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ダイジェスト

JB SPECIAL ISSUE—MULTI-SCALE PLATFORM FOR GPCR BIOLOGY

GUEST EDITORS: ASUKA INOUE AND ARUN K. SHUKLA

JB SPECIAL ISSUE—REVIEW

Morphinan Evolution: The Impact of Advances in Biochemistry and Molecular Biology

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Morphinan-based opioids, derived from natural alkaloids like morphine, codeine and thebaine, have long been pivotal in managing severe pain. However, their clinical utility is marred by significant side effects and high addiction potential. This review traces the evolution of the morphinan scaffold in light of advancements in biochemistry and molecular biology, which have expanded our understanding of opioid receptor pharmacology. We explore the development of semi-synthetic and synthetic morphinans, their receptor selectivity and the emergence of biased agonism as a strategy to dissociate analgesic properties from undesirable effects. By examining the molecular intricacies of opioid receptors and their signaling pathways, we highlight how receptor-type selectivity and signaling bias have informed the design of novel analgesics. This synthesis of historical and contemporary perspectives provides an overview of the morphinan landscape, underscoring the ongoing efforts to mitigate the problems facing opioids through smarter drug design. We also highlight that most morphinan derivatives show a preference for the G protein pathway, although detailed experimental comparisons are still necessary. This fact underscores the utility of the morphinan skeleton in future opioid drug discovery.

Keywords: opioid receptors, morphinan, selective agonist, niased ligand

Exploring Diverse Signaling Mechanisms of G Protein-Coupled Receptors through Structural Biology

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Recent advancements in structural biology have facilitated the elucidation of complexes involving G protein-coupled receptors (GPCRs) and their associated signal transducers, including G proteins and arrestins. A comprehensive analysis of these structures provides profound insights into the dynamics of signaling mechanisms. These structural revelations can potentially guide the development of drugs to minimize side effects through targeted and selective signaling. Understanding the binding modes of different signal-selective ligands is imperative for future drug research and development. Here, we conduct a comparative examination of the structural details of various GPCR—signal transducer complexes and delve into the molecular basis of the currently proposed signal selectivity.

Keywords: GPCR, bias agonism, cryo-EM single particle analysis, signal transduction, drug discovery

GPCR signaling bias: an emerging framework for opioid drug development

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Biased signaling, also known as functional selectivity, has emerged as an important concept in drug development targeting G-protein-coupled receptors (GPCRs). Drugs that provoke biased signaling are expected to offer an opportunity for enhanced therapeutic effectiveness with minimized side effects. Opioid analgesics, whilst exerting potent pain-relieving effects, have become a social problem owing to their serious side effects. For the development of safer pain medications, there has been extensive exploration of agonists with a distinct balance of G-protein and β-arrestin (βarr) signaling. Recently, several approaches based on protein–protein interactions have been developed to precisely evaluate individual signal pathways, paving the way for the comprehensive analysis of biased signals. In this review, we describe an overview of bias signaling in opioid receptors, especially the μ-opioid receptor (MOR), and how to evaluate signaling bias in the GPCR field. We also discuss future directions for rational drug development through the integration of diverse signal datasets.

Keywords: GPCRG, protein beta-arrest in biased, ligandmu-opioid, receptor assay

Rethinking c-Fos for understanding drug action in the brain

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Understanding the mechanisms of drug action in the brain, from the genetic to the neural circuit level, is crucial for the development of new agents that act upon the central nervous system. Determining the brain regions and neurons affected by a drug is essential for revealing its mechanism of action in the brain. c-Fos, a marker of neuronal activation, has been widely used to detect neurons activated by stimuli with high spatial resolution. In this review, the use of c-Fos for the visualization and manipulation of activated neurons is introduced. I also explain that a higher temporal resolution can be achieved by changing the staining method for visualization of c-Fos. Moreover, a new method that allows labeling and manipulating commonly activated neurons using two different stimuli is proposed.

