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ダイジェスト

***JB Special Issue*—Commentary**

Emerging roles of extracellular vesicles in physiology and disease

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Extracellular vesicles (EVs), such as exosomes and microvesicles, are small membrane vesicles secreted by almost all cell types and are abundant in blood, body fluids, such as urine, spinal fluid, tears and saliva, and cell culture media. From an evolutionary perspective, they are biologically significant as a means for expelling unwanted cellular contents. Recently, EVs have received considerable attention as messengers of intercellular communication networks, allowing the exchange of proteins and lipids between the cells producing them and target cells that trigger various cellular responses. EVs also carry mRNAs and microRNAs inside them, transferring genetic information among cells. In addition, the expression pattern of these molecules is related to the cellular state and the progression of diseases, and the search for biomarkers within the EV is underway in many research fields. However, the physiological and pathophysiological roles of EVs remain largely elusive. Therefore, in this special issue, we have compiled reviews of the latest research findings on EV research.

Keywords; biomarker, exosome, extracellular vesicle, intercellular communication, microvesicle

JB Special Issue—Reviews

Current understandings of the relationship between extracellular vesicles and cilia

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Mammalian cells have a tiny hair-like protrusion on their surface called a primary cilium. Primary cilia are thought to be the antennae for the cells, receiving signals from the environment. In some studies, extracellular vesicles (EVs) were found attached to the surface of the primary cilium. An idea for the phenomenon is that the primary cilium is the receptor for receiving the EVs. Meanwhile, a unicellular organism, *Chlamydomonas*, which has two long cilia, usually called flagella, release EVs termed ectosomes from the surface of the flagella. Accumulating evidence suggests that the primary cilium also functions as the 'emitter' of EVs. Physiological and pathological impacts are also elucidated for the release of EVs from primary cilia. However, the roles of released cilia-derived EVs remain to be clarified. This review introduces the historical background of the relationship between EVs and cilia, and recent progresses in the research field.

Keywords; actin, cilia, ectosome, extracellular vesicles, phosphoinositide

Senescence-associated extracellular vesicle release plays a role in senescence-associated secretory phenotype (SASP) in age-associated diseases

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Cellular senescence is an important tumour suppression mechanism that inhibits the proliferation of damaged cells. In senescent cells, irreparable DNA damage causes accumulation of genomic DNA fragments in the cytoplasm, which are recognized by the cyclic GMP-AMP synthase–stimulator of interferon gene pathway, resulting in secretion of numerous inflammatory proteins. This phenomenon is called senescence-associated secretory phenotype, and results in multiple physiological or pathological processes in the body. In addition, DNA damage also increases small extracellular vesicle release from senescent cells. This review presents recent insights into the molecular mechanisms and biological functions of senescence-associated extracellular vesicle release that is associated with age-related diseases, particularly cancer.

Keywords; cellular senescence, cGAS–STING, DNA damage, extracellular vesicle, senescence-associated secretory phenotype

The Yin and Yang of tumour-derived extracellular vesicles in tumour immunity

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Extracellular vesicles (EVs) are small particles that are naturally released from various types of cells. EVs contain a wide variety of cellular components, such as proteins, nucleic acids, lipids and metabolites, which facilitate intercellular communication in diverse biological processes. In the tumour microenvironment, EVs have been shown to play important roles in tumour progression, including immune system–tumour interactions. Although previous studies have convincingly demonstrated the immunosuppressive functions of tumour-derived EVs, some studies have suggested that tumour-derived EVs can also stimulate host immunity, especially in therapeutic conditions. Recent studies have revealed the heterogeneous nature of EVs with different structural and biological characteristics that may account for the divergent functions of EVs in tumour immunity. In this review article, we provide a brief summary of our current understanding of tumour-derived EVs in immune activation and inhibition. We also highlight the emerging utility of EVs in the diagnosis and treatment of cancers and discuss the potential clinical applications of tumour-derived EVs.

Keywords; cancer, exosomes, extracellular vesicles, immunity, tumour microenvironment

Impact of exosome-mediated feto-maternal interactions on pregnancy maintenance and development of obstetric complications

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Pregnancy is an immunological paradox, a phenomenon in which the foetus and the placenta, containing foreign antigens to the mother, develop without inducing rejection by the maternal immune system. Cell-to-cell communication between the foetus and the mother is mediated by secreted factors such as cytokines, hormones and extracellular vesicles (EVs) for a successful pregnancy and to avoid rejection. Exosomes, the smallest of EVs, are released extracellularly, where they are taken up by proximal or distant recipient cells. Here, we discuss the role of EVs, especially exosomes in feto-maternal communication during pregnancy. This review will provide an overview of the functional roles exosomes may play during embryo implantation, modulating immune responses during pregnancy and the onset of labour. Moreover, we will discuss exosomal function in obstetric pathology, and the development of pregnancy-associated complications such as preeclampsia and preterm birth as well as the biomarker potential of exosomes for detecting such conditions.

