregulated long non-coding RNA LINC01093 in liver fibrosis promotes hepatocyte apoptosis via increasing ubiquitination of SIRT1

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The apoptosis of hepatocytes contributes to the activation of hepatic stellate cells (HSCs), thus promoting the accumulation of extracellular matrix proteins and aggravating liver fibrosis. Silent information regulator 1 (SIRT1) is an anti-fibrotic protein whose downregulation induces hepatocyte apoptosis. This study aims to identify whether SIRT1 is regulated by long non-coding RNA LINC01093 and explore its underlying mechanisms. Liver fibrosis was induced in mice using CCl_4, and the differential expressions of several fibrosis-related long noncoding RNAs were detected in liver tissues. The effect of LINC01093 on cell apoptosis and viability of hepatocytes were investigated after LINC01093 overexpression or knockdown using flow cytometry and MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] assay. The anti-fibrotic effect of LINC01093 overexpression was observed *in vivo*. LncRNA LINC01093 is downregulated in CCl_4-induced liver tissues and TGF-β1-stimulated hepatocytes. Downregulated LINC01093 promoted cell apoptosis and inhibited cell viability of hepatocytes. The co-culture between LINC01093-knockdown hepatocytes and HSCs increased the expressions of pro-fibrotic proteins. Downregulated LINC01093 promoted hepatocyte apoptosis via promoting degradation and ubiquitination of SIRT1 under TGF-β1 stimulation. The injection of LINC01093-overexpressing vectors alleviated liver fibrosis *in vivo*. In liver fibrosis, the downregulated LINC01093 promoted hepatocyte apoptosis, which is mediated by increasing the degradation and ubiquitination of SIRT1.

Keywords: apoptosis, hepatocyte, liver fibrosis, lncRNA

LINC01093, ubiquitination

Protein Structure

Mimicking cotranslational folding of prosubtilisin E in vitro

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Nascent polypeptides are synthesized on ribosomes starting at the N-terminus and simultaneously begin to fold during translation. We constructed N-terminal fragments of prosubtilisin E containing an intramolecular chaperone (IMC) at N-terminus to mimic cotranslational folding intermediates of prosubtilisin. The IMC-fragments of prosubtilisin exhibited progressive enhancement of their secondary structures and thermostabilities with increasing polypeptide length. However, even the largest IMC-fragment with 72 residues truncated from the C-terminus behaved as a molten globule, indicating the requirement of the C-terminal region to have a stable tertiary structure. Furthermore, truncation of the IMC in the IMC-fragments resulted in aggregation, suggesting that the IMC plays a crucial role to prevent misfolding and aggregation of cotranslational folding intermediates during translation of prosubtilisin polypeptide.

Keywords: cotranslational folding intermediate, intramolecular chaperone, prosubtilisin E, secondary structure

Glycobiology and Carbohydrate Biochemistry

Decreased ADAM17 expression in the lungs of a-Klotho reduced mouse

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The deficiency of *α*-Klotho in mice causes phenotypes resembling human age-associated disorders at 3–4 weeks after birth and shows short lifespans of ～2 months. One of the crucial symptoms is pulmonary emphysema, although *α*-Klotho is not expressed in the lungs. *α*-Klotho secreted from the kidneys is probably involved in the pathology of emphysema because kidney-specific knockout mice exhibit emphysematous structural changes. We examined whether any glycan changes in *α*-Klotho mouse lungs were observed, because *α*-Klotho is reported to have glycosidase activity. Here, we found the accumulation of heparan sulphate in the microsomal fraction of *α*-Klotho mouse lungs. Meanwhile, a disintegrin and metalloproteinase 17 (ADAM17) expression was decreased in *α*-Klotho mice. From these results, it is thought that the increase in heparan sulphate is due to insufficient cleavage of the core protein by ADAM17. Additionally, a reduction in *α*-Klotho and a decline of ADAM17 were also observed both in normal aged mice and in senescence marker protein-30 (SMP30) knockout mice, a mouse model of premature ageing. Thus, the decrease in ADAM17 is caused by the reduction in *α*-Klotho. These may be involved in the deterioration of lung function during ageing and may be associated with the pathology of pulmonary emphysema.