Keywords: CANE, catFISH, c-Fos, Split-CANE, TAI-FISH

JB COMMENTARY

Cryo-electron microscopy reveals the impact of the nucleosome dynamics on transcription activity

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The structural biology of nucleosomes and their complexes with chromatin-associated factors contributes to our understanding of fundamental biological processes in the genome. With the advent of cryo-electron microscopy (cryo-EM), several structures are emerging with histone variants, various species and chromatin-associated proteins that bind to nucleosomes. Cryo-EM enables visualization of the dynamic states of nucleosomes, leading to the accumulation of knowledge on chromatin-templated biology. The cryo-EM structure of nucleosome in *Komagataella pastoris*, as studied by Fukushima *et al*., provided the insights into transcription ability of RNAPII with nucleosome dynamics. In this commentary, we review the recent advances in the structural biology of nucleosomes and their related biomolecules.

Keywords: Cryo-EM, nucleosome, chromatin, transcription

REGULAR PAPER

BIOCHEMISTRY

BIOCHEMISTRY GENERAL

Unusual weak and delayed GTPase activity of FtsZ from human pathogenic bacteria *Helicobacter pylori*

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Actively treadmilling FtsZ acts as the pivotal scaffold for bacterial cell divisome components, providing them with a circumferential ride along the site of future division. FtsZ from slow-growing *Helicobacter pylori* (HpFtsZ), a class I carcinogen that thrives abundantly in the acidic environment, is poorly understood. We studied HpFtsZ as a function of pH, cations and time and compared it with well-studied *Escherichia coli* FtsZ (EcFtsZ). HpFtsZ shows pH-dependent GTPase activity, which is inhibited under acidic conditions. Mg^{+2} ions play an indispensable role in its GTPase activity; however, higher Mg^{+2} levels negatively affect its activity. As compared to EcFtsZ, HpFtsZ exhibits lower and slower nucleotide hydrolysing activity. Molecular dynamics simulation studies of FtsZ reveal that GTP binding induces a rewiring of the hydrogen bond network, which results in reduction of the binding cleft volume leading to the spontaneous release of GTP. The GTPase activity is linked to the extent of reduction in the binding cleft volume, which is also supported by the binding free energy analysis. Evidently, HpFtsZ is a pH-sensitive GTPase with low efficiency that may reflect on the overall slow growth rate of *H. pylori.*

Keywords: berberine, EcFtsZ, GTP hydrolysis, HpFtsZ, pH

PROTEIN INTERACTION AND RECOGNITION

Production and characterization of an Fv-clasp of rheumatoid factor, a low-affinity human autoantibody

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Rheumatoid factor (RF) is an autoantibody against IgG that affects autoimmune diseases and inhibits the effectiveness of pharmaceuticals and diagnostic agents. Although RFs derived from various germline genes have been identified, little is known about their molecular recognition mechanisms. In this study, the Fv-clasp format was used to prepare YES8c, an RF. We developed an *Escherichia coli* secretion expression system capable of producing milligram-scale of YES8c Fv-clasp per 1 L of culture. Although YES8c is an autoantibody with very low affinity, the produced Fv-clasp maintained specific binding to IgG. Interestingly, the molecules prepared by *E. coli* secretion had a higher affinity than those prepared by refolding. In the structure of the

YES8c–Fc complex, the N-terminus of the light chain is close to Fc; therefore, it is suggested that the addition of the N-terminal methionine may cause collisions with Fc, resulting in reduced affinity. Our findings suggest that the Fv-clasp, which provides sufficient stability and a high bacterial yield, is a useful format for studying RFs with very low affinity. Furthermore, the Fv-clasp produced from a secretion expression system, which can properly process the N-terminus, would be suitable for analysis of RFs in which the N-terminus may be involved in interactions.
Keywords: antibody, Fv-clasp, molecular recognition, rheumatoid factor

GLYCOBIOLOGY AND CARBOHYDRATE BIOCHEMISTRY

Endogenous reductase activities for the generation of ribitol-phosphate, a CDP-ribitol precursor, in mammals

