CREB-associated glycosylation and function in human disease

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Abstract

The shortcomings of mRNA sequencing in explaining biological functions have resulted in proteomics gradually becoming a hotspot for research. However, the function of proteins becomes complicated as a result of post-translational modifications (PTMs) such as phosphorylation, glycosylation, acetylation, etc. Post-translational modifications do not change the physicochemical properties such as charge and solubility of the proteins, but they can have significant consequences on disease initiation in living organisms. The cyclic adenosine monophosphate (cAMP) response element-binding protein (CREB) is an important transcription regulator in eukaryotic cells. It is involved in the development of neurodegenerative diseases, diabetic complications, tumorigenesis, and neurogenesis. Previously, researchers have paid much more attention to the phosphorylation modification of CREB. However, it seems that the functional regulation-mediated glycosylation modification of CREB was just beginning to be understood. In this review, the current studies and most updated insights on how the glycosylation modification of CREB affects targeted gene expression and disease development will be comprehensively discussed. We hope to further evaluate the role of CREB glycosylation on the regulation of gene function.

Key words: CREB, function, glycosylation modification

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Introduction

In the field of genomics, a great achievement have been made, including the complete genetic sequencing of many species. With the further development of genome sequencing, more research has focused on organ complexity in higher organisms and the encoding of rare genes. However, gene sequencing is still unable to elucidate the biological function of genes. Although genome sequencing has contributed greatly to scientific research, there is no strict linear relationship between proteins and genes. Therefore, proteomics, which is the study of protein characteristics, including protein expression levels, protein–protein interactions and post-translational modifications (PTMs), has gradually become a hotspot for research. Proteomics research provides a comprehensive understanding of disease occurrence and cellular metabolism at the protein level; these issues could provide solutions for disease prevention and treatment.

Previous studies have shown that O-GlcNAc glycosylation may regulate transcription factors and other proteins in the nucleus.[1,2] For example, the O-GlcNAc glycosylation of RNA polymerase II regulates the structure of the enzyme[3] and inhibits transcriptional elongation by preventing phosphorylation of the C-terminal domain.[3] The O-GlcNAc glycosyltransferase (OGT) catalyzes the covalent bonding of O-GlcNAc to substrate in vivo, and has been shown to promote gene silencing by binding to mSin3A, a transcription inhibitor.[4] However, the understanding of the extent of O-GlcNAc glycosylation and its effects on gene regulation is still under research.

Cyclic adenosine monophosphate (cAMP) response element-binding protein (CREB) is an important transcriptional regulator in eukaryotic cells that exists in all mammalian cells. It can bind to the cAMP response element (CRE) sequence TGACGTCA, or the conserved half CRE TGACG. The CREB family members include CREB-1, CREB-2, CREB-3, CREB-5, CREB3L1, CREB3L2, CREB3L3 (CREBH), and CREB3L4. The CREB is also a DNA-binding protein member belonging to a leucine zipper family that includes the activating transcription factor (ATF). The cAMP-responsive transcription factors within the CREB/ATF family include CREB, ATF-1 and cAMP response element modulator (CREM) τ. The structure of CREB contains a signal-responsive kinase-inducible domain (KID), glutamine-rich (Q1 and Q2) constitutive activation domain(s) and a basic leucine zipper (bZIP) domain (Fig. 1A).

The differences in the molecular structures of CREBs are caused by different factors such as splicing, protein translation initiation sites, RNA molecular stability, and PTMs. The CREB plays an important regulatory role in the fields involved in, i.a., regulating gene transcription, physiological rhythm, cell development and survival, addiction, learning, and memory.[5–7] Post-translational modifications play a vital role in the dynamic regulation and complexity of biological processes including the cell cycle, transcription and programmed cell death.[8–11] Most importantly, CREB is regulated by PTMs and is subject to post-transcriptional regulation by phosphorylation, ubiquitination, SUMOylation, and miRNA regulation (Fig. 1B). In living organisms, alterations of proteins are dynamic and several methods of PTMs, such as phosphorylation, glycosylation, acetylation, lipoylation, ubiquitination, and methylation, exist. The phosphorylation of Ser133 site is the central link of CREB activation.[12] After the Ser133 site of CREB is phosphorylated, it can be recognized by the CREB-binding protein (CBP) and bind to it. This acetylates the CRE in the CREB promoter sequence and initiates the gene transcription process. In contrast, other modifications regulate the transcriptional activity of CREB by affecting the Ser133 site. In addition, the CREB