Keywords: *α*-Klotho, ADAM17, ageing, COPD, heparan sulphate

Enzyme Inhibitors

Anti-melanogenic activity of salacinol by inhibition of tyrosinase oligosaccharide processing

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Hyperpigmentation that manifests through melasma and solar lentigo (age spots), although mostly harmless for health, bothers many people. Controlling the rate-limiting activity of tyrosinase is most effective for suppressing excessive melanin formation and accordingly recent research has focused on the maturation of tyrosinase. Salacia, a medicinal plant, has been used to treat diabetes in India and Sri Lanka. Salacia extract reportedly contains components that inhibit the activity of *α*-glucosidase. Salacinol, the active ingredient in Salacia extract, has unique thiosugar sulphonium sulphate inner salt structure. Here, we observed that the salacinol component of Salacia extract possesses anti-melanogenic activity in comparison to various existing whitening agents. Although the anti-melanogenic mechanism of salacinol is presumably medicated by inhibition of tyrosinase activity, which is often found in existing whitening agents, salacinol did not inhibit tyrosinase activity *in vitro*. Analysis of the intracellular state of tyrosinase showed a decrease in the mature tyrosinase form due to inhibition of N-linked oligosaccharide processing. Salacinol inhibited the processing glucosidase I/II, which are involved in the initial stage of N-linked glycosylation. Owing to

high activity, low cytotoxicity and high hydrophilicity, salacinol is a promising candidate compound in whitening agents aimed for external application on skin.

Keywords: glucosidase, hyperpigmentation, melanogenesis, salacinol, tyrosinase

Biochemistry of Proteolysis

Histidine 131 in presenilin 1 is the pH-sensitive residue that causes the increase in Ab42 level in acidic pH

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Alzheimer disease (AD) is the most common neurodegenerative disease worldwide. The pathological hallmark of AD is the presence of senile plaques in the brain, which are accumulations of amyloid-β peptide (Aβ) ending at the 42nd residue (*i.e.* Aβ42), which is produced through multistep cleavage by γ-secretase. Thus, methods to regulate γ-secretase activity to attenuate the production of Aβ42 are in urgent demand towards the development of treatments for AD. We and others have demonstrated that γ-secretase activity is affected by its localization and ambient environment. In particular, an increase in Aβ42 production is correlated with the intracellular transport of γ-secretase and endosomal maturation-dependent luminal acidification. In this study, we focused on the mechanism by which γ-secretase affects Aβ42 production together with alterations in pH. Histidine is known to function as a pH sensor in many proteins, to regulate their activities through the protonation state of the imidazole side chain. Among the histidines facing the luminal side of presenilin (PS) 1, which is the catalytic subunit of γ-secretase, point mutations at H131 had no effect on the Aβ42 production ratio in an acidic environment. We also observed an increase in Aβ42 ratio when histidine was introduced into N137 of PS2, which is the corresponding residue of H131 in PS1. These results indicated that H131 serves as the pH sensor in PS1, which contains γ -secretase, to regulate Aβ42 production depending on the luminal pH. Our findings provide new insights into therapeutic strategies for AD targeting endosomes or the intracellular transport of γ-secretase.

Keywords: γ-secretase, Alzheimer disease, acidic pH, histidine, presenilin

MOLECULAR BIOLOGY

Protein Synthesis

Reconstitution of yeast translation elongation and termination in vitro utilizing CrPV IRES-containing mRNA

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We developed an *in vitro* translation system from yeast, reconstituted with purified translation elongation and termination factors and programmed by CrPV IGR IRES-containing mRNA, which functions in the absence of initiation factors. The system is capable of synthesizing the active reporter protein, nanoLuciferase, with a molecular weight of 19 kDa. The protein synthesis by the system is appropriately regulated by controlling its composition, including translation factors, amino acids and antibiotics. We found that a high eEF1A concentration relative to the ribosome concentration is critically required for efficient IRES-mediated translation initiation, to ensure its dominance over IRES-independent random internal translation initiation.

Keywords: CrPV IGR IRES, in vitro translation, translation elongation, translation termination, yeast

In vitro yeast reconstituted translation system reveals function of eIF5A for synthesis of long polypeptide

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We have recently developed an *in vitro* yeast reconstituted translation system, which is capable of synthesizing long polypeptides. Utilizing the system, we examined the role of eIF5A and its hypusine modification in translating polyproline sequence within long open reading frames. We found that polyproline motif inserted at the internal position of the protein arrests translation exclusively at low Mg^{2+} concentrations, and peptidylpolyproline-tRNA intrinsically destabilizes 80S ribosomes. We demonstrate that unmodified eIF5A essentially resolves such ribosome stalling; however, the hypusine modification drastically stimulates ability of eIF5A to rescue polyproline-mediated ribosome stalling and is particularly important for the efficient translation of the N-terminal or long internal polyproline motifs.