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The core M3 *O*-mannosyl glycan on *α*-dystroglycan serves as the binding epitope for extracellular matrix molecules. Defects in core M3 glycans cause congenital muscular dystrophies that are collectively known as dystroglycanopathies. The core M3 glycan contains a tandem *D*-ribitol-5-phosphate (Rbo5P) structure, which is synthesized by the Rbo5P-transferases fukutin and fukutin-related protein using CDP-ribitol (CDP-Rbo) as a donor substrate. CDP-Rbo is synthesized from CTP and Rbo5P by CDP-Rbo pyrophosphorylase A. However, the Rbo5P biosynthesis pathway has yet to be elucidated in mammals. Here, we investigated the reductase activities toward four substrates, including ribose, ribulose, ribose-phosphate and ribulose-phosphate, to identify the intracellular Rbo5P production pathway and elucidated the role of the aldo-keto reductases AKR1A1, AKR1B1 and AKR1C1 in those pathways. It was shown that the ribose reduction pathway is the endogenous pathway that contributes most to Rbo5P production in HEK293T cells and that AKR1B1 is the major reductase in this pathway.
Keywords: aldo-keto reductase, CDP-ribitol, *O*-mannosyl glycan, ribitol-5-phosphate, *α*-dystroglycan

BIOCHEMISTRY IN CELL MEMBRANES

Dissection of an ABC transporter LolCDE function analyzed by photo-crosslinking

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The envelope of ***Escherichia coli*** contains approximately 100 different species of lipoproteins, most of which are localized to the inner leaflet of the outer membrane. The localization of lipoprotein (Lol) system, consisting of five Lol proteins, is responsible for the trafficking of lipoproteins to the outer membrane. LolCDE binds to lipoproteins destined for the outer membrane and transfers them to the periplasmic chaperone LolA. Although the cryo-EM structures of ***E. coli*** LolCDE have been reported, the mechanisms by which outer membrane lipoproteins are transferred to LolA remain elusive. In this study, we investigated the interaction between LolCDE and lipoproteins using site-specific photo-crosslinking. We introduced a photo-crosslinkable amino acid into different locations across the four helices which form the central lipoprotein-binding cavity, and identified domains that crosslink with peptidoglycan-associated lipoprotein (Pal) *in vivo*. Using one of the derivatives containing the photo-crosslinkable amino acid, we developed an *in vitro* system to analyze the binding of lipoproteins to LolCDE. Our results indicate that compound 2, a LolCDE inhibitor, does not inhibit the binding of lipoproteins to LolCDE, but rather promotes the dissociation of bound lipoproteins from LolCDE.
Keywords: ABC transporter, inhibitor, lipoprotein, outer membrane, photo-crosslinking

Thermostability optimization of the aspartate/alanine exchange transporter from *Tetragenococcus halophilus*

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Aspartate/alanine exchange transporter (AspT) is a secondary transporter isolated from the lactic acid bacterium *Tetragenococcus halophilus* D10 strain. This transporter cooperates with aspartate decarboxylase to produce proton-motive force through decarboxylative phosphorylation. A method that successfully

analyzes the AspT mechanism could serve as a prototype for elucidating the substrate transport mechanism of other exchange transporters; therefore, the purpose of this study was to search for conditions that improve the thermal stability of AspT for 3D structure analysis. We used the fluorescence size-exclusion chromatography-based thermostability assay to evaluate conditions that contribute to AspT stability. We found that the AspT thermostability was enhanced at pH 5.0 to 6.0 and in the presence of Na^+ and Li^+. Pyridoxal phosphate, a coenzyme of aspartate decarboxylase, also had a thermostabilizing effect on AspT. Under the conditions obtained from these results, it was possible to increase the temperature at which 50% of dimer AspT remained by 14°C. We expect these conditions to provide useful information for future structural analysis of AspT.

