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ダイジェスト

JB Review

Efferocytosis during myocardial infarction

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Myocardial infarction is one of the major causes of death worldwide. Many heart cells die during myocardial infarction through various processes such as necrosis, apoptosis, necroptosis, autophagy-related cell death, pyroptosis and ferroptosis. These dead cells in infarcted hearts expose the so-called 'eat-me' signals, such as phosphatidylserine, on their surfaces, enhancing their removal by professional and non-professional phagocytes. Clearance of dead cells by phagocytes in the diseased hearts plays a crucial role in the pathology of myocardial infarction by inhibiting the inflammatory responses caused by the leakage of contents from dead cells. This review focuses on the rapidly growing understanding of the molecular mechanisms of dead cell phagocytosis, termed efferocytosis, during myocardial infarction, which contributes to the pathophysiology of myocardial infarction.

Keywords: apoptosis, efferocytosis, myocardial infarction, necrosis, phosphatidylserine

BIOCHEMISTRY

Biochemistry General

MicroRNA-448 targets SATB1 to reverse the cisplatin resistance in lung cancer via mediating Wnt/*β*-catenin signalling pathway

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This study aims to examine whether miR-448 reverses the cisplatin (DDP) resistance in lung cancer by modulating SATB1. QRT-PCR and immunohistochemistry were used to examine the miR-448 and SATB1 expressions in DDP-sensitive and -resistant lung cancer patients. A microarray was used to investigate the cytoplasmic/nucleic ratio (C/N ratios) of genes in A549 cells targeted by miR-448, followed by Dual-luciferase reporter gene assay. A549/DDP cells were transfected with miR-448 mimics/inhibitors with or without SATB1 siRNA followed by MTT assay, Edu staining, flow cytometry, qRT-PCR and western blotting. MiR-448 was lower but *SATB1* was increased in DDP-resistant patients and A549/DDP cells. And the patients showed low miR-448 expression or SATB1 positive expression had poor prognosis. SATB1, as a target gene with higher C/N ratios (>1), was found negatively regulated by miR-448. Besides, miR-448 inhibitors increased resistance index of A549/DDP cells, promoted cell proliferation, increased cell distribution in S phrase, declined cell apoptosis and activated Wnt/*β*-catenin pathway. However, SATB1 siRNA could reverse the above effect caused by miR-448 inhibitors. MiR-448 targeting SATB1 to counteract the DDP resistance of lung cancer cells via Wnt/*β*-catenin pathway.

Keywords: cisplatin, drug resistance, lung cancer, miR-448, SATB1

Biochemistry in Diseases and Aging

MicroRNA-1323 downregulation promotes migration and invasion of breast cancer cells by targeting tumour protein D52

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Breast cancer (BC) is one of the most common malignancies globally in women, with high mortality rate as a result of tumour metastasis. MicroRNAs play vital roles in the occurrence and development of human cancer. This study aimed to investigate the biological roles of miR-1323 in BC. The expression levels of miR-1323 were detected by quantitative real-time PCR assay. The effect of miR-1323 on BC cell proliferation was determined by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide and colony formation assay. Wound healing analysis and Matrigel Transwell assay were conducted to evaluate miR-1323-mediated BC cell migration and invasion. A luciferase reporter assay was used to test the target of miR-1323. We found that miR-1323 levels were downregulated in BC tissues and serums. Low-miR-1323 levels were associated with lymph node metastasis and advanced clinical stage. Tumour protein D52 (TPD52) was identified as a direct target of miR-1323. Low expression of miR-1323 contributed to the overexpression of TPD52 leading to enhanced BC progression. Our findings suggest that silencing of miR-1323 enhances BC development by regulating TPD52 expression, suggesting that miR-1323 and TPD52 may serve as potential therapeutic targets for BC treatment.