Keywords: eIF5A, hypusine, intrinsic ribosome destabilization, in vitro translation, polyproline

CELL

Biomembranes, Organelles, and Protein Sorting

A novel dynein-type AAAþ protein with peroxisomal targeting signal type 2

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Peroxisomal matrix proteins are imported into peroxisomes in a process mediated by peroxisomal targeting signal (PTS) type 1 and 2. The PTS2 proteins are imported into peroxisomes after binding with Pex7p. Niwa *et al*. (A newly isolated Pex7-binding, atypical PTS2 protein P7BP2 is a novel dynein-type AAA+ protein. *J Biochem* 2018;164:437–447) identified a novel Pex7p-binding protein in CHO cells and characterized the subcellular distribution and molecular properties of the human homologue, 'P7BP2'. Interestingly, P7BP2 possesses PTS2 at the NH_2 terminal and six putative AAA+ domains. Another group has suggested that the protein also possesses mitochondrial targeting signal at the NH_2 terminal. In fact, the P7BP2 expressed in mammalian cells is targeted to both peroxisomes and mitochondria. The purified protein from Sf9 cells is a monomer and has a disc-like ring structure, suggesting that P7BP2 is a novel dynein-type AAA+ family protein. The protein expressed in insect cells exhibits ATPase activity. P7BP2 localizes to peroxisomes and mitochondria, and has a common function related to dynein-type ATPases in both organelles.

Keywords: dynein-type AAA+, ATPase, mitochondria, peroxisomal targeting signal, peroxisome, Pex7p

Cell Cycle

MicroRNA-1271-5p inhibits cell proliferation and enhances radiosensitivity by targeting CDK1 in hepatocellular carcinoma

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This study aims to determine whether miR-1271-5p inhibits cell proliferation and enhances the radiosensitivity by targeting cyclin-dependent kinase 1 (CDK1) in hepatocellular carcinoma (HCC). Its expression levels in the HCC cell lines were significantly lower than those in normal human liver cell line. Bioinformatics analysis indicated CDK1 was a potential target of miR-1271-5p. Dual-Luciferase Reporter Assay confirmed that CDK1 is a direct target gene of miR-1271-5p. With overexpression of miR-1271-5p in SMMC-7721 and HuH-7 cells, cell proliferation was decreased, radiosensitivity was enhanced, cell cycle distribution was altered and the growth of transplanted tumours in nude mice was significantly reduced. miR-1271-5p overexpression enhanced radiosensitivity, which could be reduced by CDK1 overexpression. Overall, our findings suggested that miR-1271-5p inhibits cell proliferation and enhances the radiosensitivity of HCC cell lines by targeting CDK1.

Keywords: CDK1, hepatocellular carcinoma, miR-1271-5p, proliferation, radiosensitivity

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ダイジェスト

JB Review

Featured article of the month.

Roles of GPRC5 family proteins: focusing on GPRC5B and lipid-mediated signalling

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In the past decade, physiological roles and molecular functions of GPRC5 family receptors, originally identified as retinoic acid-induced gene products, have been uncovered, even though their intrinsic agonists are still a mystery. They are differentially distributed in certain tissues and cells in the body suggesting that cell-type-specific regulations and functions are significant. Molecular biological approaches and knockout mouse studies reveal that GPRC5 family proteins have pivotal roles in cancer progression and control of metabolic homeostasis pathways. Remarkably, GPRC5B-mediated tyrosine-phosphorylation signalling cascades play a critical role in development of obesity and insulin resistance through dynamic sphingolipid metabolism.

Keywords: ceramide, diacylglycerol, GPRC5B, insulin resistance, sphingomyelin

BIOCHEMISTRY

Biochemistry General

N1 -methyladenosine (m1 A) RNA modifi cation: the key to ribosome control

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RNA displays diverse functions in living cells. The presence of various chemical modifications of RNA mediated by enzymes is one of the factors that impart such functional diversity to RNA. Among more than 100 types of RNA modification, N^1-methyladenosine (m^1A) is found mainly in tRNA and rRNA of many living organisms and is known to be deeply implicated in the topology or function of the two classes of RNA. In this commentary article, we would like to deal with the functional significance of m^1A in RNA, and also to describe one methyltransferase installing m^1A in a large subunit rRNA, whose orthologue in *Caenorhabditis elegans* was discovered recently and was reported in this journal.

