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ダイジェスト

BIOCHEMISTRY

Biochemistry General

MiR-133a-3p inhibits the malignant progression of oesophageal cancer by targeting CDCA8

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The study aims to explore the interaction between miR-133a-3p and cell division cycle associated 8 (CDCA8) in oesophageal cancer (EC) and their effect on malignant behaviour of EC cells. Differential miRNAs and mRNAs were obtained from The Cancer Genome Atlas (TCGA) database. Quantitative real-time PCR (qRT-PCR) was used to detect the expression levels of miR-133a-3p and CDCA8 mRNA in EC cells. Western blot was used to detect the expression of CDCA8 protein. CCK-8, flow cytometry and Transwell assays were conducted to detect cell proliferation, cell cycle and apoptosis, as well as migration and invasion, respectively. The targeting relationship between miR-133a-3p and CDCA8 was verified by dual-luciferase reporter gene assay. In EC, miR-133a-3p expression was evidently low and CDCA8 expression was prominently high. MiR-133a-3p downregulated CDCA8 expression. A range of cell function experiments revealed that CDCA8 promoted the proliferation, migration and invasion of EC cells, reduced cell cycle arrest in G0/G1 phase and inhibited cell apoptosis, while miR-133a-3p could reverse the above effects by regulating CDCA8. MiR-133a-3p is a crucial tumour suppressor miRNA in EC, playing a tumour suppressor role by targeting CDCA8.

Key words: apoptosis, CDCA8, invasion, migration, miR-133a-3p, proliferation

Nucleosome assembly protein 1 is a regulator of histone H1 acetylation

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Acetylation of histone H1 is generally considered to activate transcription, whereas deacetylation of H1 represses transcription. However, the precise mechanism of the acetylation is unknown. Here, using chromatography, we identified nucleosome assembly protein 1 (NAP-1) as having inhibitory activity against histone H1 acetylation by acetyltransferase p300. We found that native NAP-1 interacts with H1 in a *Drosophila* crude extract. We also found that it inhibits the deacetylation of histone H1 by histone deacetylase 1. The core histones in nucleosomes were acetylated in a GAL4–VP16 transcriptional activator-dependent manner *in vitro*. This acetylation was strongly repressed by hypoacetylated H1 but to a lesser extent by hyperacetylated H1. Consistent with these findings, a micrococcal nuclease assay indicated that hypoacetylated H1, which represses activator-dependent acetylation, was incorporated into chromatin, whereas hyperacetylated H1 was not. To determine the contribution of NAP-1 to transcriptional regulation *in vivo*, we compared NAP-1 knockdown (KD) with coactivator CREB-binding protein (CBP) KD using RNA sequencing in *Drosophila* Schneider 2 cells. Most genes were downregulated rather than upregulated by NAP-1 KD, and those downregulated genes were also downregulated by CBP KD. Our results suggest that NAP-1 plays a role in transcriptional regulation by fine-tuning the acetylation of histone H1.

Key words: histone acetylationhistone deacetylase 1 (HDAC1) linker histone H1NAP-1p300

Human TRPV1 and TRPA1 are receptors for bacterial quorum sensing molecules

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In this study, we investigated the activation of TRPV1 and TRPA1 by *N*-acyl homoserine lactones, quorum sensing molecules produced by Gram-negative bacteria, and the inhibitory effect of TRPV1 and TRPA1 by autoinducing peptides (AIPs), quorum sensing molecules produced by Gram-positive bacteria, using human embryonic kidney 293T cell lines stably expressing human TRPV1 and TRPA1, respectively. As a result, we found that some *N*-acyl homoserine lactones, such as *N*-octanoyl-L-homoserine lactone (C_8-HSL), *N*-nonanoyl-L-homoserine lactone (C_9-HSL) and *N*-decanoyl-L-homoserine lactone (C_{10}-HSL), activated both TRPV1 and TRPA1. In addition, we clarified that

some *N*-acyl homoserine lactones, such as *N*-3-oxo-dodecanoyl-L-homoserine lactone (3-oxo-C_{12}-HSL), only activated TRPV1 and *N*-acyl homoserine lactones having saturated short acyl chain, such as *N*-acetyl-L-homoserine lactone (C_2-HSL) and *N*-butyryl-L-homoserine lactone (C_4-HSL), only activated TRPA1. Furthermore, we found that an AIP, simple linear peptide CHWPR, inhibited both TRPV1 and TRPA1 and peptide having thiolactone ring DICNAYF, the thiolactone ring were formed between C3 to F7, strongly inhibited only the TRPV1. Although the specificity of TRPV1 and TRPA1 for quorum sensing molecules was different, these data suggest that both TRPV1 and TRPA1 would function as receptors for quorum sensing molecule produced by bacteria.