Keywords: biomarker, breast cancer, metastasis, miR-1323, tumour protein D52

Analytical Biochemistry

Old but not obsolete: an enhanced high-speed immunoblot

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The immunoblotting technique (also known as western blotting) is an essential tool used in biomedical research to determine the relative size and abundance of specific proteins and protein modifications. However, long incubation times severely limit its throughput. We have devised a system that improves antigen binding by cyclic draining and replenishing (CDR) of the antibody solution in conjunction with an immunoreaction enhancing agent. Biochemical analyses revealed that the CDR method reduced the incubation time of the antibodies, and the presence of a commercial immunoreaction enhancing agent altered the affinity of the antibody, respectively. Combination of the CDR method with the immunoreaction enhancing agent considerably enhanced the output signal and further reduced the incubation time of the antibodies. The resulting high-speed immunoblot can be completed in 20 min without any loss in sensitivity. Further, the antibodies are fully reusable. This method is effective for both chemiluminescence and fluorescence detection. Widespread adoption of this technique could dramatically boost efficiency and productivity across the life sciences.

Keywords: chemiluminescence, fluorescence, immunoblot, immunoreaction enhancing agent, western blot

Stirring rate affects thermodynamics and unfolding kinetics in isothermal titration calorimetry

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Isothermal titration calorimetry (ITC) directly provides thermodynamic parameters depicting the energetics of intermolecular interactions in solution. During ITC experiments, a titration syringe with a paddle is continuously rotating to promote a homogeneous mixing. Here, we clarified that the shape of the paddles (flat, corkscrew and small-pitched corkscrew) and the stirring rates influence on the thermodynamic parameters of protein–ligand interaction. Stirring with the flat paddle at lower and higher rate both yielded a lower exothermic heat due to different reasons. The complete reaction with no incompetent fractions was achieved only when the stirring was performed at 500 or 750 rpm using the small-pitched corkscrew paddle. The evaluation of the protein solution after 1,500 rpm stirring indicated that proteins in the soluble fraction decreased to 94% of the initial amount, among which 6% was at an unfolded state. In addition, a significant increase of micron aggregates was confirmed. Furthermore, a new approach for the determination of the unfolding kinetics based on the time dependence of the total reaction heat was developed. This study demonstrates that a proper stirring rate and paddle shape are essential for the reliable estimation of thermodynamic parameters in ITC experiments.

Keywords: binding thermodynamics, isothermal titration calorimetry, protein–ligand interaction, stirring rate, unfolding kinetics

MOLECULAR BIOLOGY

Genes and Other Genetic Materials

JUND-dependent up-regulation of HMOX1 is associated with cisplatin resistance in muscle-invasive bladder cancer

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The standard-of-care for metastatic muscle-invasive bladder cancer (MIBC) is platinum-based chemotherapy regimens. Acquired resistance that occurs frequently through unidentified mechanisms, however, remains the major obstacle for implementing therapeutic effectiveness. Here, using data mining and analysis on clinical samples, we show that expression of JUND, a core component of activator protein-1 family, was significantly induced in cisplatin (CDDP)-resistant MIBC. Accumulation of nuclear JUND was associated with low post-chemotherapy survival in MIBC patients. In both genetically engineered cell models and murine xenograft models, we provided evidence that bladder cancer (BC) cells with excessive JUND expression were less responsive to CDDP treatment. This CDDP resistance was further demonstrated to be mediated, at least in part, by transactivation of HMOX1 [the gene encoding heme oxygenase-1 (HO-1)], one of the most important antioxidant signalling pathways of cell adaptation to stress. One mutation within the HMOX1 promoter successfully abolished oxidative stress-enhanced and JUND-driven HMOX1 promoter activation, suggesting that this unique

site synergized for maximal HO-1 induction in CDDP-challenged BC cells. Overall, our data highlight an indispensible role of JUND, both as a target as a modifier of the oxidative stress signalling, in conferring an adaptive response during the pathogenesis of CDDP resistance in MIBC.