Overexpressing microRNA-34a overcomes ABCG2-mediated drug resistance to 5-FU in side population cells from colon cancer via suppressing DLL1

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Colon cancer side population (SP) cells are a small subset of cancer cells that have cancer stemness capacity and enhanced drug resistance. ABCG2 is a multidrug resistance-related protein in SP cells and has been demonstrated to be regulated by Notch signalling pathway. Recently, microRNAs are reported to play a critical role in SP cell fate. However, their role in ABCG2-mediated drug resistance in colon cancer SP cells remains unclear. In the current study, the different expressions of miR-552, miR-611, miR-34a and miR-5000-3p were compared within SP and non-SP cells, which were separated from human colon cancer cell lines (SW480 and LoVo). We found that miR-34a was significantly down-regulated in SP cells and that overexpressing miR-34a overcame drug resistance to 5-fluorouracil (5-FU). The luciferase reporter assay indicated that miR-34a negatively regulated DLL1, a ligand of Notch signalling pathway, via binding with 3′-untranslated region of its messenger RNA. In addition, overexpressing miR-34a overcame ABCG2-mediated resistance to 5-FU via DLL1/Notch pathway *in vitro*, and suppressed tumour growth under 5-FU treatment *in vivo*. In conclusion, our findings suggest that miR-34a acts as a tumour suppressor via enhancing chemosensitivity to 5-FU in SP cells, which provides a novel therapeutic target in chemotherapy-resistant colon cancer.

Keywords: colon cancer, drug resistance, miR-34a, Notch signalling pathway, side population cells

C-Myc-activated long non-coding RNA PVT1 enhances the proliferation of cervical cancer cells by sponging miR-486-3p

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Cervical cancer is one of the most prevalent gynecological malignancies. Although the functions of long non-coding RNA (lncRNA) plasmacytoma variant translocation 1 (PVT1) and c-Myc in tumorigenesis have been acknowledged, the roles of c-Myc and lncRNA-PVT1 in the proliferation of cervical cancer are still unclear. Our study is designed to demonstrate the regulatory network involving c-Myc and lncRNA-PVT1 in cervical cancer. Quantitative real-time PCR and western blot assays were performed in our research to estimate the expression levels of RNA and proteins. CCK8 assays were applied to demonstrate the viability of HeLa and SiHa cells. Immunofluorescence assay was then used to investigate the co-localization of lncRNA-PVT1 and miR-486-3p. Binding of c-Myc to the promoter region of *PVT1* was identified by ChIP-assay. Functionally, upregulation of lncRNA-PVT1 enhanced the proliferation and viability of cervical cancer cells. Mechanistically, lncRNA-PVT1 sponged miR-486-3p and released its repression of extracellular matrix protein 1. Besides, c-Myc functioned as an activator of lncRNA-PVT1 and upregulated its expression by binding to the promoter of *PVT1* in cervical cancer cells. lncRNA-PVT1 was upregulated by c-Myc and thus enhanced the proliferation of cervical cancer cells by sponging miR-486-3p.

Keywords: cervical cancer, c-Myc, extracellular matrix protein 1 (ECM1), lncRNA plasmacytoma variant translocation 1 (PVT1), miR-486-3p

Protein Structure

X-ray dose-dependent structural changes of the [2Fe-2S] ferredoxin from Chlamydomonas reinhardtii

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Plant-type ferredoxin (Fd) is an electron transfer protein in chloroplast. Redox-dependent structural change of Fd controls its association with and dissociation from Fd-dependent enzymes. Among many X-ray structures of oxidized Fd have been reported so far, very likely a given number of them was partially reduced by strong X-ray. To understand the precise structural change between reduced and oxidized Fd, it is important to know whether the crystals of oxidized Fd may or may not be reduced during the X-ray experiment. We prepared the thin plate-shaped Fd crystals from *Chlamydomonas reinhardtii* and monitored its absorption spectra during experiment. Absorption spectra of oxidized Fd crystals were clearly changed to that of reduced form in an X-ray dose-dependent manner. In another independent experiment, the X-ray diffraction images obtained from different parts of one single crystal were sorted and merged to form two datasets with low and high X-ray doses. An Fo–Fo map calculated from the two datasets showed that X-ray reduction causes a small displacement of the iron atoms in the [2Fe-2S] cluster. Both our spectroscopic and crystallographic studies confirm X-ray dose-dependent reduction of Fd, and suggest a structural basis for its initial reduction step especially in the core of the cluster.