Key words: autoinducing peptides, *N*-acyl homoserine lactones, quorum sensing molecules, TRPA1, TRPV1, *Abbreviations*: AHL; *N*-acyl homoserine lactone; AhR; aryl hydrocarbon receptor; AIP; autoinducing peptide; HSL; homoserine lactone; IL; interleukin; [Ca^{2+}]i; intracellular Ca^{2+} concentration; IQGAP1; IQ-motif-containing GTPase-activating protein; LPS; lipopolysaccharide; PPAR; peroxisome proliferator activated receptor; T2R; taste receptor type 2; TRPA1; transient receptor potential cation channel; subfamily A; member 1; TRPM3; transient receptor potential cation channel; subfamily M; member 3; TRPM8; transient receptor potential cation channel; subfamily M; member 8; TRPV1; transient receptor potential cation channel; subfamily V; member 1; TRPV2; transient receptor potential cation channel; subfamily V; member 2; TRPV4; transient receptor potential cation channel; subfamily V; member 4; C_2-HSL; *N*-acetyl-L-homoserine lactone; C_4-HSL; *N*-butyryl-L-homoserine lactone; C_6-HSL; *N*-hexanoyl-L-homoserine lactone; C_7-HSL; *N*-heptanoyl-L-homoserine lactone; C_8-HSL; *N*-octanoyl-L-homoserine lactone; C_9-HSL; *N*-nonanoyl-L-homoserine lactone; C_{10}-HSL; *N*-decanoyl-L-homoserine lactone; C_{11}-HSL; *N*-undecanoyl-L-homoserine lactone; C_{12}-HSL; *N*-dodecanoyl-L-homoserine lactone; C_{13}-HSL; *N*-tridecanoyl-L-homoserine lactone; C_{14}-HSL; *N*-tetradecanoyl-L-homoserine lactone; C_{15}-HSL; *N*-pentadecanoyl-L-homoserine lactone; C_{16}-HSL; *N*-hexadecanoyl-L-Homoserine lactone; 3-oxo-C_6-HSL; *N*-(*β*-ketocaproyl)-L-homoserine lactone; 3-oxo-C_8-HSL; *N*-3-oxo-octanoyl-L-homoserine lactone; 3-oxo-C_{10}-HSL; *N*-3-oxo-decanoyl-L-homoserine lactone; 3-oxo-C_{12}-HSL; *N*-3-oxo-dodecanoyl-L-homoserine lactone; 3-oxo-C_{14}-HSL; *N*-3-oxo-tetradecanoyl-L-homoserine lactone; 3-oxo-C_{16}-HSL; *N*-3-oxo-hexadecanoyl-L-homoserine lactone; OH-C_8-HSL; *N*-3-hydroxyoctanoyl-L-homoserine lactone; OH-C_{10}-HSL; *N*-3-hydroxydecanoyl-L-homoserine lactone; C_{14}:1-Δ^9-cis-HSL; *N*-cis-tetradec-9Z-enoyl-L-homoserine lactone; C_{16}:1-Δ^9-cis-HSL; *N*-cis-hexadec-9Z-enoyl-L-homoserine lactone; C_{18}:1-Δ^9-cis-HSL; *N*-cis-octadec-9*Z*-enoyl-L-homoserine lactone; 3-oxo-C_{14}:1-Δ^7-cis-HSL; *N*-3-oxo-tetradec-7(*Z*)-enoyl-L-homoserine lactone; 3-oxo-C_{16}:1-Δ^{11}-cis-HSL; *N*-3-oxo-hexadec-11(*Z*)-enoyl-L-homoserine lactone

Protein Structure

Structure of the Acinetobacter baumannii PmrA receiver domain and insights into clinical mutants aff ecting DNA binding and promoting colistin resistance

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Acinetobacter baumannii is an insidious emerging nosocomial pathogen that has developed resistance to all available antimicrobials, including the last resort antibiotic, colistin. Colistin resistance often occurs due to mutations in the PmrAB two-component regulatory system. To better understand the regulatory mechanisms contributing to colistin resistance, we have biochemically characterized the *A. baumannii* PmrA response regulator. Initial DNA-binding analysis shows that *A. baumannii* PmrA bound to the *Klebsiella pneumoniae* PmrA box motif. This prompted analysis of the putative *A. baumannii* PmrAB regulon that indicated that the *A. baumannii* PmrA consensus box is 5′-HTTAAD N_5 HTTAAD. Additionally, we provide the first structural information for the *A. baumannii* PmrA N-terminal domain through X-ray crystallography and we present a full-length model using molecular modelling. From these studies, we were able to infer the effects of two critical PmrA mutations, PmrA::I13M and PmrA::P102R, both of which confer increased colistin resistance. Based on these data, we suggest structural and dynamic reasons for how these mutations can affect PmrA function and hence encourage resistive traits. Understanding these mechanisms will aid in the development of new targeted antimicrobial therapies.

