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ダイジェスト

JB SPECIAL ISSUE: PHYSIOLOGICAL AND PATHOLOGICAL ORGAN REMODELING AND PLASTICITY GUEST EDITOR: FUMIKO TOYOSHIMA SPECIAL ISSUE ARTICLES

Cardiac remodeling: novel pathophysiological mechanisms and therapeutic strategies

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Morphological and structural remodeling of the heart, including cardiac hypertrophy and fibrosis, has been considered as a therapeutic target for heart failure for approximately three decades. Groundbreaking heart failure medications demonstrating reverse remodeling effects have contributed significantly to medical advancements. However, nearly 50% of heart failure patients still exhibit drug resistance, posing a challenge to the healthcare system. Recently, characteristics of heart failure resistant to ARBs and *β*-blockers have been defined, highlighting preserved systolic function despite impaired diastolic function, leading to the classification of heart failure with preserved ejection fraction (HFpEF). The pathogenesis and aetiology of HFpEF may be related to metabolic abnormalities, as evidenced by its mimicry through endothelial dysfunction and excessive intake of high-fat diets. Our recent findings indicate a significant involvement of mitochondrial hyper-fission in the progression of heart failure. This mitochondrial pathological remodeling is associated with redox imbalance, especially hydrogen sulphide accumulation due to abnormal electron leak in myocardium. In this review, we also introduce a novel therapeutic strategy for heart failure from the current perspective of mitochondrial redox-metabolic remodeling.

Keywords: cardiac remodeling, mitochondria, redox/energy metabolism, supersulphide, transient receptor potential., Abbreviations: CTGF, connective tissue growth factor, GEF-H1, guanine nucleotide exchange factor, HFpEF, heart failure with preserved ejection fraction, MHC, myosin heavy chain, MMP, matrix metalloproteinase, MRTF, myocardin-related transcription factor, NFAT, nuclear factor of activated T cell, PICP, procollagen type 1 carboxy-terminal peptide, PIIINP, procollagen type III amino-terminal, SMA, smooth muscle actin, TGF, transforming growth factor, TRPC, transient receptor potential canonical

Molecular mechanisms of mechanosensing and plasticity of tendons and ligaments

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Tendons and ligaments, crucial components of the musculoskeletal system, connect muscles to bones. In the realm of sports, tendons and ligaments are vulnerable tissues, with injuries such as Achilles tendon rupture and anterior cruciate ligament tears directly impacting an athlete's career. Furthermore, repetitive trauma and tissue degeneration can lead to conditions like secondary osteoarthritis, ultimately affecting the overall quality of life. Recent research highlights the pivotal role of mechanical stress in maintaining homeostasis within tendons and ligaments. This review delves into the latest insights on the structure of tendons and ligaments and the plasticity of tendon tissue in response to mechanical loads.

Keywords: collagen fibres, developmental mechanisms, mechanical stimuli, tendon and ligament tissues, tissue plasticity, transcription factors

Spatial heterogeneity and functional zonation of living tissues and organs in situ

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In most organs, resources such as nutrients, oxygen and physi-

ologically active substances are unevenly supplied within the tissue spaces. Consequently, different tissue functions are exhibited in each space. This spatial heterogeneity of tissue environments arises depending on the spatial arrangement of nutrient vessels and functional vessels, leading to continuous changes in the metabolic states and functions of various cell types from regions proximal to these vessels to distant regions. This phenomenon is referred to as 'zonation'. Traditional analytical methods have made it difficult to investigate this zonation in detail. However, recent advancements in intravital imaging, spatial transcriptomics and single-cell transcriptomics technologies have facilitated the discovery of 'zones' in various organs and elucidated their physiological roles. Here, we outline the spatial differences in the immune system within each zone of organs. This information provides a deeper understanding of organs' immune systems.

Keywords: immune system, intravital imaging, single-cell transcriptomics, spatial transcriptomics, zonation

The Hox-based positional memory in muscle stem cells

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The skeletal muscle is a contractile tissue distributed throughout the body with various anatomical sizes, shapes and functions. In pathological conditions, such as muscular dystrophy, age-related sarcopenia and cancer cachexia, skeletal muscles are not uniformly affected throughout the body. This region-specific vulnerability cannot be fully explained by known physiological classifications, including muscle fiber types. Accumulating evidence indicates that the expression patterns of topographic homeobox (Hox) genes provide a molecular signature of positional memory, reflecting the anatomical locations and embryonic history of muscles and their associated muscle stem cells in adult mice and humans. Hox-based positional memory is not merely a remnant of embryonic development but is expected to be an intrinsic determinant controlling muscle function because recent studies have shown that aberrant Hox genes affect muscle stem cells. In this review, we discuss the concept of Hox-based positional memory, which may offer a new perspective on the region-specific pathophysiology of muscle disorders.

