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ダイジェスト

JB Review

Featured article of the month

Post-transcriptional regulation of infl ammation by RNA-binding proteins via cis-elements of mRNAs

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In human genome, there are approximately 1,500 RNA-binding proteins (RBPs). They can regulate mRNA stability or translational efficiency via ribosomes and these processes are known as 'post-transcriptional regulation'. Accumulating evidences indicate that post-transcriptional regulation is the determinant of the accurate levels of cytokines mRNAs. While transcriptional regulation of cytokines mRNAs has been well studied and found to be important for the rapid induction of mRNA and regulation of the acute phase of inflammation, post-transcriptional regulation by RBPs is essential for resolving inflammation in the later phase, and their dysfunction may lead to severe autoimmune diseases such as rheumatoid arthritis or systemic lupus erythematosus. For post-transcriptional regulation, RBPs recognize and directly bind to *cis*-regulatory elements in 3′ untranslated region of mRNAs such as AU-rich or constitutive decay elements and play various roles. In this review, we summarize the recent findings regarding the role of RBPs in the regulation of inflammation.

Keywords: chronic inflammation, cis-regulatory element, post-transcriptional regulation, RNA-binding protein, translation

BIOCHEMISTRY

Biochemistry General

MicroRNA-449b-5p suppresses cell proliferation, migration and invasion by targeting TPD52 in nasopharyngeal carcinoma

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Nasopharyngeal carcinoma (NPC) is an important type of head and neck malignant cancer with geographical distribution. MicroRNA-449b-5p (miR-449b-5p) is related to the development of various cancers, while its function in NPC remains unknown. The present study aimed to investigate the role and target gene of miR-449b-5p in NPC. Expressions of miR-449b-5p in NPC cell lines and clinical tissues were detected by quantitative real-time polymerase chain reaction (qRT-PCR). Cell proliferation was determined by MTT and colony formation assays. Migration and invasion abilities after different treatment were evaluated by wound healing and Transwell assays, respectively. Dual-luciferase reporter assay was performed to explore the relationship between miR-449b-5p and tumour protein D52 (TPD52). TPD52 expression was determined by qRT-PCR and western blot assay. miR-449b-5p was significantly downregulated in NPC cell lines and clinical tissues than the matched control. Overexpression of miR-449b-5p inhibited proliferation, migration and invasion of NPC cells. Dual-luciferase reporter assay indicated that miR-449b-5p directly targeted TPD52. Furthermore, shRNA-mediated downregulation of TPD52 rectified the promotion of cell migration and invasion by miR-449b-5p inhibition. In conclusion, the present study suggests that miR-449b-5p, as a novel tumour-suppressive miRNA against NPC, inhibits proliferation, migration and invasion of NPC cells via inhibiting TPD52 expression.

Keywords: invasion, microRNA-449b-5p, migration, nasopharyngeal carcinoma, tumour protein D52

Protein Interaction and Recognition

Multifunctional transcriptional coactivator PC4 is a global co-regulator of p53-dependent stress response and gene regulation

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Human positive coactivator 4 (PC4), a multifunctional chromatin-associated protein, is known to directly interact with p53 and modulate expressions of a few p53-dependent genes. However, the role of PC4 in p53′s myriad of other regulatory functions is not known. The p53–PC4 interaction was selectively perturbed by a small peptide which led to abrogation of genotoxic stress-induced up-regulation of many p53-dependent genes and reduction of apoptosis in A549 cells. Over-expression of a PC4 point mutant, incapable of binding p53, recapitulated many of the effects of the peptide. Global gene expression profiling in A549 cells, upon peptide treatment, revealed PC4′s involvement in the regulation of many p53-dependent pathways, including the Hip-

po pathway. Introduction of the peptide in neuronal cells significantly reduced its amyloid-β-induced death. Thus, PC4 emerges as a global co-regulator of p53 and a therapeutic target against pathogeneses where the p53-dependent cell death process plays a crucial role.