Key words: *Acinetobacter baumannii*, colistin resistance, PmrA, response regulator, two-component system

Enzymology

Characterization of recombinant murine GDE4 and GDE7, enzymes producing lysophosphatidic acid and/or cyclic phosphatidic acid

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GDE4 and GDE7 are membrane-bound enzymes that exhibit lysophospholipase D activities. We found that GDE7 produced not only lysophosphatidic acid (LPA) but also cyclic phosphatidic acid (cPA) from lysophospholipids by a transphosphatidylation reaction. In contrast, GDE4 produced only LPA. The analysis of substrate specificity showed that 1-alkyl-lysophosphospholipids were preferred substrates for both enzymes rather than 1-alkyl-lysophospholipids and 1-alkenyl-lysophospholipids. Among the various lysophospholipids with different polar head groups that were tested, lysophosphatidylglycerol and lysophosphatidylserine were preferred substrates for GDE4 and GDE7, respectively. The detailed analysis of the dependency of the enzyme activities of GDE4 and GDE7 on divalent cations suggested multiple divalent cations were bound in the active sites of both enzymes. Taken together, these results suggest the possibility that GDE7 functions as a cPA-producing enzyme in the body.

Key words: cyclic phosphatidic acid, GDE4, GDE7, lysophosphatidic acid, lysophospholipase D

Analytical Biochemistry

SI-MOIRAI: a new method to identify and quantify the metabolic fate of nucleotides

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Since the discovery of nucleotides over 100 years ago, extensive studies have revealed the importance of nucleotides for homeostasis, health and disease. However, there remains no established method to investigate quantitatively and accurately intact nucleotide incorporation into RNA and DNA. Herein, we report a new method, Stable-Isotope Measure Of Influxed Ribonucleic Acid Index (SI-MOIRAI), for the identification and quantification of the metabolic fate of ribonucleotides and their precursors. SI-MOIRAI, named after Greek goddesses of fate, combines a stable isotope-labelling flux assay with mass spectrometry to enable quantification of the newly synthesized ribonucleotides into r/m/tRNA under a metabolic stationary state. Using glioblastoma (GBM) U87MG cells and a patient-derived xenograft (PDX) GBM mouse model, SI-MOIRAI analyses showed that newly synthesized GTP was particularly and disproportionally highly utilized for rRNA and tRNA synthesis but not for mRNA synthesis in GBM *in vitro* and *in vivo*. Furthermore, newly synthesized pyrimidine nucleotides exhibited a significantly lower utilization rate for RNA synthesis than newly synthesized purine nucleotides. The results reveal the existence of discrete pathways and compartmentalization of purine and pyrimidine metabolism designated for RNA synthesis, demonstrating the capacity of SI-MOIRAI to reveal previously unknown aspects of nucleotide biology.

Key words: cancer metabolism, flux analysis, glioblastoma (GBM), mass spectrometry, metabolomics, nucleotide metabolism

MOLECULAR BIOLOGY

Molecular Biology General

Circ_0000514 promotes breast cancer progression by regulating the miR-296-5p/CXCL10 axis

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The biological function of circular RNA 0000514 (circ_0000514) in breast cancer (BC) is still unknown. In this study, we downloaded the microarray dataset GSE101123 from Gene Expression Omnibus database and then analysed the differentially expressed circular RNAs in BC tissues compared with adjacent tissues, and we demonstrated that circ_0000514 was up-regulated in BC tis-

sues. Circ_0000514, miR-296-5p and CXC chemokine ligand10 (CXCL10) expressions in BC tissues and cell lines were probed by quantitativereal-time polymerase chain reaction and Western blot. Cell counting kit-8, 5-bromo-2′-deoxyuridine and transwell assays were adopted to determine the cell viability, proliferation, migration and invasion. The targeting relationship between miR-296-5p and circ_0000514 or CXCL10 3′-UTR was predicted by bioinformatics and validated by dual-luciferase reporter assay. We demonstrated that circ_0000514 and CXCL10 expressions were raised in BC tissues and cell lines while miR-296-5p expression was declined. Circ_0000514 knockdown could inhibit the proliferation, migration and invasion of BC cells and miR-296-5p overexpression also suppressed the malignant phenotypes of BC cells. Mechanistically, miR-296-5p was identified as the downstream target of circ_0000514 and could be inhibited by circ_0000514. Moreover, CXCL10 was the target of miR-296-5p, whose expression could be indirectly and positively regulated by circ_0000514. In conclusion, circ_0000514 is involved in BC progression via regulating miR-296-5p/CXCL10 axis.

Key words: proliferation, miR-296-5p, migration, invasion, CXCL10, circ_0000514

CELL

Muscles

Electrical polarity-dependent gating and a unique subconductance of RyR2 induced by S-adenosyl methionine via the ATP binding site

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S-Adenosyl-L-methionine (SAM) was used to probe the functional effects exerted via the cardiac RyR isoform (RyR2) adenine nucleotide binding site. Single channel experiments revealed that SAM applied to the cytoplasmic face of RyR2 had complex voltage dependent effects on channel gating and conductance. At positive transmembrane holding potentials, SAM caused a striking reduction in channel openings and a reduced channel conductance. In contrast, at negative potentials, SAM promoted a clearly resolved subconductance state. At membrane potentials between −75 and −25 mV, the open probability of the subconductance state was independent of voltage. ATP, but not the non-adenosine-based ryanodine receptor (RyR) activator 4-chloro-m-cresol, interfered with the effects of SAM at both negative and positive potentials. This suggests that ATP and SAM interact with a common binding site. Molecular docking showed SAM bound to the adenine nucleotide binding site and formed a hydrogen bond to Glu4886 in the C-terminal end of the S6 alpha helix. In this configuration, SAM may alter the conformation of the RyR2 ion conduction pathway. This work provides novel insight into potential functional outcomes of ligand binding to the RyR adenine nucleotide binding site.