Keywords: absorption spectroscopy, ferredoxin, protein–protein interaction, redox-dependent structural change, X-ray crystallography

Structure of HIRAN domain of human HLTF bound to duplex DNA provides structural basis for DNA unwinding to initiate replication fork regression

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Replication fork regression is a mechanism to rescue a stalled fork by various replication stresses, such as DNA lesions. Helicase-like transcription factor, a SNF2 translocase, plays a central role in the fork regression and its N-terminal domain, HIRAN (HIP116 and Rad5 N-terminal), binds the 3′-hydroxy group of single-stranded DNA. Furthermore, HIRAN is supposed to bind double-stranded DNA (dsDNA) and involved in strand separation in the fork regression, whereas structural basis for mechanisms underlying dsDNA binding and strand separation by HIRAN are still unclear. Here, we report the crystal structure of HIRAN bound to duplex DNA. The structure reveals that HIRAN binds the 3′-hydroxy group of DNA and unexpectedly unwinds three nucleobases of the duplex. Phe-142 is involved in the dsDNA binding and the strand separation. In addition, the structure unravels the mechanism underlying sequence-independent recognition for purine bases by HIRAN, where the N-glycosidic bond adopts syn conformation. Our findings indicate direct involvement of HIRAN in the fork regression by separating of the daughter strand from the parental template.

Keywords: crystal structure, DNA damage response, protein–DNA interaction, replication fork regression, template switching

Protein Interaction and Recognition

The constitutive high-affi nity Met-binding site in the kringle domain is dispensable for the signalling activity of hepatocyte growth factor

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Activation of a tyrosine kinase receptor Met by hepatocyte growth factor (HGF) requires binding of proteolytically activated, two-chain (tc) HGF, but the biochemical detail of this ligand–receptor interaction specificity remains elusive because biologically inactive single chain (sc) HGF can also bind to Met with high affinity. We found that this proteolysis-independent Met binding can be eliminated by mutagenesis introduced in the kringle domain without losing the ability to bind and activate cellular Met receptor after proteolytic activation, arguing against this site's involvement in the physiological signalling. This non-signal producing Met–HGF interaction can also be eliminated by addition of a heparin mimetic sucrose octasulphate (SOS). By including SOS in the running buffer, we succeeded in detecting cleavage-dependent tcHGF–Met complex formation by size exclusion chromatography.

Keywords: hepatocyte growth factor, kringle domain, ligand/receptor interaction, Met receptor, size exclusion chromatography

Glycobiology and Carbohydrate Biochemistry

Galectin-lattice sustains function of cationic amino acid transporter and insulin secretion of pancreatic *β* cells

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Maintenance of cell surface residency and function of glycoproteins by lectins are essential for regulating cellular functions. Galectins are β-galactoside-binding lectins and form a galectin-lattice, which regulates stability, clustering, membrane sub-domain localization and endocytosis of plasmalemmal glycoproteins. We have previously reported that galectin-2 (Gal-2) forms a complex with cationic amino acid transporter 3 (CAT3) in pancreatic β cells, although the biological significance of the molecular interaction between Gal-2 and CAT3 has not been elucidated. In this study, we demonstrated that the structure of N-glycan of CAT3 was either tetra- or tri-antennary branch structure carrying β-galactosides, which works as galectin-ligands. Indeed, CAT3 bound to Gal-2 using β-galactoside epitope. Moreover, the disruption of the glycan-mediated bindings between galectins and CAT3 significantly reduced cell surface expression levels of CAT3. The reduced cell surface residency of CAT3 attenuated the cellular arginine uptake activities and subsequently reduced nitric oxide production, and thus impaired the arginine-stimulated insulin secretion of pancreatic β cells. These results indicate that galectin-lattice stabilizes CAT3 by preventing endocytosis to sustain the arginine-stimulated insulin secretion of pancreatic β cells. This provides a novel cell biological insight into the endocrinological mechanism of nutrition metabolism and homeostasis.

Keywords: cationic amino acid transporter, galectin-lattice, insulin secretion, N-glycan, NO production

CELL

Cell General

Bicarbonate enhances the infl ammatory response by activating JAK/STAT signalling in LPS±IFN-γ-stimulated macrophages