Keywords: Hox, muscle regeneration, muscle stem cells, positional memory, skeletal muscle

Cutting-edge skin ageing research on tissue stem cell

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In developed economies, the growing number of older individuals is a pressing issue. As a result, research progress into ageing has emphasized the significance of staying healthy in one's later years. Stem cells have a fundamental role to play in fostering diverse cell types and necessary processes for tissue repair and regeneration. Stem cells experience the effects of ageing over time, which is caused by their functional deterioration. Changes to stem cells, their niches and signals from other tissues they interact with are crucial factors in the ageing of stem cells. Progress in single-cell RNA sequencing (scRNA-seq) technology has greatly advanced stem cell research. This review examines the mechanisms of stem cell ageing, its impact on health and investigates the potential of stem cell therapy, with a special emphasis on the skin.

Keywords: ageing, homeostasis, regeneration, single cell RNA sequencing, stem cell

REGULAR PAPERS

BIOCHEMISTRY

Protein Interaction and Recognition

Sequential post-translational modifications regulate damaged DNA binding protein DDB2 function

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Nucleotide excision repair (NER) is a major DNA repair system and hereditary defects in this system cause critical genetic diseases (*e.g.* xeroderma pigmentosum, Cockayne syndrome and trichothiodystrophy). Various proteins are involved in the eukaryotic NER system and undergo several post-translational modifications. Damaged DNA-binding protein 2 (DDB2) is a DNA damage recognition factor in the NER pathway. We previously demonstrated that DDB2 was SUMOylated in response to UV irradiation; however, its physiological roles remain unclear. We herein analysed several mutants and showed that the N-terminal tail of DDB2 was the target for SUMOylation; however, this region did not contain a consensus SUMOylation sequence. We found a SUMO-interacting motif (SIM) in the N-terminal tail that facilitated SUMOylation. The ubiquitination of a SUMOylation-deficient DDB2 SIM mutant was decreased, and its retention of chromatin was prolonged. The SIM mutant showed impaired NER, possibly due to a decline in the timely handover of the lesion site to XP complementation group C. These results suggest that the SUMOylation of DDB2 facilitates NER through

enhancements in ubiquitination.

Keywords: DDB2, DNA damage response, nucleotide excision repair (NER), SUMOylation, ubiquitination

Glycobiology and Carbohydrate Biochemistry

Dyslipidemia and hyperglycemia induce overexpression of Syndecan-3 in erythrocytes and modulate erythrocyte adhesion

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Erythrocytes are important vascular components that play vital roles in maintaining vascular homeostasis, in addition to carrying oxygen. Previously, we reported that the changes in the internal milieu (e.g. hyperglycemia or hypercholesterolemia) increase erythrocyte adhesion to various extracellular matrix components, potentially through altering glycosaminoglycans (GAGs). In this study, we have investigated the expression of syndecan (Sdc) family members that could be involved in mediating cytoadherence under conditions of dyslipidemia and hyperglycemia. Among the Sdc family members analysed, we found significant overexpression of Sdc-3 in erythrocyte membranes harvested from high-fat-fed control and diabetic animals. Animal studies revealed a positive correlation between Sdc-3 expression, blood sugar levels and erythrocyte adhesion. In the human study, diabetic cohorts with body mass index ＞24.9 showed significantly increased expression of Sdc-3. Interestingly, blocking the Sdc-3 moiety with an anti-Sdc-3 antibody revealed that the core protein might not be directly involved in erythrocyte adhesion to fibronectin despite the GAGs bringing about adhesion. Lastly, Nano liquid chromatography-mass spectrometry/MS verified the presence of Sdc-3 in erythrocyte membranes. In conclusion, the high-fat diet and diabetes modulated Sdc-3 expression in the erythrocyte membrane, which may alter its adhesive properties and promote vascular complications.