Key words: adenine nucleotides; calcium channel; excitation–contraction coupling; ryanodine receptor, sarcoplasmic reticulum

Neurobiology

Lemur tail kinase 1 (LMTK1) regulates the endosomal localization of *β*-secretase BACE1

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Lemur tail kinase 1 (LMTK1), previously called apoptosis-associated tyrosine kinase (AATYK), is an endosomal Ser/Thr kinase. We recently reported that LMTK1 regulates axon outgrowth, dendrite arborization and spine formation via Rab11-mediated vesicle transport. Rab11, a small GTPase regulating recycling endosome trafficking, is shown to be associated with late-onset Alzheimer's disease (LOAD). In fact, genome-wide association studies identified many proteins regulating vesicle transport as risk factors for LOAD. Furthermore, LMTK1 has been reported to be a risk factor for frontotemporal dementia. Then, we hypothesized that LMTK1 contributes to AD development through vesicle transport and examined the effect of LMTK1 on the cellular localization of AD-related proteins, amyloid precursor protein (APP) and *β*-site APP cleaving enzyme 1 (BACE1). The *β*-cleavage of APP by BACE1 is the initial and rate-limiting step in A*β* generation. We found that LMTK1 accumulated BACE1, but not APP, to the perinuclear endosomal compartment, whereas the kinase-negative(kn) mutant of LMTK1A did not. The *β*-C-terminal fragment was prone to increase under overexpression of LMTK1A kn. Moreover, the expression level of LMTK1A was reduced in AD brains. These results suggest the possibility that LMTK1 is involved in AD development through the regulation of the proper endosomal localization of BACE1.

Key words: AATYK, Alzheimer's disease, BACE1, LMTK1, Rab11

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ダイジェスト

JB Special Issue—Review

Fifty years of Protein Data Bank in the Journal of Biochemistry

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Protein Data Bank (PDB), jointly founded in 1971 by Brookhaven National Laboratory, USA, and the Cambridge Crystallographic Data Centre, UK, is the single global archive of experimentally determined biological macromolecular structures. PDB deposition is mandatory for publication in most scientific journals, which means 'no PDB deposition, no structural publication'. The current PDB archive contains more than 180,000 entries and includes many structures from Asian institutions. The first protein structure from Japan was that of cytochrome *c* determined by Prof Masao Kakudo's group at the Institute for Protein Research, Osaka University, in 1971 at a resolution of 4 Å, and a subsequent atomic structure at 2.3 Å resolution was deposited to PDB in 1976 as the 1st Asian and 21st entry of the entire PDB archive. Since then, 317 protein structures whose primary citation was the *Journal of Biochemistry* (*J. Biochem*.) have been deposited to PDB. Based on this long history between PDB and *J. Biochem*., a statistical analysis of all structural reports in *J. Biochem*. has been carried out using the relational database system at PDBj (https://pdbj.org) and reviewed the yearly distribution, resolution, quality of structure, type of target protein, number of citations and comparison against other major journals.

Key words: X-ray crystallography, The Journal of Biochemistry, Protein Data Bank Japan, Protein Data Bank, nuclear magnetic resonance

JB Special Issue—Commentaries

Crystallographic studies of cytochrome c and cytochrome c oxidase

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I started on crystallographic studies of cytochrome c (Cyt.c) in the later 1960s at the Institute for Protein Research, Osaka University. The institute successfully built the structural model of ferro-Cyt.c by the multiple heavy atom replacement method in the early 1970s. In the early 1990s, crystals of cytochrome c oxidase (CcO) from bovine heart were obtained by using polyethylene glycol 4000 (Sigma) as the precipitant. We reported the first structure of a mammalian membrane protein at 2.8 Å resolution in 1995. High-resolution crystallography of CcO is in progress to understand the coupling mechanism of O_2 reduction and proton pumping. We determined the structure of the mammalian Cyt.c–CcO complex at 2.0 Å resolution and identified the 'soft and specific' interaction between Cyt.c and CcO, which affected high-efficiency inter-molecular electron transfer.

A retrospect of the structure determination of Taka-amylase A

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The 3D structure of Taka-amylase A was determined by X-ray crystal analysis at 3 Å resolution by Masao Kakudo's laboratory at the Institute for Protein Research, Osaka University, in 1980. Seven kinds of heavy atom derivatives were used for phase determination. There are three copies of Taka-amylase molecules in the asymmetric unit, which improved the quality of electron density maps, leading to the completion of a molecular model with 478 amino acids. The structure determination process in those days is described briefly.