Keywords: cisplatin (CDDP), heme oxygenase-1 (HO-1), JUND, muscle-invasive

Gene Expression

MicroRNA-191 regulates endometrial cancer cell growth via TET1-mediated epigenetic modulation of APC

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Endometrial cancer (EC) is a common gynecological malignancy with relatively favourable prognosis, although alternative diagnostic and therapeutic options remain to be explored for advanced disease. Recent studies enabled to apply microRNAs (miRs) to clinical cancer management as promising diagnostic and therapeutic biomarkers. We here aimed to identify proliferation-associated miRNAs and characterize their functions in EC cells. Our small RNA-sequencing analysis showed that miR-191 is abundantly expressed in HEC-1A and Ishikawa EC cells along with the high expression of miR-182, which was previously characterized as an EC proliferation-related miRNA in EC. We showed that miR-191 was upregulated in EC tissues than in adjacent normal tissues and its knockdown repressed EC cell proliferation. *In silico* miRNA target screening identified that ten–eleven translocation 1 (*TET1*) is one of the putative miR-191 targets. *TET1* expression could be downregulated by miR-191 through the mRNA–miRNA interaction in the 3′-untranslated region of *TET1*. In line with TET1 functions as a methylcytosine dioxygenase, which removes genome-wide DNA methylation marks, decreased TET1 expression resulted in hypermethylation in the promotor region of tumour suppressor *adenomatous polyposis coli*. Taken together, miR-191 could function as an oncogenic miRNA in EC and serve as a prospective diagnostic and therapeutic target for advanced disease.

Keywords: 5-hydroxymethylcytosine, endometrial cancer, epigenetic regulation, miRNA-191, ten–eleven translocation 1

CELL

Cell General

Differential roles of two DDX17 isoforms in the formation of membraneless organelles

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The RNA helicase, DDX17 is a member of the DEAD-box protein family. DDX17 has two isoforms: p72 and p82. The p82 isoform has additional amino acid sequences called intrinsically disordered regions (IDRs), which are related to the formation of membraneless organelles (MLOs). Here, we reveal that p72 is mostly localized to the nucleoplasm, while p82 is localized to the nucleoplasm and nucleoli. Additionally, p82 exhibited slower intranuclear mobility than p72. Furthermore, the enzymatic mutants of both p72 and p82 accumulate into the stress granules. The enzymatic mutant of p82 abolishes nucleolar localization of p82. Our findings suggest the importance of IDRs and enzymatic activity of DEAD-box proteins in the intracellular distribution and formation of MLOs.

Keywords: DDX17, intrinsically disordered regions, membraneless organelles, nucleolus, stress granules

Biomembranes / Organelles and Protein Sorting

Isoform-dependent subcellular localization of LMTK1A and LMTK1B and their roles in axon outgrowth and spine formation

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Lemur kinase 1 (LMTK1) is a membrane-bound Ser/Thr kinase that is expressed in neurons. There are two splicing variants of

LMTK1 with different membrane binding modes, viz., cytosolic LMTK1A that binds to membranes through palmitoylation at the N-terminal cysteines and LMTK1B, an integral membrane protein with transmembrane sequences. We recently reported that LMTK1A regulates axon outgrowth and spine formation in neurons. However, data about LMTK1B are scarce. We analysed the expression and cellular localization of LMTK1B along with its role in axon and spine formation. We found that both LMTK1B and LMTK1A were expressed equally in the cerebral cortex and cerebellum of the mouse brain. Similar to LMTK1A, the wild type of LMTK1B was localized to Rab11-positive pericentrosomal compartment. The kinase negative (kn) mutant of LMTK1B was found to be associated with an increase in the tubular form of endoplasmic reticulum (ER), which was not the case with LMTK1A kn. Furthermore, unlike LMTK1A kn, LMTK1B kn did not stimulate the axon outgrowth and spine formation. These results suggest that while LMTK1A and LMTK1B share a common function in recycling endosomal trafficking at the pericentrosomal compartment, LMTK1B has an additional unique function in vesicle transport in the ER region.