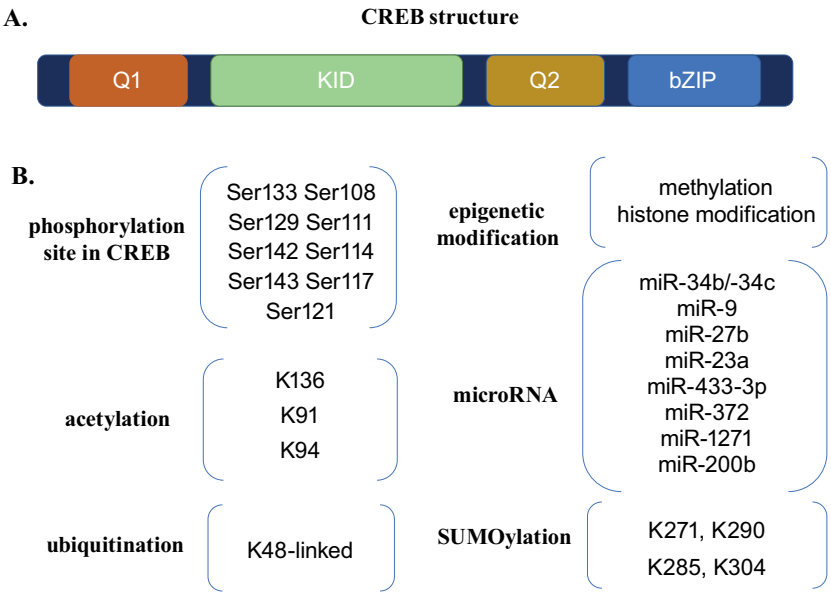

Fig. 1. A. Structure of cyclic adenosine monophosphate (cAMP) response element-binding protein (CREB); B. Post-translational modifications and post-transcriptional regulation of CREB and related amino acid residues in CREB

KID – kinase-inducible domain; bZIP – basic leucine zipper; Q1 – glutamine (Q) domain 1; Q2 – glutamine (Q) domain 2.

molecule is the phosphorylation substrate of other protein kinases such as protein kinase A (PKA), mitogen-activated protein kinase (MAPK) and protein kinase C (PKC). Therefore, CREB acquires diverse biological functions through orderly PTMs that can be affected by different signaling pathways and various regulatory factors to induce CREB activation.

In our review, we summarized studies published between January 1, 2000 and December 31, 2021, relevant to glycosylation-mediated CREB regulation, that promoted a comprehensive understanding of glycosylation-mediated CREB transcriptional activation and the effect of other factors on glycosylation modification. These findings will further elucidate the role of CREB glycosylation in the regulation of gene function.

Objectives

In this review, we aimed to summarize studies involving the regulating model of CREB-associated glycosylation and develop a comprehensive insight into its functional regulation pathway.

Methodology

Literature search strategy

In our review, we followed the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines. Seven databases were systematically searched including PubMed, Scopus, ScienceDirect, ProQuest, EBSCO, Wiley Online, and Taylor & Francis Online from January 1, 2000 to December 31, 2021.

Study selection criteria

Researchers examined the titles for relevance to our study. Additionally, based on the abstracts, we evaluated the eligibility for inclusion. If the abstract was ambiguous, the researchers examined the full text in order to determine whether the report met the inclusion criteria. All references were imported into an Endnote X9 library (Clarivate Analytics, Philadelphia, USA) and duplicates were removed. All citations were included if they met the following criteria: 1) the study pertained to the glycosylation of CREB; 2) the study concerned the glycosylation of CREB cofactors; 3) the study referred to the biological function of CREB or to the CREB-related diseases; and 4) the full text of the study was published in English. Reports were excluded if: 1) they did not describe original research; or 2) the abstract screening demonstrated that the research did not concern CREB-associated glycosylation.

Types of CREB glycosylation modifications

O-GlcNAc glycosylation of CREB

Protein O-GlcNAc glycosylation refers to the dynamic PTM occurring in the cytoplasm and nucleus where only one N-acetylglucosamine (G1cNAc) is connected to serine or threonine (Ser/Thr) hydroxyl groups through O-glycosidic bonds. This protein modification is abundant in eukaryotic cells. Its distribution in nuclear pore complexes and chromatin is the highest, followed by a large number of proteins distributed in the cytoplasm, mitochondria and membrane.[12] As a critical eukaryotic transcription factor, CREB is also subject to the O-GlcNAc glycosylation, which is the main form of CREB protein glycosylation.