Key words: crystal, X-ray Crystallography Methods, amylase, Catalysis Enzyme, Glycoproteins

Forty years of the structure of plant-type ferredoxin

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The X-ray structure of a [2Fe–2S]-type ferredoxin (Fd) from *Spirulina platensis*, solved by a collaborative group led by Profs Masao Kakudo, Yukiteru Katsube and Hiroshi Matsubara, was the first high-resolution structure of a plant-type Fd deposited in the Protein Data Bank. The main chain structure, comprising a [2Fe-2S] cluster ligated by four conserved cysteine residues, together with a molecular evolutionary study based on a series of amino acid sequence determinations, was reported in *Nature* in 1980. The consequent detailed crystallographic analysis, including crystallization, heavy atom derivatization, data collection, phase calculation and model building, was published by the same group in the *Journal of Biochemistry* in 1981. The pioneering X-ray analysis of *S. platensis* Fd at 2.5 Å resolution

was a key milestone in structural research on the photosynthetic electron transport chain, informing related and challenging studies on other components of the photosynthetic electron transfer chain.

Key words: X-ray crystallography, Protein Data Bank, photosynthesis, ferredoxin, electron transport

Crystal structure of endocrine-disrupting chemical bisphenol A and estrogen-related receptor γ

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The human estrogen-related receptor γ (ERRγ) is an orphan nuclear receptor. The ERRγ behaves as a constitutive activator of transcription and plays a key role in controlling mitochondrial energy production and energy metabolism. Bisphenol A (BPA) is used mainly in producing polycarbonate plastics and epoxy resins, but it is known as an endocrine disruptor and strongly binds to ERRγ. We determined the crystal structure of ERRγ in complex with BPA. Our structure revealed the molecular mechanism of BPA recognition by ERRγ, in which BPA is well anchored to its ligand-binding pocket. Our structure is the first report of the complex between a nuclear receptor and endocrine disruptor BPA. This structural analysis had a profound impact on subsequent studies of endocrine disruptors.

Key words: X-ray crystallography, Protein Data Bank, estrogen-related receptor γ, endocrine-disrupting chemical, bisphenol A

The crystal structure of D-amino acid oxidase with a substrate analog, *o*-aminobenzoate

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Since the discovery of D-amino acid oxidase (DAO) in 1935, many studies have been conducted without clarifying its 3D structure for a long time. In 1996, the crystal structure of DAO was determined, and it was shown that the catalytic bases required for the two catalytic mechanisms were not present in the active site. The crystal structure of DAO in complex with *o*-aminobenzoate was solved and is used for modeling Michaelis complex. The Michaelis complex model provided structural information leading to a new mechanism for reductive half-reaction of DAO. Currently, DAO is being researched for medical and applied purposes.

Key words: D-amino acid oxidase, enzyme mechanism, flavin adenine dinuclotide, Michaelis complex model, x-ray crystallography

BIOCHEMISTRY

Protein Structure

Structural insights into the enhanced thermostability of cysteine substitution mutants of L-histidine decarboxylase from Photobacterium phosphoreum

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Enzymatic amino acid assays are important in physiological research and clinical diagnostics because abnormal amino acid concentrations in biofluids are associated with various diseases. L-histidine decarboxylase from *Photobacterium phosphoreum* (PpHDC) is a pyridoxal 5′-phosphate-dependent enzyme and a candidate for use in an L-histidine quantitation assay. Previous cysteine substitution experiments demonstrated that the PpHDC C57S mutant displayed improved long-term storage stability and thermostability when compared with those of the wild-type enzyme. In this study, combinational mutation experiments of single cysteine substitution mutants of PpHDC were performed, revealing that the PpHDC C57S/C101V/C282V mutant possessed the highest thermostability. The stabilizing mechanism of these mutations was elucidated by solving the structures of PpHDC C57S and C57S/C101V/C282V mutants by X-ray crystallography. In the crystal structures, two symmetry-related PpHDC molecules form a domain-swapped homodimer. The side chain of S57 is solvent exposed in the structure, indicating that the C57S mutation eliminates chemical oxidation or disulfide bond formation with a free thiol group, thereby providing greater stability. Residues 101 and 282 form hydrophobic interactions with neighboring hydrophobic residues. Mutations C101V and C282V enhanced thermostability of PpHDC by filling a cavity present in the hydrophobic core (C101V) and increasing hydrophobic interactions.