Keywords: AspT, cation, fluorescence size-exclusion chromatography–based thermostability assay, pyridoxal phosphate, thermal stability

CdbC: a disulfide bond isomerase involved in the refolding of mycoloyltransferases in *Corynebacterium glutamicum* cells exposed to oxidative conditions

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In *Corynebacterium glutamicum* cells, *cdbC*, which encodes a protein containing the CysXXCys motif, is regulated by the global redox-responsive regulator OsnR. In this study, we assessed the role of the periplasmic protein CdbC in disulfide bond formation and its involvement in mycomembrane biosynthesis. Purified CdbC efficiently refolded scrambled RNaseA, exhibiting prominent disulfide bond isomerase activity. The transcription of *cdbC* was decreased in cells grown in the presence of the reductant dithiothreitol (DTT). Moreover, unlike wild-type and *cdbC*-deleted cells, *cdbC*-overexpressing (P_{180}-*cdbC*) cells grown in the presence of DTT exhibited retarded growth, abnormal cell morphology, increased cell surface hydrophobicity and altered mycolic acid composition. P_{180}-*cdbC* cells cultured in a reducing environment accumulated trehalose monocorynomycolate, indicating mycomembrane deformation. Similarly, a two-hybrid analysis demonstrated the interaction of CdbC with the mycoloyltransferases MytA and MytB. Collectively, our findings suggest that CdbC, a periplasmic disulfide bond isomerase, refolds misfolded MytA and MytB and thereby assists in mycomembrane biosynthesis in cells exposed to oxidative conditions.

Keywords: disulfide bond isomerase, gram-positive, mycolic acid, mycomembrane biosynthesis, oxidative stress

MOLECULAR BIOLOGY

Gene Expression

Distinctive physical properties of DNA shared by RNA polymerase II gene promoters and 5′-flanking regions of tRNA genes

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Numerous noncoding (nc)RNAs have been identified. Similar to the transcription of protein-coding (mRNA) genes, long noncoding (lnc)RNA genes and most of micro (mi)RNA genes are transcribed by RNA polymerase II (Pol II). In the transcription of mRNA genes, core promoters play an indispensable role; they support the assembly of the preinitiation complex (PIC). However, the structural and/or physical properties of the core promoters of lncRNA and miRNA genes remain largely unexplored, in contrast with those of mRNA genes. Using the core promoters of human genes, we analyzed the repertoire and population ratios of residing core promoter elements (CPEs) and calculated the following five DNA physical properties (DPPs): duplex DNA free energy, base stacking energy, protein-induced deformability, rigidity and stabilizing energy of Z-DNA. Here, we show that their CPE and DPP profiles are similar to those of mRNA gene promoters. Importantly, the core promoters of these three classes of genes have two highly distinctive sites in their DPP profiles around the TSS and position −27. Similar characteristics in DPPs are also found in the 5′-flanking regions of tRNA genes, indicating their common essential roles in transcription initiation over the kingdom of RNA polymerases.

Keywords: Core promoter, lncRNA, miRNA, physical properties of DNA, protein-coding gene, tRNA

BIOTECHNOLOGY

Biotechnology General

DF-Phos: Prediction of Protein Phosphorylation Sites by Deep Forest

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Phosphorylation is the most important and studied post-transla-

tional modification (PTM), which plays a crucial role in protein function studies and experimental design. Many significant studies have been performed to predict phosphorylation sites using various machine-learning methods. Recently, several studies have claimed that deep learning-based methods are the best way to predict the phosphorylation sites because deep learning as an advanced machine learning method can automatically detect complex representations of phosphorylation patterns from raw sequences and thus offers a powerful tool to improve phosphorylation site prediction. In this study, we report DF-Phos, a new phosphosite predictor based on the Deep Forest to predict phosphorylation sites. In DF-Phos, the feature vector taken from the CkSAApair method is as input for a Deep Forest framework for predicting phosphorylation sites. The results of 10-fold cross-validation show that the Deep Forest method has the highest performance among other available methods. We implemented a Python program of DF-Phos, which is freely available for non-commercial use at https://github.com/zahiriz/DF-Phos. Moreover, users can use it for various PTM predictions.
Keywords: Deep Forest, Feature Extraction, Machine Learning, Prediction, Protein Phosphorylation