Keywords: axon outgrowth, endoplasmic reticulum, endosome trafficking, LMTK1, Rab

BIOTECHNOLOGY

Gene and Protein Engineering

PCR-based approach for site-specifi c conjugation of long ouble-stranded DNA to a single-domain VHH antibody

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Site-specific conjugation of double-stranded DNA using antibodies enables the development of unique applications for antibody–drug conjugates utilizing recent advances in nucleic acid medicines. Here, we describe a novel method to conjugate a camelid-derived single-domain VHH (variable domain of a heavy chain antibody) antibody with arbitrarily sized double-stranded DNA by PCR. Cysteine in anti-human epidermal growth factor receptor (EGFR) VHH was replaced by alanine, and an unpaired cysteine was introduced at the carboxyl terminus. These modifications enabled site-specific labelling with a maleimide-modified DNA oligo via thioether bond formation; the ensuing product—single-stranded DNA conjugated at the carboxyl terminus of VHH—retained its affinity for EGFR. To investigate whether this VHH–single-stranded DNA conjugate might be used as a forward primer, we subjected it to PCR, producing 100–500 bp DNA. We confirmed the amplification of the VHH–double-stranded DNA conjugate by examining its mobility on acrylamide gel; retention of the binding affinity of the conjugate for EGFR was identified by immuno-PCR.

Keywords: disulphide, DNA conjugation, heat resistance, PCR, VHH antibody

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ダイジェスト

JB Review

The coupling of translational control and stress responses

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The translation of messenger RNA (mRNA) into protein is a multistep process by which genetic information transcribed into an mRNA is decoded to produce a specific polypeptide chain of amino acids. Ribosomes play a central role in translation by coordinately working with various translation regulatory factors and aminoacyl-transfer RNAs. Various stresses attenuate the ribosomal synthesis in the nucleolus as well as the translation rate in the cytosol. To efficiently reallocate cellular energy and resources, mammalian cells are endowed with mechanisms that directly link the suppression of translation-related processes to the activation of stress adaptation programmes. This review focuses on the integrated stress response (ISR) and the nucleolar stress response (NSR) both of which are activated by various stressors and selectively upregulate stress-responsive transcription factors. Emerging findings have delineated the detailed molecular mechanisms of the ISR and NSR and expanded their physiological and pathological significances.

Keywords: integrated stress response, nucleolar stress response, protein synthesis, ribosome biosynthesis, translation initiation

BIOCHEMISTRY

Biochemistry General

DNMT1 promotes cell proliferation via methylating hMLH1 and hMSH2 promoters in EGFR-mutated non-small cell lung cancer

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Aberrant DNA methylation is a common form of epigenetic alterations and it has been proved to be closely related to many cancers, while its role in epidermal growth factor receptor (EGFR)-mutated non-small cell lung cancer (NSCLC) is not clear. This study focuses on the role of DNA methyltransferase 1 (DNMT1) in EGFR-mutated NSCLC pathogenesis. First, the expression of DNMT1 was up-regulated, while the expressions of human mutL homolog 1(hMLH1) and human mutS homolog 2 (hMSH2) were down-regulated in EGFR-mutated NSCLC patients and cell line HCC827. The results of the correlation analysis showed that DNMT1 expression was inversely correlated with the expressions of hMLH1 and hMSH2. Then, we found that DNMT1 enhanced the promoter methylation levels of hMLH1 and hMSH2, thus suppressing their expressions. DNMT1 knockdown inhibited the proliferation of HCC827 cells, while both hMLH1 knockdown and hMSH2 knockdown could eliminate its inhibitory effect on cell proliferation. In xenograft mouse models, lentiviral vector-sh-DNMT1 could significantly reduce tumor volumes, confirmed that DNMT1 inhibited tumor cell proliferation *in vivo*. In conclusion, DNMT1 suppressed the expressions of hMLH1 and hMSH2 via elevating their promoter methylation, thus promoting cell proliferation in EGFR-mutated NSCLC.