Key words: thermostable mutant, thermostability, protein engineering, histidine decarboxylase, crystal structure, Abbreviations: AADCs, amino acid decarboxylase; ASA, accessible surface area; CL, catalytic loop; HDC, L-histidine decarboxylase; HDH, histamine dehydrogenase; HsHDC, HDC from humans; LL, long loop; 1-methoxy PMS, 1-methoxy-5-methylphenazinium methylsulfate; MjTDC, tyrosine decarboxylase from Methanocaldococcus jannaschii; MR, molecular replacement; PLP, pyridoxal 5′-phosphate; PpHDC, L-histidine decarboxylase from Photobacterium phosphoreum; PpHDC-SM, PpHDC C57S mutant; PpHDC-TM, PpHDC C57S/C101V/C282V mutant; (WST)-8, water-soluble tetrazolium salt; WT, wild-type

Reactive Oxygen and Nitrogen Species

Development of cisplatin resistance in breast cancer MCF7 cells by up-regulating aldo-keto reductase 1C3 expression, glutathione synthesis and proteasomal proteolysis

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Cisplatin (CDDP) is widely prescribed for the treatment of various cancers including bladder cancers, whereas its clinical use for breast cancer chemotherapy is restricted owing to easy acquisition of the chemoresistance. Here, we established a highly CDDP-resistant variant of human breast cancer MCF7 cells and found that procuring the resistance aberrantly elevates the expression of aldo-keto reductase (AKR) 1C3. Additionally, MCF7 cell sensitivity to CDDP was decreased and increased by overexpression and knockdown, respectively, of AKR1C3, clearly inferring that the enzyme plays a crucial role in acquiring the CDDP resistance. The CDDP-resistant cells suppressed the formation of cytotoxic reactive aldehydes by CDDP treatment, and the suppressive effects were almost completely abolished by pretreating with AKR1C3 inhibitor. The resistant cells also exhibited the elevated glutathione amount and 26S proteasomal proteolytic activities, and their CDDP sensitivity was significantly augmented by pretreatment with an inhibitor of glutathione synthesis or proteasomal proteolysis. Moreover, the combined treatment with inhibitors of AKR1C3, glutathione synthesis and/or proteasomal proteolysis potently overcame the CDDP resistance and docetaxel cross-resistance. Taken together, our results suggest that the combination of inhibitors of AKR1C3, glutathione synthesis and/or proteasomal proteolysis is effective as an adjuvant therapy to enhance CDDP sensitivity of breast cancer cells.

Key words: aldo-keto reductase 1C3, breast cancer, chemoresistance, cisplatin, glutathione

Biochemistry in Cell Membranes

Differential use of p24 family members as cargo receptors for the transport of glycosylphosphatidylinositol-anchored proteins and Wnt1

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Complexes of p24 proteins act as cargo receptors for the transport of COPII vesicles from the endoplasmic reticulum (ER). The major cargos of p24 complexes are hydrophilic proteins tethered to the ER membrane via a covalently attached glycosylphosphatidylinositol (GPI) or fatty acid. Each p24 complex is known to contain members from all four p24 subfamilies (p24 α, p24β, p24γ and p24δ). However, it remains unclear how the cargo specificities of p24 complexes are influenced by member stoichiometry. Here, we report the subunit compositions of mammalian p24 complexes involved in the transport of GPI-anchored proteins and Wnt1. We show that at least one p24α is required for the formation of p24 complexes and that a p24 complex consisting of p24α2, p24β1, p24γ2 and p24δ1 is required for the efficient transport of GPI-anchored proteins. On the other hand, a p24 complex containing p24α2, p24α3, p24β1, p24γ and p24δ1 is involved in the transport of Wnt1. Further, interactions between p24α2 and p24α3 are critical for Wnt1 transport. Thus, p24α and p24γ subfamily members are important for cargo selectivity. Lastly, our data fit with an octamer, rather than a tetramer, model of p24 complexes, where each complex consists of two proteins from each p24 subfamily.

Key words: GPI-anchored proteins, p24 complex, subunit composition, transport, Wnt1

Biochemistry in Diseases and Aging

Capn4 aggravates angiotensin II-induced cardiac hypertrophy by activating the IGF-AKT signalling pathway

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Capn4 belongs to a family of calpains that participate in a wide variety of biological functions, but little is known about the role of Capn4 in cardiac disease. Here, we show that the expression of Capn4 was significantly increased in Angiotensin II (Ang II)-treated cardiomyocytes and Ang II-induced cardiac hypertrophic mouse hearts. Importantly, in agreement with the Capn4 expression patterns, the maximal calpain activity measured in heart homogenates was elevated in Ang II-treated mice and oral coadministration of SNJ-1945 (calpain inhibitor) attenuated the total calpain activity measured *in vitro*. Functional assays indicated that overexpression of Capn4 obviously aggravated Ang II-in-

duced cardiac hypertrophy, whereas Capn4 knockdown resulted in the opposite phenotypes. Further investigation demonstrated that Capn4 maintained the activation of the insulin-like growth factor (IGF)-AKT signalling pathway in cardiomyocytes by increasing c-Jun expression. Mechanistic investigations revealed that Capn4 directly bound and stabilized c-Jun and knockdown of Capn4 increased the ubiquitination level of c-Jun in cardiomyocytes. Additionally, our results demonstrated that the antihypertrophic effect of Capn4 silencing was partially dependent on the inhibition of c-Jun. Overall, these data suggested that Capn4 contributes to cardiac hypertrophy by enhancing the c-Jun-mediated IGF-AKT signalling pathway and could be a potential therapeutic target for hypertrophic cardiomyopathy.