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Macrophages, which develop by changing their functions according to various environmental conditions and stimuli, defend against the pathogens and play roles in homoeostasis and disease states. Bicarbonate (HCO_3^-) is important in the maintenance of intracellular and extracellular pH in the body. However, the effects of bicarbonate on macrophage function have not been examined. In this study, we investigated the effects of bicarbonate on macrophage activation in lipopolysaccharide (LPS) and interferon (IFN)-γ (LPS＋IFN-γ)-stimulated murine macrophage-like RAW264.7 cells. The expression of the interleukin (IL)-6, inducible nitric oxide (NO) synthase and cyclooxygenase-2 genes was enhanced by sodium bicarbonate ($NaHCO_3$**)** in a concentration-dependent manner in LPS＋IFN-γ-stimulated RAW264.7 cells. The production of IL-6, **NO-2NO2-** and prostaglandin E_2 was also increased by treatment with $NaHCO_3$ in these cells. Moreover, $NaHCO_3$-mediated elevation of inflammatory gene expression was abrogated by solute carrier (SLC) transporter inhibitors. Furthermore, its $NaHCO_3$-mediated activation was negated by a JAK inhibitor, tofacitinib. $NaHCO_3$-enhanced phosphorylation of STAT1, and its enhancement was abrogated by pre-treating with SLC transporter inhibitors in LPS＋IFN-γ-stimulated RAW264.7 cells. In addition, similar results were obtained in murine bone marrow-derived macrophages. These results indicate that bicarbonate enhanced the inflammatory response through the JAK/STAT signalling in LPS＋IFN-γ-stimulated macrophages.

Keywords: bicarbonate, inflammation, JAK/STAT, macrophage, SLC transporter

Differentiation, Development and Aging

High glucose inhibits osteogenic differentiation of bone marrow mesenchymal stem cells via regulating miR-493-5p/ZEB2 signalling

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Diabetic osteoporosis (DOP) is attributed to the aberrant physiological function of bone marrow mesenchymal stem cells (BMSCs) under high glucose (HG) environment. MicroRNAs (miRNAs) are involved in the pathological processes of DOP. We aimed to explore the underlying mechanism of miRNA in DOP. BMSCs were cultured in osteogenic medium with HG to induce osteogenic differentiation, and the interaction between miR-493-5p and ZEB2 was assessed by luciferase assay. Herein, we found miR-493-5p is gradually reduced during osteogenic differentiation in BMSCs. HG treatment inhibits osteogenic differentiation

and induces an up-regulation of miR-493-5p leading to reduced level of its downstream target ZEB2. Inhibition of miR-493-5p attenuates HG-induced osteogenic differentiation defects by upregulation of ZEB2. Mechanistically, miR-493-5p/ZEB2 signalling mediates HG-inhibited osteogenic differentiation by inactivation of Wnt/*β*-catenin signalling. More importantly, knockdown of miR-493-5p therapeutically alleviated the DOP condition in mice. HG prevents BMSCs osteogenic differentiation via up-regulation of miR-493-5p, which results in reduced level of ZEB2 by directly targeting its 3′-untranslated region of mRNA. Thus, miR-493-5p/ZEB2 is a potential therapeutic target and provides novel strategy for the treatment and management of DOP.

Keywords: bone marrow mesenchymal stem cells, high glucose, miR-493-5p osteogenic differentiation, ZEB2

Tumor and Immunology

Overexpression of RSK4 reverses doxorubicin resistance in human breast cancer cells via PI3K/AKT signalling pathway

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Doxorubicin (DOX) is one of the most effective chemotherapy drugs for the treatment of metastatic breast cancer (BC), but drug resistance becomes an obstacle to treatment. This study aims to investigate the role of Ribosomal S6 protein kinase 4 (RSK4) in regulating BC resistance to DOX. We first used Kaplan–Meier Plotter to identify the prognostic roles of RSK4 in BC. DOX-resistant BC cells (MCF-7/DOX) were constructed and the expression of RSK4 was determined by reverse transcript polymerase chain reaction and western blot. Subsequently, we overexpressed the RSK4 in MCF-7/DOX cells, and measured drug resistance, colony formation, cell migration, invasion ability and cell apoptosis after transfection. In addition, western blot was used to explore the expression of apoptosis-related proteins and BC-resistance protein. Effects of RSK4 on activation of the PI3K/AKT signalling pathway were also tested. Furthermore, tumour xenograft in nude mice was constructed to observe the effect of RSK4 overexpression on tumour growth *in vivo*. In conclusion, RSK4 was positively correlated with survival rate in BC patients, which is lowly expressed in MCF-7/DOX. Meanwhile, the overexpression of RSK4 may inhibit drug resistance, cell migration, invasion, apoptosis and tumour growth. RSK4 may effectively attenuate DOX resistance in BC by inhibiting the PI3K/AKT signalling pathway.

Keywords: breast cancer, doxorubicin, drug-resistance, PI3K/AKT signalling pathway, RSK4