Keywords: and Sdc-3, diabetes, erythrocyte adhesion, glycosaminoglycans, HFD, HSPGs

VP1 of human and murine noroviruses recognizes glycolipid sulfatide via the P domain

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Noroviruses are a prevalent cause of human viral gastroenteritis, yet the precise mechanisms underlying their infection cycle, particularly their interactions with and entry into cells, remain poorly understood. Human norovirus (HuNoV) primarily targets human small intestinal epithelial cells, within which 3-*O*-sulfogalactosylceramide (sulfatide) ranks among the most abundant glycosphingolipids (GSLs). While sulfatide involvement in the binding and infection mechanism of several viruses has been documented, its interaction with noroviruses remains underexplored. This study investigated whether noroviruses interact with sulfatide. We found that the recombinant viral capsid protein VP1 of HuNoV (genogroups I and II) and murine norovirus (genogroup V) exhibited robust binding to sulfatide compared with other tested GSLs using enzyme-linked immunosorbent assay, thin-layer chromatography binding assay and real-time quantitative reverse transcription polymerase chain reaction binding assay. VP1 also bound 3-*O*-sulfated lactosylceramide, which shares the 3-*O*-sulfated galactose moiety with sulfatide. However, both VP1 and its P domain, identified as the sulfatide-binding domain, exhibited limited binding to structural analogues of sulfatide and other sulfated compounds. These findings suggest a specific recognition of the 3-*O*-sulfated galactose moiety. Notably, we found that sulfatide is a novel binding target for norovirus particles. Overall, our findings reveal a previously unknown norovirus–sulfatide interaction, proposing sulfatide as a potential candidate for norovirus infection receptors.

Keywords: binding protein, carbohydrate-mediated interaction, glycolipids, norovirus, sulfatide

CELL

Tumor and Immunology

CRABP2 promotes cell migration and invasion by activating PI3K/AKT and MAPK signalling pathways via upregulating LAMB3 in prostate cancer

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Prostate cancer (PCa) has become a worldwide health burden among men. Previous studies have suggested that cellular retinoic acid binding protein 2 (CRABP2) significantly affects the regulation of cell proliferation, motility and apoptosis in multiple cancers; however, the effect of CRABP2 on PCa is poorly reported. CRABP2 expression in different PCa cell lines and its effect on different cellular functions varied. While CRABP2 promotes cell migration and invasion, it appears to inhibit cell pro-

liferation specifically in PC-3 cells. However, the proliferation of DU145 and 22RV1 cells did not appear to be significantly affected by CRABP2. Additionally, CRABP2 had no influence on the cell cycle distribution of PCa cells. The RNA-seq assay showed that overexpressing CRABP2 upregulated laminin subunit beta-3 (LAMB3) mRNA expression, and the enrichment analyses revealed that the differentially expressed genes were enriched in the phosphoinositide 3-kinase (PI3K)/activated protein kinase B (AKT) and mitogen-activated protein kinase (MAPK) signalling pathways. The following western blot experiments also confirmed the upregulated LAMB3 protein level and the activation of the PI3K/AKT and MAPK signalling pathways. Moreover, overexpressing CRABP2 significantly inhibited tumour growth *in vivo*. In conclusion, CRABP2 facilitates cell migration and invasion by activating PI3K/AKT and MAPK signalling pathways through upregulating LAMB3 in PCa.

Keywords: CRABP2, LAMB3, MAPK signalling pathway, PI3K/AKT signalling pathway, prostate cancer

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JB REVIEW

New insights into the regulation and roles of phosphatidylinositol 3,4-bisphosphate

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Phosphoinositides (PIPs) are phospholipids and components of the cellular membrane. In mammals, seven phosphorylated derivatives of PIPs have been identified. Among them, phosphatidylinositol 3,4-bisphosphate [PI (3,4) P_2] is produced by lipid phosphatases (e.g., SHIP2) or by lipid kinases PI3KC2α and PI3KC2β. Although PI (3,4) P_2 is undetectable in normal mouse or human tissues and common cell lines, it appears in a mouse prostate cancer model and in cells exposed to oxidative stress, indicating that PI (3,4) P_2 is involved in the pathogenesis of some diseases. Here, I summarize recent findings on the cellular roles and pathophysiological significance of PI (3,4) P_2.