Key words: IGF-AKT pathway, deubiquitination, c-Jun, cardiac hypertrophy, Capn4, Abbreviations: Angiotensin II (Ang II), insulin-like growth factor (IGF), Neonatal rat ventricular cardiomyocyte (NRCM), co-immunoprecipitation (co-IP), brain natriuretic peptide (BNP), *β*-myosin heavy chain (*β*-MHC), cycloheximide (CHX)

CREG1 administration stimulates BAT thermogenesis and improves diet-induced obesity in mice

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Brown and beige adipocytes, which express thermogenic uncoupling protein-1 (UCP1), stimulate glucose and lipid metabolism, improving obesity and metabolic diseases such as type 2 diabetes and hyperlipidemia. Overexpression of cellular repressor of E1A-stimulated genes 1 (CREG1) promotes adipose tissue browning and inhibits diet-induced obesity (DIO) in mice. In this study, we investigated the effects of CREG1 administration on DIO inhibition and adipose browning. Subcutaneous administration of recombinant CREG1 protein to C57BL/6 mice stimulated UCP1 expression in interscapular brown adipose tissue (IBAT) and improved DIO, glucose tolerance and fatty liver compared with those in phosphate-buffered saline-treated mice. Injection of *Creg1*-expressing adenovirus into inguinal white adipose tissue (IWAT) significantly increased browning and mRNA expression of beige adipocyte marker genes compared with that in mice injected with control virus. The effect of *Creg1* induction on beige adipocyte differentiation was supported in primary culture using preadipocytes isolated from IWAT of Creg1-transgenic mice compared with that of wild-type mice. Our results indicate a therapeutic effect of CREG1 on obesity and its associated pathology and a potential of CREG1 to stimulate brown/beige adipocyte formation.

Key words: UCP1, obesity, CREG1, beige adipocyte, BAT

MOLECULAR BIOLOGY

Molecular Biology General

The dedicated chaperones of eL43, Puf6 and Loc1 can also bind RPL43 mRNA and regulate the production of this ribosomal protein

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The level of ribosome biogenesis is highly associated with cell growth rate. Because many ribosomal proteins have extraribosomal functions, overexpression or insufficient supply of these proteins may impair cellular growth. Therefore, the supply of ribosomal proteins is tightly controlled in response to rRNA syntheses and environmental stimuli. In our previous study, two RNA-binding proteins, Puf6 and Loc1, were identified as dedicated chaperones of the ribosomal protein eL43, with which they associate to maintain its protein level and proper loading. In this study, we demonstrate that Puf6 and Loc1 interact with *RPL43* mRNA. Notably, Puf6 and Loc1 usually function as a dimeric complex to bind other mRNAs; however, in this instance, the individual proteins, but not the complex form, can bind *RPL43* mRNA. Thus, Puf6 or Loc1 could bind *RPL43* mRNA in *loc1*Δ or *puf6*Δ, respectively. The binding of Puf6 or Loc1 caused negative effects for eL43 production: decreased RNA stability and translation of *RPL43A/B* mRNA. The present results suggest that these dedicated chaperones control the protein levels of eL43 from the standpoint of stability and through regulating its production.

Key words: trans-acting factors, RNA binding proteins, ribosome biogenesis, post-transcriptional regulation, chaperone

Genes and Other Genetic Materials

Cloning and characterization of Thioredoxin 1 from the Cnidarian Hydra

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Thioredoxins, small disulphide-containing redox proteins, play an important role in the regulation of cellular thiol redox balance through their disulfide reductase activity. In this study, we have identified, cloned, purified and characterized thioredoxin 1 (*Hv*Trx1) from the Cnidarian *Hydra vulgaris* Ind-Pune. Bioinfor-

matics analysis revealed that *Hv*Trx1 contains an evolutionarily conserved catalytic active site Cys-Gly-Pro-Cys and shows a closer phylogenetic relationship with vertebrate Trx1. Optimum pH and temperature for enzyme activity of purified *Hv*Trx1 was found to be pH 7.0 and 25°C, respectively. Enzyme activity decreased significantly at acidic or alkaline pH as well as at higher temperatures. *Hv*Trx1 was found to be expressed ubiquitously in whole mount *in situ* hybridization.

Treatment of *Hydra* with hydrogen peroxide (H_2O_2), a highly reactive oxidizing agent, led to a significant increase in gene expression and enzyme activity of Trx1. Further experiments using PX12, an inhibitor of Trx1, indicated that Trx1 plays an important role in regeneration in *Hydra*. Finally, by using growth assay in *Escherichia coli* and wound healing assay in human colon cancer cells, we demonstrate that *Hv*Trx1 is functionally active in both prokaryotic and eukaryotic heterologous systems.