The O-GlcNAc glycosylation of CREB is similar to that of most proteins, mainly because O-linked GlcNAc transferase includes OGT and O-GlcNAcase (OGA). Namely, OGT is responsible for adding the glycosyl group and OGA is responsible for removing it.[13]

N-glycosylation of CREB

N-glycosylation refers to the glycosylation of an asparagine (Asn) amide group. The N-terminal α-amino or arginine ω-amino groups are the connecting points for N-linked glycosylation and are the most common forms in eukaryotes. N-glycosylation is generally carried out in the endoplasmic reticulum (ER) and catalyzed by oligosaccharyltransferase (OST). The N-glycosylation modification site has the conserved amino acid sequence, Asn-X-Ser/Thr, in which X is an amino acid other than proline and the oligosaccharide chain is relatively conserved. Finally, the core sugar chain is transferred to Asn of the target protein.[14]

The ATF6β, a subtype of the ER transmembrane glycoprotein ATF6 within the ATF/CREB family, undergoes N-glycosylation and participates in the regulation of the subtype, ATFα, and downstream genes.[15] Additionally, CBP can interact with BRCA2 and affect the N-linked post-translational glycosylation of BRCA2.[16] N-linked glycosylation is required for the optimal proteolytic activation of the membrane-bound transcription factor CREBH.[17] In general, N-glycosylation plays a pivotal role in protein folding, stabilization and degradation processes (Table 1).

Table 1. Glycosylation modification effect on cyclic adenosine monophosphate (cAMP) response element-binding protein (CREB) cofactors and binding protein

CREB cofactors	Modification way	Mechanism
CRTC	O-GlcNAc	Glycosylation represses CREB-dependent transcription by impairing its association with CREB-regulated transcription coactivator.
CBP	N-glycosylation	The binding of CBP to the N-terminal region of BRCA2 is necessary for N-glycosylation of residue 272 on BRCA2.
CBP	O-linked N-acetylglucosamine	Ser147 and Ser236 sites of CREB-binding protein are O-glycosylated.

CREB – cyclic adenosine monophosphate (cAMP) response element binding protein; CRTC – CREB transcription coactivator; CBP – CREB-binding protein.

Glycosylation of CREB and its cofactors

Glycosylation affects CREB and its family members

The CREB is a key regulator in a variety of neuronal processes, such as brain development, circadian rhythm and long-term memory. Multiple studies have suggested that CREB-1 can be glycosylated by O-O-GlcNAc in the Q2 domain, of which the main glycosylation sites are Ser260, Thr256, Thr259, and Thr261. After analyzing the relationship between glycosylation levels and the nuclear extracts of glycosylated CREB-1, it was demonstrated that glycosylated CREB-1 transcriptional activity is 41.5% higher than that of nonglycosylated CREB-1. An in vitro assay validated that glycosylation affects the interaction between CREB-1 and TAFI130 by inhibiting the transcriptional activity of CREB-1.[18] These studies indicate that O-glycosylation is involved in gene regulation and provides a link between O-GlcNAc and information storage processes occurring in the brain.

Rexach et al. explored the functional role the glycosylation of CREB plays in neurons using chemical enzyme quantification.[12] They demonstrated that CREB is dynamically modified with an O-linked β-N-acetyl-D-glucosamine sugar in response to neuronal activity and that glycosylation represses CREB-dependent transcription by impairing its association with CREB-regulated transcription coactivator (CRTC). In another study, it was demonstrated that by blocking the glycosylation of CREB, the function and behavior of cells changed and the growth of neuronal axons and dendrites was enhanced, improving long-term memory.[19] This finding suggested a novel role of O-glycosylation of CREB in memory formation.

In diabetes, researchers used a high-glucose microenvironment in order to induce proinflammatory cytokine signaling, and demonstrated that high glucose levels increased the recruitment of the interleukin-1β (IL-1β) promoter to CBP. This promoted chromatin remodeling and transcription. Phenolic acid treatment interfered with chromatin remodeling and monocyte transcription, weakening protein glycosylation. High glucose levels stimulated the inflammatory response by acting as an anti-glycosylation agent and signaling pathway modifier.[20]