Keywords: DNA methylation, DNA methyltransferase 1, EGFR-mutated NSCLC, human mutL homolog 1, human mutS homolog 2

LncRNA SDHAP1 confers paclitaxel resistance of ovarian cancer by regulating EIF4G2 expression via miR-4465

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Ovarian cancer has ranked as one of the leading causes of female morbidity and mortality around the world, which affects ~ 239,000 patients and causes 152,000 deaths every year. Chemotherapeutic resistance of ovarian cancer remains a devastating actuality in clinic. The aberrant upregulation of long non-coding RNA succinate dehydrogenase complex flavoprotein subunit A pseudogene 1 (lncRNA SDHAP1) in the Paclitaxel (PTX)-resistant ovarian cancer cell lines has been reported. However, studies focussed on SDHAP1 in its regulatory function of chemotherapeutic resistance in ovarian cancer are limited, and the detailed mechanisms remain unclear. In this study, we demonstrated that SDHAP1 was upregulated in PTX-resistant SKOV3 and Hey-8 ovarian cancer cell lines while the level of miR-4465 was downregulated. Knocking-down SDHAP1 induced reacquirement of chemo-sensitivity to PTX in ovarian cancer cells *in vitro*. Mechanically, SDHAP1 upregulated the expression of EIF4G2 by sponging miR-4465 and thus facilitated the PTX-induced apoptosis in ovarian cancer cells. The regulation network involving SDHAP1, miR-4465 and EIF4G2 could be a potential therapy target for the PTX-resistant ovarian cancer.

Keywords: eukaryotic translation initiation factor 4 F (EIF4F), lncRNA succinate dehydrogenase complex flavoprotein subunit A pseudogene 1 (SDHAP1), miR-4465, Paclitaxel (PTX)

Long non-coding RNA MIAT promotes cervical cancer proliferation and migration

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Cervical cancer is one of the most common cancers in the world while its pathological mechanisms are not well-elucidated. Long non-coding RNA (lncRNA) has been implicated in cancer development. The dysregulation of lncRNA myocardial infarction-associated transcript (MIAT) has been reported in several cancers while its role in cervical cancer is not described yet. In this study, the role of MIAT in cervical cancer was explored. We evaluated the expression of MIAT in cervical cancer tissues and cell lines. Furthermore, we explored the effects of MIAT on proliferation and invasion of cervical cancer using cell model and animal transplantation model. We also evaluated the effects of MIAT on activation of PI3K/Akt/mTOR signalling pathway. Our results show that MIAT was up-regulated in cervical cancer tissues and cell lines. Knocking down MIAT resulted in decreased cell proliferation, migration and invasion of cervical cancer cells and suppression of tumour growth in mice. Mechanically, knocking down MIAT suppressed the activation of PI3K/Akt signalling pathway. In conclusion, MIAT promotes cell proliferation and invasion in cervical cancer.

Keywords: cervical cancer, long non-coding RNA, MIAT, proliferation

Protein Structure

Structure, dynamics and function of the evolutionarily changing biliverdin reductase B family

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Biliverdin reductase B (BLVRB) family members are general flavin reductases critical in maintaining cellular redox with recent findings revealing that BLVRB alone can dictate cellular fate. However, as opposed to most enzymes, the BLVRB family remains enigmatic with an evolutionarily changing active site and unknown structural and functional consequences. Here, we applied a multi-faceted approach that combines X-ray crystallography, NMR and kinetics methods to elucidate the structural and functional basis of the evolutionarily changing BLVRB active site. Using a panel of three BLVRB isoforms (human, lemur and hyrax) and multiple human BLVRB mutants, our studies reveal a novel evolutionary mechanism where coenzyme 'clamps' formed by arginine side chains at two co-evolving positions within the active site serve to slow coenzyme release (Positions 14 and 78). We find that coenzyme release is further slowed by the weaker binding substrate, resulting in relatively slow turnover numbers. However, different BLVRB active sites imposed by either evolution or mutagenesis exhibit a surprising inverse relationship between coenzyme release and substrate turnover that is independent of the faster chemical step of hydride transfer also measured here. Collectively, our studies have elucidated the role of the evolutionarily changing BLVRB active site that serves to modulate coenzyme release and has revealed that coenzyme release is coupled to substrate turnover.
Keywords: biliverdin reductase B, coenzyme, dynamics, enzyme