Key words: Evolutionary conservation, Hydra, redox regulation, regeneration, Trx1

Gene Expression

Genome-wide distribution of 5hmC in the dental pulp of mouse molars and incisors

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The dental pulp is critical for the production of odontoblasts to create reparative dentin. In recent years, dental pulp has become a promising source of mesenchymal stem cells that are capable of differentiating into multiple cell types. To elucidate the transcriptional control mechanisms specifying the early phases of odontoblast differentiation, we analysed the DNA demethylation pattern associated with 5-hydroxymethylcytosine (5hmC) in the primary murine dental pulp. 5hmC plays an important role in chromatin accessibility and transcriptional control by modelling a dynamic equilibrium between DNA methylation and demethylation. Our research revealed 5hmC enrichment along genes and non-coding regulatory regions associated with specific developmental pathways in the genome of mouse incisor and molar dental pulp. Although the overall distribution of 5hmC is similar, the intensity and location of the 5hmC peaks significantly differs between the incisor and molar pulp genome, indicating cell type-specific epigenetic variations. Our study suggests that the differential DNA demethylation pattern could account for the distinct regulatory mechanisms underlying the tooth-specific ontogenetic programs.

Key words: TET enzymes, promoter, gene body, dental pulp, 5-hydroxymethylcysteine (5hmC)

CELL

Muscles

DA-Raf and the MEK inhibitor trametinib reverse skeletal myocyte diff erentiation inhibition or muscle atrophy caused by myostatin and GDF11 through the non-Smad Ras–ERK pathway

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Myostatin (Mstn) and GDF11 are critical factors that are involved in muscle atrophy in the young and sarcopenia in the elderly, respectively. These TGF-β superfamily proteins activate not only Smad signalling but also non-Smad signalling including the Ras-mediated ERK pathway (Raf–MEK–ERK phosphorylation cascade). Although Mstn and GDF11 have been shown to induce muscle atrophy or sarcopenia by Smad2/3-mediated Akt inhibition, participation of the non-Smad Ras–ERK pathway in atrophy and sarcopenia has not been well determined. We show here that both Mstn and GDF11 prevented skeletal myocyte differentiation but that the MEK inhibitor U0126 or trametinib restored differentiation in Mstn- or GDF11-treated myocytes. These MEK inhibitors induced the expression of DA-Raf1 (DA-Raf), which is a dominant-negative antagonist of the Ras–ERK pathway. Exogenous expression of DA-Raf in Mstn- or GDF11-treated myocytes restored differentiation. Furthermore, administration of trametinib to aged mice resulted in an increase in myofiber size or recovery from muscle atrophy. The trametinib administration downregulated ERK activity in these muscles. These results imply that the Mstn/GDF11-induced Ras–ERK pathway plays critical roles in the inhibition of myocyte differentiation and muscle regeneration, which leads to muscle atrophy. Trametinib and similar approved drugs might be applicable to the treatment of muscle atrophy in sarcopenia or cachexia.

Key words: muscle atrophy, muscle differentiation, non-Smad signalling, Ras–ERK pathway, sarcopenia

Cell Death

Long non-coding RNA AFAP1-AS1 promotes thyroid cancer progression by sponging miR-204-3p and upregulating DUSP4

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Long non-coding RNA actin filament-associated protein 1-antisense RNA 1 (AFAP1-AS1) shows crucial regulatory function in tumor progression. Nonetheless, the biological function and underlying mechanism of AFAP1-AS1 in the progression of thyroid cancer is still unclear. Expressions of AFAP1-AS1, miR-204-3p and DUSP4 were quantified utilizing quantitative real-time polymerase chain reaction and/or western blot. In loss-of-function and gain-of-function assays, cell proliferation, migration and invasion were appraised by CCK-8 assay, wound healing assay, Transwell migration and invasion assays, respectively. Luciferase reporter assay was employed for validating the interaction between miR-204-3p and AFAP1-AS1 or the 3′UTR of dual specificity phosphatase 4 (DUSP4). AFAP1-AS1 was highly expressed in thyroid cancer tissues and cell lines. Highly expressed AFAP1-AS1 was in association with advanced TNM stage and positive lymph node metastasis. Knockdown of AFAP1-AS1 suppressed the proliferation, migration and invasion of thyroid cancer cells, and overexpression of AFAP1-AS1 induced a reversed effect. MiR-204-3p was targetedly repressed by AFAP1-AS1, and miR-204-3p could negatively regulate DUSP4 expression. AFAP1-AS1 augmented the expression of DUSP4 via repressing miR-204-3p, and the effects of AFAP1-AS1 overexpression on thyroid cancer cells were also partly abolished by miR-204-3p restoration. In summary, AFAP1-AS1 facilitates thyroid cancer cell proliferation, migration and invasion by regulating miR-204-3p/DUSP4 axis.

Key words: thyroid cancer, proliferation, MiR-204-3p, metastasis, DUSP4, AFAP1-AS1