Keywords: cell death, gene regulation, neuron, PC4, p53

Lipid Biochemistry

Glycosylinositol phosphoceramide-specific phospholipase D activity catalyzes transphosphatidylation

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Glycosylinositol phosphoceramide (GIPC) is the most abundant sphingolipid in plants and fungi. Recently, we detected GIPC-specific phospholipase D (GIPC-PLD) activity in plants. Here, we found that GIPC-PLD activity in young cabbage leaves catalyzes transphosphatidylation. The available alcohol for this reaction is a primary alcohol with a chain length below C4. Neither secondary alcohol, tertiary alcohol, choline, serine nor glycerol serves as an acceptor for transphosphatidylation of GIPC-PLD. We also found that cabbage GIPC-PLD prefers GIPC containing two sugars. Neither inositol phosphoceramide, mannosylinositol phosphoceramide nor GIPC with three sugar chains served as substrate. GIPC-PLD will become a useful catalyst for modification of polar head group of sphingophospholipid.

Keywords: glycosylinositol phosphoceramide, phospholipase D, phytoceramide 1-phosphate, sphingolipid

Biochemistry in Cell Membranes

Propofol alleviates oxidative stress via upregulating lncRNA-TUG1/ Brg1 pathway in hypoxia/reoxygenation hepatic cells

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Reducing oxidative stress is an effective method to prevent hepatic ischaemia/reperfusion injury (HIRI). This study focuses on the role of propofol on the oxidative stress of hepatic cells and the involved lncRNA-TUG1/Brahma-related gene 1 (Brg1) pathway in HIRI mice. The mouse HIRI model was established and was intraperitoneally injected with propofol postconditioning. Hepatic injury indexes were used to evaluate HIRI. The oxidative stress was indicated by increasing 8-isoprostane concentration. Mouse hepatic cell line AML12 was treated with hypoxia and subsequent reoxygenation (H/R). The targeted regulation of lncRNA-TUG1 on Brg1 was proved by RNA pull-down, RIP (RNA-binding protein immunoprecipitation) and the expression level of Brg1 responds to silencing or overexpression of lncRNA-TUG1. Propofol alleviates HIRI and induces the upregulation of lncRNA-TUG1 in the mouse HIRI model. Propofol increases cell viability and lncRNA-TUG1 expression level in H/R-treated hepatic cells. In H/R plus propofol-treated hepatic cells, lncRNA-TUG1 silencing reduces cell viability and increased oxidative stress. LncRNA-TUG1 interacts with Brg1 protein and keeps its level via inhibiting its degradation. Brg1 overexpression reverses lncRNA-TUG1 induced the reduction of cell viability and the increase in oxidative stress. LncRNA-TUG1 silencing abrogates the protective role of propofol against HIRI in the mouse HIRI model. LncRNA-TUG1 has a targeted regulation of Brg1, and thereby affects the oxidative stress induced by HIRI. This pathway mediates the protective effect of propofol against HIRI of hepatic cell.

Keywords: hepatic ischaemia/reperfusion injury (HIRI), lncRNA-TUG1, oxidative stress, propofol

Plasmalogen mediates integration of adherens junction

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Ether glycerolipids, plasmalogens are found in various mammalian cells and tissues. However, physiological role of plasmalogens in epithelial cells remains unknown. We herein show that synthesis of ethanolamine-containing plasmalogens, plasmenylethanolamine (PlsEtn), is deficient in MCF7 cells, an epithelial cell line, with severely reduced expression of alkyl-dihydroxyacetonephosphate synthase (ADAPS), the second enzyme in the PlsEtn biosynthesis. Moreover, expression of ADAPS or supplementation of PlsEtn containing C18-alkenyl residue delays the migration of MCF7 cells as compared to that mock-treated MCF7 and C16-alkenyl-PlsEtn-supplemented MCF7 cells. Localization of E-cadherin to cell–cell junctions is highly aug-

mented in cells containing C18-alkenyl-PlsEtn. Together, these results suggest that PlsEtn containing C18-alkenyl residue plays a distinct role in the integrity of E-cadherin-mediated adherens junction.