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ダイジェスト

***JB SPECIAL ISSUE*—NEW FRONTIERS IN UBIQUITIN RESEARCH**

GUEST EDITORS: YASUSHI SAEKI AND SHIGEO MURATA

JB SPECIAL ISSUE—REVIEW

Relationships between protein degradation, cellular senescence, and organismal aging

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Aging is a major risk factor for many diseases. Recent studies have shown that age-related disruption of proteostasis leads to the accumulation of abnormal proteins and that dysfunction of the two major intracellular proteolytic pathways, the ubiquitin-proteasome pathway, and the autophagy-lysosome pathway, is largely responsible for this process. Conversely, it has been shown that activation of these proteolytic pathways may contribute to lifespan extension and suppression of pathological conditions, making it a promising intervention for anti-aging. This review provides an overview of the important role of intracellular protein degradation in aging and summarizes how the disruption of proteostasis is involved in age-related diseases.
Keywords: aging, autophagy, proteasome, proteostasis, senescence

Protein homeostasis and degradation in quiescent neural stem cells

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Tissue stem cells are maintained in the adult body throughout life and are crucial for tissue homeostasis as they supply newly functional cells. Quiescence is a reversible arrest in the G0/G1 phase of the cell cycle and a strategy to maintain the quality of tissue stem cells. Quiescence maintains stem cells in a self-renewable and differentiable state for a prolonged period by suppressing energy consumption and cell damage and depletion. Most adult neural stem cells in the brain maintain the quiescent state and produce neurons and glial cells through differentiation after activating from the quiescent state to the proliferating state. In this process, proteostasis, including proteolysis, is essential to transition between the quiescent and proliferating states associated with proteome remodeling. Recent reports have demonstrated that quiescent and proliferating neural stem cells have different expression patterns and roles as proteostatic molecules and are affected by age, indicating differing processes for protein homeostasis in these two states in the brain. This review discusses the multiple regulatory stages from protein synthesis (protein birth) to proteolysis (protein death) in quiescent neural stem cells.
Keywords: adult mouse brain, lysosomes, neural stem cells, proteostasis, quiescence

Mitochondrial quality control via organelle and protein degradation

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Mitochondria are essential eukaryotic organelles that produce ATP as well as synthesize various macromolecules. They also participate in signalling pathways such as the innate immune response and apoptosis. These diverse functions are performed by >1,000 different mitochondrial proteins. Although mitochondria are continuously exposed to potentially damaging conditions such as reactive oxygen species, proteases/peptidases localized in different mitochondrial subcompartments, termed mitoproteases, maintain mitochondrial quality and integrity. In

addition to processing incoming precursors and degrading damaged proteins, mitoproteases also regulate metabolic reactions, mitochondrial protein half-lives and gene transcription. Impaired mitoprotease function is associated with various pathologies. In this review, we highlight recent advances in our understanding of mitochondrial quality control regulated by autophagy, ubiquitin–proteasomes and mitoproteases.

Keywords: autophagy, mitophagy, peptidase, protease, ubiquitin

Molecular structure and function of mysterin/RNF213

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Mysterin is a large intracellular protein harboring a RING finger ubiquitin ligase domain and is also referred to as RING finger protein 213 (RNF213). The author performed the first molecular cloning of the *mysterin* gene as the final step in genetic exploration of cerebrovascular moyamoya disease (MMD) and initiated the next round of exploration to understand its molecular and cellular functions. Although much remains unknown, accumulating findings suggest that mysterin functions in cells by targeting massive intracellular structures, such as lipid droplets (LDs) and various invasive pathogens. In the latter case, mysterin appears to directly surround and ubiquitylate the surface of pathogens and stimulate cell-autonomous antimicrobial reactions, such as xenophagy and inflammatory response. To date, multiple mutations causing MMD have been identified within and near the RING finger domain of *mysterin*; however, their functional relevance remains largely unknown. Besides the RING finger, mysterin harbors a dynein-like ATPase core and an RZ finger, another ubiquitin ligase domain unique to mysterin, while functional exploration of these domains has also just commenced. In this review, the author attempts to summarize the core findings regarding the molecular structure and function of the mysterin protein, with an emphasis on the perspective of MMD research.