MOLECULAR BIOLOGY
Molecular Biology General

MicroRNA-760 inhibits cell viability and migration through down-regulating BST2 in gastric cancer

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Gastric cancer is one of the most common types of carcinoma with a threat to global health. MicroRNA-760 (miR-760) was significantly down-regulated in the primary tumour of patients with advanced gastric cancer. However, the role of miR-760 in gastric cancer is still unclear. Herein, miR-760 was down-regulated in gastric cancer tissues. Moreover, miR-760 overexpression and knockdown were conducted in gastric cancer cells (MGC-803 and SGC-7901) *in vitro*. The *in vitro* functional assays proved that miR-760 overexpression reduced cell viability, cell cycle, migration and invasion, promoted apoptosis and suppressed MMP activity in MGC-803 cells. Conversely, miR-760 knockdown led to the opposite in SGC-7901 cells. Notably, bone marrow stromal antigen 2 (BST2) was verified as a target gene of miR-760. MiR-760 mimics down-regulated BST2 level in gastric cancer tissues and in MGC-803 cells, whereas miR-760 inhibitor up-regulated its level in SGC-7901 cells. MiR-760-regulated cell properties through reduction of BST2. In addition, miR-760 inhibited tumourigenesis in a nude mouse xenograft model *in vivo*. In conclusion, our results demonstrated that miR-760 exhibited a suppressive role in gastric cancer via inhibiting BST2, indicating that miR-760/BST2 axis may provide promising therapeutic target for gastric cancer.
Keywords: BST2, gastric cancer, migration, miR-760, viability

Protein Synthesis

Translation effi ciency affects the sequence-independent +1 ribosomal frameshifting by polyamines

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Antizyme (AZ) interacts with ornithine decarboxylase, which catalyzes the first step of polyamine biosynthesis and recruits it to the proteasome for degradation. Synthesizing the functional AZ protein requires transition of the reading frame at the termination codon. This programmed +1 ribosomal frameshifting is induced by polyamines, but the molecular mechanism is still unknown. In this study, we explored the mechanism of polyamine-dependent +1 frameshifting using a human cell-free translation system. Unexpectedly, spermidine induced +1 frameshifting in the mutants replacing the termination codon at the shift site with a sense codon. Truncation experiments showed that +1 frameshifting occurred promiscuously in various positions of the AZ sequence. The probability of this sequence-independent +1 frameshifting increased in proportion to the length of the open reading frame. Furthermore, the +1 frameshifting was induced in some sequences other than the AZ gene in a polyamine-

dependent manner. These findings suggest that polyamines have the potential to shift the reading frame in the +1 direction in any sequence. Finally, we showed that the probability of the sequence-independent +1 frameshifting by polyamines is likely inversely correlated with translation efficiency. Based on these results, we propose a model of the molecular mechanism for AZ +1 frameshifting.

Keywords: human cell-free translation system, polyamine, ribosomal frameshifting, spermidine, translation control

Genetic Diseases

Aminoglycosides are effi cient reagents to induce readthrough of premature termination codon in mutant B4GALNT1 genes found in families of hereditary spastic paraplegia

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The readthrough of premature termination codon (PTC) by ribosome sometimes produces full-length proteins. We previously reported a readthrough of PTC of glycosyltransferase gene *B4GALNT1* with hereditary spastic paraplegia (HSP). Here we featured the readthrough of *B4GALNT1* of two mutants, M4 and M2 with PTC by immunoblotting and flow cytometry after transfection of *B4GALNT1* cDNAs into cells. Immunoblotting showed a faint band of full-length mutant protein of M4 but not M2 at a similar position with that of wild-type B4GALNT1. AGC sequences at immediately before and after the PTC in M4 were critical for the readthrough. Treatment of cells transfected with mutant M4 cDNA with aminoglycosides resulted in increased readthrough of PTC. Furthermore, treatment of transfectants of mutant M2 cDNA with G418 also resulted in the induction of readthrough of PTC. Both M4 and M2 cDNA transfectants showed increased/induced bands in immunoblotting and GM2 expression in a dose-dependent manner of aminoglycosides. Results of mass spectrometry supported this effect. Here, we showed for the first time the induction and/or enhancement of the readthrough of PTCs of *B4GALNT1* by aminoglycoside treatment, suggesting that aminoglycosides are efficient for patients with HSP caused by PTC of *B4GALNT1*, in which gradual neurological disorders emerged with aging.