Keywords; exosomes, extracellular vesicles, feto-maternal communication, preeclampsia, pregnancy

Stimulation of exosome biogenesis by adiponectin, a circulating factor secreted from adipocytes

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Adiponectin is an adipocyte-derived circulating factor that protects various organs and tissues. Such a pleiotropic action mechanism has not yet been fully explained. Clinically important multimer adiponectin existing in serum bound to cells expressing T-cadherin, a glycosylphosphatidylinositol-anchored cadherin, but not to the cells expressing other known receptors, AdipoRs or calreticulin. Adiponectin bound to the cell-surface, accumulated inside of multivesicular bodies through T-cadherin, and increased exosome biogenesis and secretion from the cells. Such increased exosome production accompanied the reduction of cellular ceramides in endothelial cells and mouse aorta, and enhanced skeletal muscle regeneration. Significantly lower plasma exosome levels were found in mice genetically deficient in either adiponectin or T-cadherin. Therapeutic effects of mesenchymal stem cells (MSCs) for a pressure overload-induced heart failure in mice required the presence of adiponectin in plasma, T-cadherin expression and exosome biogenesis in MSCs themselves, accompanying an increase of plasma exosomes. Essentially all organs seem to have MSCs and/or their related somatic stem cells expressing T-cadherin. Our recent studies suggested the importance of exosome-stimulation by multimer adiponectin in its well-known pleiotropic organ protections.

Keywords; adiponectin, ceramide, exosome, mesenchymal stem cells, T-cadherin

Pathogenic and protective roles of extracellular vesicles in neurodegenerative diseases

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Neurodegenerative diseases such as Alzheimer's disease, Parkinson's disease, amyotrophic lateral sclerosis and polyglutamine diseases are caused by aggregation and abnormal accumulation of the disease-causative proteins in brain and spinal cord. Recent studies have suggested that proteins associated with neurodegen-

erative diseases are secreted and transmitted intercellularly via extracellular vesicles (EVs), which may be involved in propagation of abnormal protein accumulation and progressive degeneration in patient brains. On the other hand, it has been also reported that EVs have neuroprotective roles in these diseases, which potentially contribute to preventing aggregation formation and aberrant accumulation of the disease-associated proteins. In this review, I summarize the current understanding of the roles of EVs in neurodegenerative diseases, especially focussing on the pathogenic and neuroprotective aspects. Elucidation of these two aspects of EVs would provide insight into not only potential therapeutic targets for treatment of neurodegenerative diseases but also development of EV-based biomarkers for disease diagnostics.

Keywords; extracellular vesicle, neurodegenerative disease, pathogenic, propagation, proteostasis

BIOCHEMISTRY

Protein Structure

Multiple structural states of Ca^{2+}-regulated PET hydrolase, Cut190, and its correlation with activity and stability

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An enzyme, Cut190, from a thermophilic isolate, *Saccharomonospora viridis* AHK190 could depolymerize polyethylene terephthalate (PET). The catalytic activity and stability of Cut190 and its S226P/R228S mutant, Cut190*, are regulated by Ca^{2+} binding. We previously determined the crystal structures of the inactive mutant of Cut190*, Cut190*S176A, in complex with metal ions, Ca^{2+} and Zn^{2+}, and substrates, monoethyl succinate and monoethyl adipate. In this study, we determined the crystal structures of another mutant of Cut190*, Cut190**, in which the three C-terminal residues of Cut190* are deleted, and the inactive mutant, Cut190**S176A, in complex with metal ions. In addition to the previously observed closed, open and engaged forms, we determined the ejecting form, which would allow the product to irreversibly dissociate, followed by proceeding to the next cycle of reaction. These multiple forms would be stable or sub-stable states of Cut190, regulated by Ca^{2+} binding, and would be closely correlated with the enzyme function. Upon the deletion of the C-terminal residues, we found that the thermal stability increased while retaining the activity. The increased stability could be applied for the protein engineering of Cut190 for PET depolymerization as it requires the reaction above the glass transition temperature of PET.

Keywords; crystal structure, enzyme activity, multiple forms, polyethylene terephthalate, thermal stability

Protein Interaction and Recognition

Identification of PTPRσ-interacting proteins by proximity-labelling assay

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Receptor protein tyrosine phosphatases (RPTPs) are type-I transmembrane proteins and involved in various biological and pathological processes. Their functions are supposed to be exerted through tyrosine dephosphorylation of their specific substrates. However, our comprehensive understanding of specific substrates or interacting proteins for RPTPs is poor. PTPRσ belongs to class 2a RPTP family, dephosphorylates cortactin, and leads to autophagy flux disruption and axonal regeneration inhibition in response to its ligand chondroitin sulphate. Here, we applied proximity-dependent biotin identification (BioID) assay, a proximity-labelling assay, to PTPRσ and reproducibly identified the 99 candidates as interactors for PTPRσ including already-known interactors such as Liprin-α and Trio. Of note, cortactin was also listed up in our assay. Our results suggest that the BioID assay is a powerful and reliable tool to identify RPTP-interacting proteins including its specific substrate.