Keywords: AAA+, moyamoya disease, mysterin, RNF213, ubiquitin

Protein degraders—from thalidomide to new PROTACs

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Recently, the development of protein degraders (protein-degrading compounds) has prominently progressed. There are two remarkable classes of protein degraders: proteolysis-targeting chimeras (PROTACs) and molecular glue degraders (MGDs). Almost 70 years have passed since thalidomide was initially developed as a sedative-hypnotic drug, which is currently recognized as one of the most well-known MGDs. During the last two decades, a myriad of PROTACs and MGDs have been developed, and the molecular mechanism of action (MOA) of thalidomide was basically elucidated, including identifying its molecular target cereblon (CRBN). CRBN forms a Cullin Ring Ligase 4 with Cul4 and DDB1, whose substrate specificity is controlled by its binding ligands. Thalidomide, lenalidomide and pomalidomide, three CRBN-binding MGDs, were clinically approved to treat several intractable diseases (including multiple myeloma). Several other MGDs and CRBN-based PROTACs (ARV-110 and AVR-471) are undergoing clinical trials. In addition, several new related technologies regarding PROTACs and MGDs have also been developed, and achievements of protein degraders impact not only therapeutic fields but also basic biological science. In this article, I introduce the history of protein degraders, from the development of thalidomide to the latest PROTACs and related technologies.

Keywords: cereblon, molecular glue degrader, PROTACs, protein degrader, thalidomide, ubiquitin

REGULAR PAPER

JB COMMENTARY

On the pursuit to reconstitute the Escherichia coliribo some from purified components

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The ribosome, the protein synthesizing machinery composed of dozens of proteins and several ribosomal RNAs (rRNAs), is essential for life. In vitro reconstitution of the ribosome holds significance for understanding biosynthesis, applications in biotechnology and potential contributions to synthetic biology. There is a long history of in vitro reconstitution of bacterial ribosomes, originating in the 1970s when the 30S ribosome of *Escherichia coli* was reconstituted from the protein and rRNA components prepared from native ribosome. Since then, the reconstitution using in vitro transcribed rRNAs has been established, and more recently, the reconstitution using recombinant ribosomal proteins has also become possible. A recent report by Aoyama *et al.* (*J. Biochem.* 2022; 171: 227–237), the reconstitution of the 50S ribosome using 33 recombinant ribosomal proteins, is a new leap toward complete reconstitution of the holo ribosome complex from recombinant proteins and in vitro transcribed rRNAs. This commentary also discusses future challenges.

Keywords: 30S, 50S, reconstitution, ribosome

JB REVIEW

Therapeutic strategies targeting cellular senescence for cancer and other diseases

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Cellular senescence occurs in response to endogenous or exogenous stresses and is characterized by stable cell cycle arrest, alterations in nuclear morphology and secretion of proinflammatory factors, referred to as the senescence-associated secretory phenotype (SASP). An increase of senescent cells is associated with the development of several types of cancer and aging-related diseases. Therefore, senolytic agents that selectively remove senescent cells may offer opportunities for developing new therapeutic strategies against such cancers and aging-related diseases. This review outlines senescence inducers and the general characteristics of senescent cells. We also discuss the involvement of senescent cells in certain cancers and diseases. Finally, we describe a series of senolytic agents and their utilization in therapeutic strategies.