Keywords: cancer, membrane ruffle, membrane trafficking, phosphatidylinositol 3,4-bisphosphate, phosphoinositides

REGULAR PAPERS
BIOCHEMISTRY
Biochemistry General

Biochemical characterizations of the central fragment of human Reelin and identification of amino acid residues involved in its secretion

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Secreted protein Reelin is implicated in neuropsychiatric disorders and its supplementation ameliorates neurological symptoms in mouse disease models. Recombinant human Reelin protein may be useful for the treatment of human diseases, but its properties remain uncharacterized. Here, we report that full-length human Reelin was well secreted from transfected cells and was able to induce Dab1 phosphorylation. Unexpectedly, the central fragment of human Reelin was much less secreted than that of mouse Reelin. Three residues in the sixth Reelin repeat contributed to the secretion inefficiency, and their substitutions with mouse residues increased the secretion without affecting its biological activity. Our findings help efficient production of human Reelin protein for the supplementation therapy.

Keywords: Dab1, neuron, phosphorylation, Reelin, secretion

Metabolism and Bioenergetics

Dietary methionine functions in proliferative zone maintenance and egg production via sams-1 in Caenorhabditis elegans

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The maintenance of germ cells is critical for the prosperity of offspring. The amount of food consumption is known to be closely related to reproduction, *i.e.* the number of eggs decreases under calorie-restricted conditions in various organisms. Previous studies in *Caenorhabditis elegans* have reported that calorie

restriction reduces the number of eggs and the reduction can be rescued by methionine. However, the effect of methionine on the reproductive process has not been fully understood. In this study, to assess the gonadal function of methionine metabolism, we firstly demonstrated that a depletion in dietary methionine resulted in reduced levels of *S*-adenosyl-l-methionine (SAM) and *S*-adenosyl homocysteine in wild-type N2, but not in *glp-1* mutants, which possess only a few germ cells. Second, we found no recovery in egg numbers upon methionine administration in SAM synthase (*sams*)*-1* mutants. Furthermore, a reduced number of proliferative zone nuclei exhibited in the *sams-1* mutants was not rescued *via* methionine. Thus, our results have shown that dietary methionine is required for the normal establishment of both the germline progenitor pool and fecundity, mediated by *sams-1*.

Keywords: Methionine, *Caenorhabditis elegans*, germ cells

Biochemistry in Diseases and Aging

Variations associated with neurodevelopmental disorders affect ARF1 function and cortical development

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ADP-ribosylation factors (ARFs) are a family of small GTPases that regulate vesicle trafficking and actin dynamics in cells. Recent genetic analyses have revealed associations between variations in *ARF* genes and neurodevelopmental disorders, although their pathophysiological significance remains unclear. In this study, we conducted biochemical, cell biological and *in vivo* analyses of ARF1 variants linked to neurodevelopmental disorders. The mant-GDP dissociation assay revealed that ARF1-p. R19C, -p. F51 L, -p. R99C and -p. R99H exhibit higher GDP/GTP exchange activity compared to ARF1 wild type (WT). The GTPase-activating protein (GAP) increased the GTPase activity of WT, p.R19C, p.Y35H, p.F51 L, p.P131 L and p.P131R, but not of p.Y35D, p.T48I, p.R99C and p.R99H. The transient expression of p.R99C, p.R99H and p.K127E in mammalian cells resulted in the disruption of the Golgi apparatus. *In utero* electroporation-mediated gene transfer into the cortical neurons of embryonic mice demonstrated that p.R99C, p.R99H and p.K127E cause a migration defect. Expression of these variants resulted in the expansion of the Golgi apparatus in migrating cortical neurons. These findings suggest that the ARF1 variants linked to neurodevelopmental disorders, specifically p.R99C, p.R99H and p.K127E, disrupt the structure of the Golgi apparatus, thereby leading to a developmental defect of cortical neurons.

Keywords: ARF1, brain, Golgi apparatus, neurodevelopmental disorder, small GTPase

Neurochemistry

The NRF2 inducer CDDO-2P-Im provokes a reduction in amyloid *β* levels in Alzheimer's disease model mice