Keywords: adherens junction, cell migration, epithelial cells, peroxisome, plasmalogen

Biochemistry in Diseases and Aging

Cooperative action of APJ and α1A-adrenergic receptor in vascular smooth muscle cells induces vasoconstriction

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The apelin receptor (APJ), a receptor for apelin and elabela/apela, induces vasodilation and vasoconstriction in blood vessels. However, the prolonged effects of increased APJ-mediated signalling, involving vasoconstriction, in smooth muscle cells have not been fully characterized. Here, we investigated the vasoactive effects of APJ gain of function under the control of the smooth muscle actin (SMA) gene promoter in mice. Transgenic overexpression of APJ (SMA-APJ) conferred sensitivity to blood pressure and vascular contraction induced by apelin administration *in vivo*. Interestingly, *ex vivo* experiments showed that apelin markedly increased the vasoconstriction of isolated aorta induced by noradrenaline (NA), an agonist for α- and β-adrenergic receptors, or phenylephrine, a specific agonist for α1-adrenergic receptor (α1-AR). In addition, intracellular calcium influx was augmented by apelin with NA in HEK293T cells expressing APJ and α1A-AR. To examine the cooperative action of APJ and α1A-AR in the regulation of vasoconstriction, we developed α1A-AR deficient mice using a genome-editing technique, and then established SMA-APJ/α1A-AR-KO mice. In the latter mouse line, aortic vasoconstriction induced by a specific agonist for α1A-AR, A-61603, were significantly less than in SMA-APJ mice. These results suggest that the APJ-enhanced response requires α1A-AR to contract vessels coordinately.

Keywords: apelin, APJ, α1A-AR, vasoconstriction, GPCRs

BIOTECHNOLOGY

Biotechnology General

Production and characterization of monoclonal antibodies against the DNA binding domain of the RE1-silencing transcription factor

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The use of monoclonal antibodies for the detection of cellular biomarkers during carcinogenesis provides new strategies for cancer diagnosis or prognosis in patients. Loss of the Restrictive Element 1-Silencing Transcription (REST) factor has been observed in previous molecular and immunological approaches in aggressive breast cancer, small cell lung cancer, liver carcinoma, and colo-rectal cancer; however, for clinic diagnosis, monoclonal antibodies for REST recognition are unavailable. The goal of this work was to design, produce and characterize monoclonal antibodies against the REST DNA binding damain (DBD) that would be suitable for immunoassays. We searched for conserved domains, and immunogenic and antigenic sites in the REST structure via *in silico* analysis. For mice immunization, we used a recombinant REST DBD purified by affinity chromatography, and then Hybridomas were generated by mouse spleen fusion with myeloma cells. Finally, for monoclonal antibody characterization, we performed enzyme-linked immunosorbent (ELISA), western blot, dot blot, immunocytochemistry (ICC) and immunoprecipitation assays. Results showed that the DBD is conserved in REST isoforms and contains immunogenic and antigenic sites. We generated three clones producing monoclonal

antibodies against REST DBD, one of them specifically recognized native REST and was suitable for ICC in samples from patients.

Keywords: DNA binding domain, immunoassays, monoclonal antibodies, NRSF, REST

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ダイジェスト

Thanking All Peer Reviewers
The JB Award

BIOCHEMISTRY
Protein Structure

Deciphering the anti-Parkinson's activity of sulphated polysaccharides from Chlamydomonas reinhardtii on the α-Synuclein mutants A30P, A53T, E46K, E57K and E35K

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Parkinsonism-linked mutations in alanine and glutamic acid residues of the pre-synaptic protein α-Synuclein (α-Syn) affect specific tertiary interactions essential for stability of the native state and make it prone to more aggregation. Many of the currently available drugs used for the treatment of Parkinson's disease (PD) are not very effective and are associated with multiple side effects. Recently, marine algae have been reported to have sulphated polysaccharides which offers multiple pharmaceutical properties. With this background, we have isolated sulphated polysaccharides from *Chlamydomonas reinhardtii* (Cr-SPs) and investigated their effects on inhibition of fibrillation/aggregation of α-Syn mutants through a combination of spectroscopic and microscopic techniques. The kinetics of α-Syn fibrillation establishes that Cr-SPs are very effective in inhibiting fibrillation of α-Syn mutants. The morphological changes associated with the fibrillation/aggregation process have been monitored by transmission electron microscopy. Sodium dodecyl sulphate-polyacrylamide gel electrophoresis gel image suggests that Cr-SPs increase the amount of soluble protein after completion of the fibrillation/aggregation process. The circular dichroism results showed that Cr-SPs efficiently delay the conversion of native protein into β-sheet-rich structures. Thus, the current work has considerable therapeutic implications towards deciphering the potential of Cr-SPs to act against PD and other protein aggregation-related disorders.