Keywords: B4GALNT1, ganglioside, glycosyltransferase, hereditary spastic paraplegia, premature termination codon

CELL

Receptors and Signal Transduction

Regulation of epidermal growth factor receptor expression and morphology of lung epithelial cells by interleukin-1*β*

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Accumulating evidences suggested that the overactivation of epidermal growth factor receptor (EGFR) was involved in the development of adult respiratory distress syndrome and pulmonary fibrosis. Elucidation of the mechanisms that regulate EGFR residence on the plasma membrane during inflammatory lung conditions is important for identifying potential therapies. We have demonstrated that flagellin phosphorylated EGFR at Ser1047 and induced transient EGFR internalization. In this study, we examined the molecular pathway and effect of interleukin 1 beta (IL-1*β*) on EGFR in alveolar epithelial cells. Treatment of A549 cells with IL-1*β* induced the activation of p38 mitogen-activated protein kinase (MAP kinase) and MAP kinase-activated protein kinase-2 (MAPKAPK-2), as well as EGFR phosphorylation at serine 1047. Both MAPKAPK-2 activation and EGFR phosphorylation were inhibited by SB203580, a p38 MAP kinase inhibitor. In addition, MK2a inhibitor (a MAPKAPK-2 inhibitor) suppressed EGFR phosphorylation. Assessment of the biotinylation of cell surface proteins indicated that IL-1*β* induced EGFR internalization. Furthermore, long-term treatment of A549 cells with IL-1*β* caused morphological changes and loss of cell–cell contact. Moreover, IL-1*β* augmented the effect of transforming growth factor beta 1 on the epithelial–mesenchymal transition. These results suggested that IL-1*β* regulates EGFR functions and induces morphological changes of alveolar epithelial cells.

Keywords: alveolar epithelial cells, EGF receptor, IL-1 beta, MAPKAPK-2, p38 MAP kinase

Stress Proteins and Molecular Chaperones

Dynamic localization of *α*B-crystallin at the microtubule cytoskeleton network in beating heart cells

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αB-crystallin is highly expressed in the heart and slow skeletal muscle; however, the roles of αB-crystallin in the muscle are obscure. Previously, we showed that αB-crystallin localizes at the sarcomere Z-bands, corresponding to the focal adhesions of cultured cells. In myoblast cells, αB-crystallin completely colocalizes with microtubules and maintains cell shape and adhesion. In this study, we show that in beating cardiomyocytes α-tubulin and αB-crystallin colocalize at the I- and Z-bands of the myocardium, where it may function as a molecular chaperone for tubulin/microtubules. Fluorescence recovery after photobleaching (FRAP) analysis revealed that the striated patterns of GFP-αB-crystallin fluorescence recovered quickly at 37°C. FRAP mobility assay also showed αB-crystallin to be associated with nocodazole-treated free tubulin dimers but not with taxol-treated microtubules. The interaction of αB-crystallin and free tubulin was further confirmed by immunoprecipitation and microtubule sedimentation assay in the presence of 1–100 μM calcium, which destabilizes microtubules. Förster resonance energy transfer analysis showed that αB-crystallin and tubulin were at 1–10 nm apart from each other in the presence of colchicine. These results suggested that αB-crystallin may play an essential role in microtubule dynamics by maintaining free tubulin in striated muscles, such as the soleus or cardiac muscles.

Keywords: αB-crystallin, dynamics (cytoskeleton), FRAP, microtubules, small heat shock protein