Keywords: aging, cancer, cellular senescence, premature aging (progeria) syndromes, senescence- and aging-related diseases, senolytic agents

REGULAR PAPER

BIOCHEMISTRY

Glycobiology and Carbohydrate Biochemistry

Comparative Analysis of Site-Specific N-glycosylation of LAMP1 from Breast Cancer Tissues

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Glycosylation changes in cancer proteins have been associated with malignant transformation. However, techniques for analyzing site-specific glycosylation changes in target proteins obtained from clinical tissue samples are insufficient. To overcome these problems, we developed a targeted N-glycoproteomic approach consisting of immunoprecipitation, glycopeptide enrichment, LC/MS/MS and structural assignment using commercially available analytical software followed by manual confirmation. This approach was applied to the comparative site-specific glycosylation analysis of lysosome-associated membrane glycoprotein 1 (LAMP1) between breast cancer (BC) tumors and normal tissues adjacent to tumors. Extensive determination of glycan heterogeneity from four N-glycosylation sites (Asn84/103/249/261) in LAMP1 identified 262 glycoforms and revealed remarkable diversity in tumor glycan structures. A significant increase in N-glycoforms with multiple fucoses and sialic acids at Asn84/249 and high-mannose-type glycans at Asn103/261 were observed in the tumor. Principal component analysis revealed that tumors of different subtypes have independent distributions. This approach enables site-specific glycopeptide analysis of target glycoprotein in breast cancer tissue and become a powerful tool for characterizing tumors with different pathological features by their glycan profiles.

Keywords: breast cancer, LAMP1, LC/MS/MS, site-specific glycosylation, tissue

MOLECULAR BIOLOGY

Genetic Diseases

Perturbed collagen metabolism underlies lymphatic recanalization failure in Gata2 heterozygous deficient mice

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Lymphedema has become a global health issue following the growing number of cancer surgeries. Curative or supportive therapeutics have long been awaited for this refractory condition. Transcription factor GATA2 is crucial in lymphatic development and maintenance, as GATA2 haploinsufficient disease often manifests as lymphedema. We recently demonstrated that *Gata2* heterozygous deficient mice displayed delayed lymphatic recanalization upon lymph node resection. However, whether GATA2 contributes to lymphatic regeneration by functioning in the damaged lymph vessels' microenvironment remains explored. In this study, our integrated analysis demonstrated that dermal collagen fibers were more densely accumulated in the *Gata2* heterozygous deficient mice. The collagen metabolism-related transcriptome was perturbed, and collagen matrix contractile activity was aberrantly increased in *Gata2* heterozygous embryonic fibroblasts. Notably, soluble collagen placement ameliorated delayed lymphatic recanalization, presumably by modulating the stiffness of the extracellular matrix around the

resection site of *Gata2* heterozygous deficient mice. Our results provide valuable insights into mechanisms underlying GATA2-haploinsufficiency-mediated lymphedema and shed light on potential therapeutic avenues for this intractable disease.

Keywords: collagen, *Gata2*, haploinsufficiency, lymphatic vessels, recanalization

CELL Extracellular

Matrices and Cell Adhesion Molecule

Notch signaling pathway induces expression of type IV collagen in angiogenesis

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Mural cell adhesion is important for the localization of basement membrane components during angiogenesis, and cell–cell interactions are thought to be critical for basement membrane formation. Type IV collagen, a component of the basement membrane, and non-triple helical type IV collagen α1 chain (NTH α1(IV)) co-localize in the basement membrane of neovascular vessels. However, it remains unclear how type IV collagen and NTH α1(IV) are produced around the basement membrane. In the present study, we developed a *de novo* angiogenesis model using human umbilical vein endothelial cell spheroids and TIG-1 fibroblast cells and demonstrated that NTH α1(IV), probably with α1(IV) chain before forming triple helix molecule, was localized in the fibroblasts in contact with vascular endothelial cells. This localization was disrupted by DAPT, a Notch signaling inhibitor. DAPT treatment also reduced type IV collagen and NTH α1(IV) secretion in TIG-1 fibroblasts, along with diminished *COL4A1* and *COL4A2* gene expression. Downregulation of Notch3 in TIG-1 fibroblasts decreased the secretion of type IV collagen and NTH α1(IV). Taken together, these findings suggest that heterogeneous and homogeneous intercellular Notch signaling *via* Notch3 induces type IV collagen and NTH α1(IV) expression in fibroblasts and contributes to basement membrane formation in neovascular vessels.

Keywords: angiogenesis, basement membrane, Notch signaling, NTH α1(IV), type IV collagen