Keywords: fibrillation inhibition, Parkinson's disease, sulphated polysaccharides, therapeutics, α-Synuclein mutants

Structural and functional characterization of a glycoside hydrolase family 3 β-N-acetylglucosaminidase from Paenibacillus sp. str. FPU-7

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Chitin, a β-1,4-linked homopolysaccharide of *N*-acetyl-D-glucosamine (GlcNAc), is one of the most abundant biopolymers on Earth. *Paenibacillus* sp. str. FPU-7 produces several different chitinases and converts chitin into *N*,*N*′-diacetylchitobiose ($(GlcNAc)_2$) in the culture medium. However, the mechanism by which the *Paenibacillus* species imports $(GlcNAc)_2$ into the cytoplasm and divides it into the monomer GlcNAc remains unclear. The gene encoding *Paenibacillus* β-*N*-acetyl-D-glucosaminidase (PsNagA) was identified in the *Paenibacillus* sp. str. FPU-7 genome using an expression cloning system. The deduced amino acid sequence of PsNagA suggests that the enzyme is a part of the glycoside hydrolase family 3 (GH3). Recombinant PsNagA was successfully overexpressed in *Escherichia coli* and purified to homogeneity. As assessed by gel permeation chromatography, the enzyme exists as a 57-kDa monomer. PsNagA specifically hydrolyses chitin oligosaccharides, $(GlcNAc)_{2–4}$, 4-nitrophenyl *N*-acetyl β-D-glucosamine (pNP-GlcNAc) and pNP-$(GlcNAc)_{2–6}$, but has no detectable activity against 4-nitrophenyl β-D-glucose, 4-nitrophenyl β-D-galactosamine and colloidal chitin. In this study, we present a 1.9 Å crystal structure of PsNagA bound to GlcNAc. The crystal structure reveals structural features related to substrate recognition and the catalytic mechanism of PsNagA. This is the first study on the structural and functional characterization of a GH3 β-*N*-acetyl-D-glucosaminidase from *Paenibacillus* sp.

Keywords: β-N-acetylglucosaminidases, chitin, chitinolytic bacteria, glycoside hydrolase family 3, Paenibacillus sp. str. FPU-7

Protein Interaction and Recognition

Observation of unexpected molecular binding activity for Mu phage tail fibre chaperones

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In the history of viral research, one of the important biological features of bacteriophage Mu is the ability to expand its host

range. For extending the host range, the Mu phage encodes two alternate tail fibre genes. Classical amber mutation experiments and genome sequence analysis of Mu phage suggested that gene products (gp) of geneS (gpS = gp49) and gene S′ (gpS′ = gp52) are tail fibres and that gene products of geneU (gpU = gp50) and geneU′ (gpU′ = gp51) work for tail fibre assembly or tail fibre chaperones. Depending on the gene orientation, a pair of genes 49–50 or 52–51 is expressed for producing different tail fibres that enable Mu phage to recognize different host cell surface. Since several fibrous proteins including some phage tail fibres employ their specific chaperone to facilitate folding and prevent aggregation, we expected that gp50 or gp51 would be a specific chaperone for gp49 and gp52, respectively. However, heterologous overexpression results for gp49 or gp52 (tail fibre subunit) together with gp51 and gp50, respectively, were also effective in producing soluble Mu tail fibres. Moreover, we successfully purified non-native gp49–gp51 and gp52–gp50 complexes. These facts showed that gp50 and gp51 were fungible and functional for both gp49 and gp52 each other.

Keywords: bacteriophage, tail fibre, chaperone, binding specificity

Enzymology

Characterization of six recombinant human RNase H2 bearing Aicardi-Goutiéres syndrome causing mutations

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Mammalian RNase H2 is a heterotrimeric enzyme consisting of one catalytic subunit (A) and two accessory subunits (B and C). RNase H2 is involved in the removal of a single ribonucleotide embedded in genomic DNA and removal of RNA of RNA/DNA hybrids. In humans, mutation of the RNase H2 gene causes a severe neuroinflammatory disorder Aicardi-Goutières syndrome (AGS). Here, we examined the activity and stability of six recombinant human RNase H2 variants bearing one AGS-causing mutation, A-G37S (Gly37 in the A subunit is replaced with Ser), A-N212I, A-R291H, B-A177T, B-V185G, or C-R69W. The activity of A-G37S was 0.3–1% of that of the wild-type RNase H2 (WT), while those of other five variants were 51–120%. In circular dichroism measurement, the melting temperatures of variants were 50–53°C, lower than that of WT (56°C). These results suggested that A-G37S had decreased activity and stability than WT, while other five variants had decreased stability but retained activity. In gel filtration chromatography of the purified enzyme preparation, WT migrated as a heterotrimer, while A-R291H eluted in two separate peaks containing either the heterotrimer or only the A subunit, suggesting that some AGS-causing mutations affect the heterotrimer-forming stability of RNase H2.