Keywords; axon regeneration, protein–protein interaction, proteomics, proximity-labelling, PTPRσ

MOLECULAR BIOLOGY

Molecular Genetics

Molecular and functional characaterization of the novel odorantbinding protein gene AccOBP10 from Apis cerana cerana

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Odorant-binding proteins (OBPs) play an important role in odour perception and transport in insects. However, little is known about whether OBPs perform other functions in insects, particularly in *Apis cerana cerana*. Within this study, an OBP gene (*AccOBP10*) was isolated and identified from *A. c. cerana*. Both homology and phylogenetic relationship analyses indicated that the amino acid sequence of AccOBP10 had a high degree of sequence identity with other members of the gene family. Analy-

sis of quantitative real-time PCR (qRT-PCR) showed that *AccOBP10* mRNA was expressed at higher levels in the venom gland than in other tissues. The mRNA transcript expression of *AccOBP10* was upregulated by low temperature (4°C), hydrogen peroxide (H_2O_2), pyridaben, methomyl and imidacloprid but downregulated by heat (42°C), ultraviolet light, vitamin C, mercuric chloride, cadmium chloride, paraquat and phoxim. Expression of AccOBP10 under abiotic stress was analysed by western blotting, and the results were consistent with those of qRT-PCR. And as a further study of AccOBP10 function, we demonstrated that knockdown of *AccOBP10* by RNA interference could slightly increase the expression levels of some stress-related genes. Collectively, these results suggest that *AccOBP10* is mainly involved in the response to stress conditions.

Keywords; Abiotic stress, Apis cerana cerana, expression analysis, molecular characterization, odorant-binding protein

CELL

Neurobiology

miR-98-5p protects against cerebral ischemia/reperfusion injury through anti-apoptosis and anti-oxidative stress in mice

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Cerebral ischemia/reperfusion (I/R) injury is an obstacle in treating ischemic stroke effectively. miR-98-5p has been reported to have the ability of reducing myocardial I/R injury. To explore the function of miR-98-5p in cerebral I/R, we established mice model of middle cerebral artery occlusion and reperfusion (MCAO/R). The level of miR-98-5p was found to be downregulated in serum of stroke patients and brain tissues of MCAO/R mice. Examination of brain tissues indicated that upregulating miR-98-5p level alleviated the infarction in MCAO/R mice. Moreover, the upregulation of miR-98-5p reduced reactive oxygen species production and enhanced superoxide dismutase activity in brain tissues of MCAO/R mice. These results indicating that miR-98-5p could protect against oxidative stress. Further study showed that miR-98-5p inhibited apoptosis by reducing the levels of death-associated protein kinase 1, B cell lymphoma/leukaemia-2 associated x protein and cleaved caspase-3, as well as increasing the level of B cell lymphoma/leukaemia-2. In addition, miR-98-5p was found to protect against oxidative stress through downregulating the level of BTB domain and CNC homology 1 and upregulating the levels of NAD(P)H: quinone oxidoreductase 1 and heme oxygenase 1. Therefore, miR-98-5p might be a potential target to treat cerebral I/R injury.

Keywords; apoptosis, cerebral ischemia/reperfusion, mice, miR-98-5p, oxidative stress

BIOTECHNOLOGY

Biotechnology General

Quantitative nascent proteome profi ling by dual-pulse labelling with O-propargyl-puromycin and stable isotope-labelled amino ac,ids

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Monitoring translational regulation in response to environmental signals is crucial for understanding cellular proteostasis. However, only limited approaches are currently available for quantifying acute changes in protein synthesis induced by stimuli. Recently, a clickable puromycin analogue, *O*-propargyl-puromycin (OPP), was developed and applied to label the C-termini of nascent polypeptide chains (NPCs). Following affinity purification via a click reaction, OPP allows for a proteomic analysis of NPCs. Despite its advantage, the affinity purification of NPCs using magnetic beads or resins inherently suffers from significant non-specific protein binding, which hinders accurate quantification of the nascent proteins. To address this issue, we employed dual-pulse labelling of NPCs with both OPP and stable isotope-labelled amino acids to distinguish *bona fide* NPCs from non-specific proteins, thereby enabling the accurate quantitative profiling of NPCs. We applied this method to dissecting translation responses upon transcriptional inhibition and quantified ～3,000 nascent proteins. We found that the translation of a subset of ribosomal proteins (*e.g.* RPSA, RPLP0) as well as signalling proteins (*e.g.* BCAR3, EFNA1, DUSP1) was significantly repressed by transcription inhibition. Together, the present method provides an accurate and broadly applicable nascent proteome profiling for many biological applications at the level of translation.

Keywords; gene, mass spectrometry, proteomics, ribosome function, translation

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