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Alzheimer's disease (AD) is the most common aetiology of dementia. The transcription factor NF-E2-related factor 2 (NRF2) induces the expression of genes encoding phase II detoxification and antioxidant genes. NRF2 is regulated by Kelch-like ECH-associated protein 1 (KEAP1), and the KEAP1-NRF2 system is the key regulatory system involved in cytoprotection. To examine whether pharmacological induction of NRF2 expression alleviates AD phenotypes *in vivo*, we employed two AD mouse models, *i.e. App NL-G-F/NL-G-F* (*App NLGF*) and *APPV717I: : TAUP301L* (APP/TAU) mice. As the synthetic oleanane triterpenoid 1-[2-cyano-3,12-dioxooleana-1,9(11-dien-28-oyl)] (CDDO)-4(−pyridin-2-yl)-imidazole (CDDO-2P-Im) exhibits strong NRF2-inducing activity, we treated AD model mice with CDDO-2P-Im. We found that Aβ42 levels were markedly greater in the brains of *App NLGF* mice than in those of APP/TAU mice. CDDO-2P-Im treatment significantly decreased Aβ42 levels, but not Aβ40 levels, in APP/TAU mice. Consequently, CDDO-2P-Im also decreased the ratio of Aβ42/Aβ40, a vital marker of amyloid plaque formation. LC–MS/MS analyses revealed that CDDO-2P-Im was delivered to the brains of the APP/TAU mice. CDDO-2P-Im induced the expression of detoxification and antioxidant gene targets of NRF2 and elevated re-

duced glutathione (GSH) levels in the mouse brain. These results support the notion that CDDO-2P-Im ameliorates AD-related pathologic changes.

Keywords: Alzheimer's disease, amyloid β, CDDO-2P-Im, GSH, NRF2

MOLECULAR BIOLOGY
Molecular Biology General

Identification of APBB1 as a substrate for anaplastic lymphoma kinase

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Anaplastic lymphoma kinase (ALK) is a well-known oncogene involved in various malignancies such as anaplastic large cell lymphoma, lung cancer and neuroblastoma. Several substrates for fused ALK have been identified and their biological functions have been described. However, the lack of a comprehensive identification of ALK substrates limits our understanding of the biological roles of receptor ALK. Thus, this study aimed to identify novel ALK substrates and characterize their biological functions. We screened the interactors of the kinase domain of receptor ALK using proximity-dependent biotin identification and identified 43 interactors. We narrowed down the candidates by evaluating whether these interactors were downstream of ALK in a neuroblastoma cell line, NB-1. Amongst these, we identified amyloid beta precursor protein-binding family B member 1 (APBB1) as an ALK downstream molecule involved in NB-1 cell viability. Finally, we assessed the kinase-substrate relationship between ALK and APBB1 and found that ALK phosphorylated multiple tyrosine residues in APBB1 both in-cell and in-tube assays, with tyrosine 269 as a major target. In conclusion, we successfully identified a new substrate for receptor ALK. Our results may help further elucidate the molecular mechanism of ALK downstream signalling in neuroblastoma.

Keywords: ALK, BioID, kinase-substrate relationship, protein–protein interaction, proteomics

CELL
Receptors and Signal Transduction

Ciliary length variations impact cilia-mediated signaling and biological responses

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Primary cilia are thin hair-like organelles that protrude from the surface of most mammalian cells. They act as specialized cell antennas that can vary widely in response to specific stimuli. However, the effect of changes in cilia length on cellular signaling and behavior remains unclear. Therefore, we aimed to characterize the elongated primary cilia induced by different chemical agents, lithium chloride (LiCl), cobalt chloride ($CoCl_2$) and rotenone, using human retinal pigmented epithelial 1 (hRPE1) cells expressing ciliary G protein-coupled receptor (GPCR), melanin-concentrating hormone (MCH) receptor 1 (MCHR1). MCH induces cilia shortening mainly via MCHR1-mediated Akt phosphorylation. Therefore, we verified the proper functioning of the MCH-MCHR1 axis in elongated cilia. Although MCH shortened cilia that were elongated by LiCl and rotenone, it did not shorten $CoCl_2$-induced elongated cilia, which exhibited lesser Akt phosphorylation. Furthermore, serum readdition was found to delay cilia shortening in $CoCl_2$-induced elongated cilia. In contrast, rotenone-induced elongated cilia rapidly shortened via a chopping mechanism at the tip of the cilia. Conclusively, we found that each chemical exerted different effects on ciliary GPCR signaling and serum-mediated ciliary structure dynamics in cells with elongated cilia. These results provide a basis for understanding the functional consequences of changes in ciliary length.

Keywords: Akt pathway, G protein-coupled receptor, hTERT-RPE1 cells, melanin-concentrating hormone, primary cilia