Keywords: activity, AGS, human RNase H2, mutation, stability

MOLECULAR BIOLOGY

Gene Expression

Gene expression profi ling of the bone trabecula in patients with osteonecrosis of the femoral head by RNA sequencing

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Early diagnosis and treatment of osteonecrosis of the femoral head (ONFH) is challenging. Bone trabecula play a vital role in the severity and progression of ONFH. In the present study, the investigators used gene expression profiling of bone trabecula to investigate gene alterations in ONFH patients. Osteonecrotic bone trabecula (ONBT) such as necrosis, fibrosis, and lacuna were confirmed by histological examination in the patients. The adjacent 'normal' bone trabecula (ANBT) did not show any pathological changes. Gene sequencing data revealed that although ANBT showed no significant histological changes, alteration of mRNA profiling in ANBT was observed, similar to that in ONBT. Our results indicated that the alteration of mRNA profiling in ANBT may cause normal bone tissue to develop into necrotic bone. RNA-seq data indicated that 2,297 differentially abundant mRNAs were found in the ONBT group (1,032 upregulated and 1,265 downregulated) and 1,523 differentially abundant mRNAs in the ANBT group (744 upregulated and 799 downregulated) compared with the healthy control group. Gene ontology (GO) enrichment analysis suggested that fatty acid metabolism and degradation were the main zones enriched with differentially expressed genes (DEG). Kyoto encyclopedia of genes and genomes (KEGG) pathway enrichment analysis indicated that peroxisome proliferator-activated receptor γ (PPAR-γ) pathway was the most significantly regulated pathway. Lipocalin-2 (LCN2), an osteoblast-enriched secreted protein, was significantly decreased in ONBT suggesting that downregulation of LCN2 might affect lipid metabolism and lead to hyperlipidemia, and thus promote pathogenesis of ONFH.

Keywords: lipid metabolism, Lipocalin-2, osteonecrosis of the femoral head, RNA seq, trabecula bone

Molecular Genetics

miR-211-5p contributes to chondrocyte differentiation by suppressing Fibulin-4 expression to play a role in osteoarthritis

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MicroRNAs (miRNAs) serve as key regulators in human disorders. Previous research reported that miR-211-5p is down-regulated in osteoarthritis (OA) and that *Fibulin-4* inhibits chondrocyte differentiation. However, the role of miR-211-5p in the development of OA has not been clarified, and its down-stream target has not been studied. This study aimed to explore the effect of miR-211-5p on chondrocyte differentiation and its influence on OA pathogenesis, as well as the interaction between miR-211-5p and *Fibulin-4*. In this study, we found that miR-211-5p is significantly down-regulated in articular cartilage tissues in an OA rat model, whereas it is clearly up-regulated during chondrocyte differentiation of ATDC5 cells. Silencing miR-211-5p in ATDC5 cells had an adverse effect on chondrocyte differentiation. *Fibulin-4* was identified as a target of miR-211-5p, and miR-211-5p participated in chondrocyte differentiation by negatively regulating *Fibulin-4* expression. In the OA rat model, miR-211-5p overexpression facilitated chondrocyte differentiation, along with the reduced pro-inflammatory cytokines level and the level of proteinases responsible for cartilage matrix degradation. In summary, miR-211-5p promotes chondrocyte differentiation by negatively regulating *Fibulin-4* expression, and represses the expression of pro-inflammatory cytokines and proteinases responsible for cartilage matrix degradation in OA. miR-211-5p may serve as a promising target for OA treatment.

Keywords: chondrocyte differentiation, Fibulin-4, miR-211-5p, osteoarthritis, pro-inflammatory cytokines

CELL

Cell Death

miR-324-5p upregulation potentiates resistance to cisplatin by targeting FBXO11 signalling in non-small cell lung cancer cells

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Dysregulation of microRNAs (miRNAs) plays a key role during the pathogenesis of chemoresistance in lung cancer (LCa). Previous study suggests that miR-324-5p may serve as a unique miRNA signature for LCa, but its role and the corresponding molecular basis remain largely explored. Herein, we report that miR-324-5p expression was significantly increased in cisplatin (CDDP)-resistant LCa tissues and cells, and this upregulation predicted a poor post-chemotherapy prognosis in LCa patients. miR-324-5p was further shown to impact CDDP response: Ectopic miR-324-5p expression in drug-naïve LCa cells was sufficient to attenuate sensitivity to CDDP and to confer more robust tumour growth in CDDP-challenged nude mice. Conversely, ablation of miR-324-5p expression in resistant cells effectively potentiated CDDP-suppressed cell growth *in vitro* and *in vivo*. Using multiple approaches, we further identified the tumour suppressor FBXO11 as the direct down-stream target of miR-324-5p. Stable expression of FBXO11 could abrogate the pro-survival effects of miR-324-5p in CDDP-challenged LCa cells. Together, these findings suggest that miR-324-5p upregulation mediates, at least partially, the CDDP resistance by directly targeting FBXO11 signalling in LCa cells. In-depth elucidation of the molecular basis underpinning miR-324-5p action bears potential implications for mechanism-based strategies to improve CDDP responses in LCa.

Keywords: apoptosis, cisplatin, FBXO11, lung cancer, miRNA

Tumor and Immunology

ZBTB7A promotes migration, invasion and metastasis of human breast cancer cells through NF-κB-induced epithelial–mesenchymal transition in vitro and in vivo

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It has been generally confirmed that zinc finger and BTB domain containing 7A (ZBTB7A) plays an important role in the occurrence and progression of malignant tumours, but the promotion or inhibition effect is related to tumour type. The mechanism between ZBTB7A and breast cancer is not well understood, so further research is needed. In this study, we first investigated the expression of ZBTB7A in tissue samples of clinical breast cancer patients, MDA-MB-231, MCF-7 and MCF-10A cells. Second, we overexpressed the ZBTB7A in MCF-7 cells and silenced the ZBTB7A in MDA-MB-231 cells using lentivirus transfection technology, respectively, and verified the effect of ZBTB7A on migration and invasion of breast cancer cell lines through *in*

vitro cell function experiments, such as wound-healing assay, migration and invasion assay, quantitative real time reverse transcriptase (qRT-PCR) and western blot. Then, the correlation between the above influences, epithelial–mesenchymal transition (EMT) and NF-κB was analysed. Finally, *in vivo* tumour transplantation model in nude mice was established to verified the effect of ZBTB7A on metastasis of breast cancer MDA-MB-231 cells. In conclusion, ZBTB7A is highly expressed in cancer tissue, breast cancer cell line MDA-MB-231 and MCF-7. Meanwhile, the high expression of ZBTB7A may promote cell migration, invasion and tumour metastasis, which may be related to EMT events by regulating NF-κB.

Keywords: breast cancer, EMT, NF-κB, ZBTB7A

BIOTECHNOLOGY

Biotechnology General

Preparation of single-chain Fv antibodies in the cytoplasm of Escherichia coli by simplifi ed and systematic chaperone optimization

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A single-chain variable fragment (scFv) antibody is a recombinant protein in which a peptide linker connects the variable regions of the heavy chain and light chain. Due to its smaller molecular size, an scFv can be expressed using *Escherichia coli*. The presence of two disulphide bonds in the molecule often prevents expression of correctly folded scFv in the *E. coli* cytoplasm, making a refolding process necessary to regenerate scFv activity. The refolding process is time-consuming and requires large amounts of expensive reagents, such as guanidine hydrochloride, L-arginine and glutathione. Here, to conveniently obtain scFv proteins, we devised a simple and systematic method to optimize the co-expression of chaperone proteins and to combine them with specially engineered *E. coli* strains that permit the formation of stable disulphide bonds within the cytoplasm. Several scFv proteins were successfully obtained in a soluble form from *E. coli* cytoplasm. Thermal denaturation experiments and/or surface plasmon resonance measurements revealed that the thus-obtained scFvs possessed a stable tertiary structure and antigen-binding activity. The combined use of engineered *E. coli* with the simplified and systematic chaperone optimization can be useful for the production of scFv proteins.

Keywords: disulphide bond, molecular chaperone, protein expression, scFv, SHuffle strain