The CREBH is a member of the CREB3 transcription factor subfamily with a bZIP domain, and is a transmembrane transcription factor anchored to the ER. It is cleaved by dictyosome protease when adapting to ER stress and then transferred to the nucleus. It was found that only 3 out of 4 N-glycosylation sites in the ER lumen region of CREBH located at the CREBH C-terminus were conserved in humans and mice, namely Thr413, Thr420 and Thr427. The CREBH undergoes glycosylation in response to ER stress and then induces proteasome hydrolyzation. Next, it is transferred to the nucleus to play a role in transcriptional regulation. However, using site-directed mutations, the conserved Thr residue can be converted to isoleucine, which weakens or destroys the N-linked glycosylation of CREBH. The CREBH remains inactive in the ER and exhibits a significantly reduced ability to drive the unfolded protein response (UPR)/cAMP receptor protein promoter. Collectively, N-glycosylation modifications are necessary for proteolysis-dependent activation of CREBH.[21] The CREBH can also undergo deglycosylation and degradation via ER-related degradation pathways, enhancing the clearance of CREBH and nuclear transport of N-terminal truncated products.[22] In addition, Zhong et al. reported that the inhibition of protein glycosylation by hexosamine D-mannosamine (ManN) is a novel pro-angiogenic strategy that acts via activation of stress pathways in endothelial cells.[23] Although ManN activated extracellular signal-regulated kinase (ERK), AKT (also known as protein kinase B), the mammalian target of rapamycin (mTOR), and CREB at 40 μM, they hypothesized that a unique mechanism may be implicated in the endothelial cell mitogenic effects of ManN. Several studies have shown that the glycosylation process corresponds to protein degradation and proteolysis, the extent of which depends on the level of cellular stress and dynamic environmental changes.

Activated transcription factor 6 (ATF6) is a member of the transcription factor ATF/CREB family and a heterodimer of CREBH. The ATF6β contains 5 conserved N-linked glycosylation sites and is a key transcriptional inhibitor of ATF6α, which assists in regulating the intensity and duration of the ATF6-dependent ER stress response. Nonglycosylated ATF6β has been shown

to directly elevate levels of *ERSR* gene expression through the loss of its inhibitory function on ATF6α.[15,24] However, low levels of glycosylation of ATF6 allow it to act as a balancing receptor in the ER and induce the activation of ATF6. Because of this, when the ER stress response is absent, nonglycosylated ATF6 is transferred to the dictyosome complex more rapidly and is cleaved by S1P and S2P proteases, leading to the constitutive nuclear localization and transcriptional activation.[25] In contrast, nonglycosylated CREBH inhibits the activation of proteolysis and reduces transcriptional activation,[21] revealing the reverse effect of N-glycosylation on CREBH and ATF6.

The *Tisp40* is a spermatid gene expressed during spermiogenesis in mice. It encodes a CREB family transcription factor and contains 2 isoforms including Tisp40α and Tisp40β. The C-terminus of Tisp40α/β is glycosylated.[26] The functions of Tisp40 are changed by the binding of unfolded protein response elements (UPREs) and are activated via the RIP pathway.

Effect of glycosylation on CREB cofactors and binding protein

CREB transcription coactivator (CRTC) was first discovered by genome high-throughput screening technology in 2003. It is a protein family that can regulate the activity of transcription factors.[27] As a coactivator of CREB, 3 members of the CRTC family have been identified, namely CRTC1, CRTC2 and CRTC3. Their C-terminals were found to bind to transcription activators and enhance the transcriptional activity of CREB.[28]

The synthesis of O-GlcNAc mainly occurs through the hexosamine biosynthetic pathway (HBP), which utilizes glucose, acetyl-CoA, glutamine, and uridine triphosphate (UTP) to produce uridine dipho-sphate N-acetylglucosamine (UDP-GlcNAc). Approximately 2–3% of fructose 6-phosphate enters the HBP, and HBP reflects the nutritional status of the cells and plays an important role in biological regulation. Due to its reversible regulation of target protein activities, it is also called the hexosamine cycle or hexosamine (O-GlcNAc) signaling. The CRTC2 is modified by O-glycosylation in the cytoplasm through a phosphorylation-dependent mechanism. The glycosylated form activates hepatic gluconeogenesis, whereas the deglycosylated O-glycosyltransferase inhibits gluconeogenesis, revealing a novel mechanism of chronic hyperglycemia, and connects the interaction between HBP and CREB O-GlcNAc (Fig. 2).[29]

The CREB-binding protein is a transcription coactivator with highly conserved sequence, considered to be an important factor in the regulation of mammalian gene transcription.[30] The CREB-binding protein was found to specifically bind phosphorylated CREB to promote its transcriptional activation.[31] In addition, CBP regulates CREB-dependent gene transcription through CRTC.[32] Some studies have identified O-linked GIcNAc-modified sites of CBP on osteoblasts by means of electron-transfer dissociation tandem mass spectrometry (ETD-MS). This shows that the Ser147 and Ser236 sites of CBP, as well

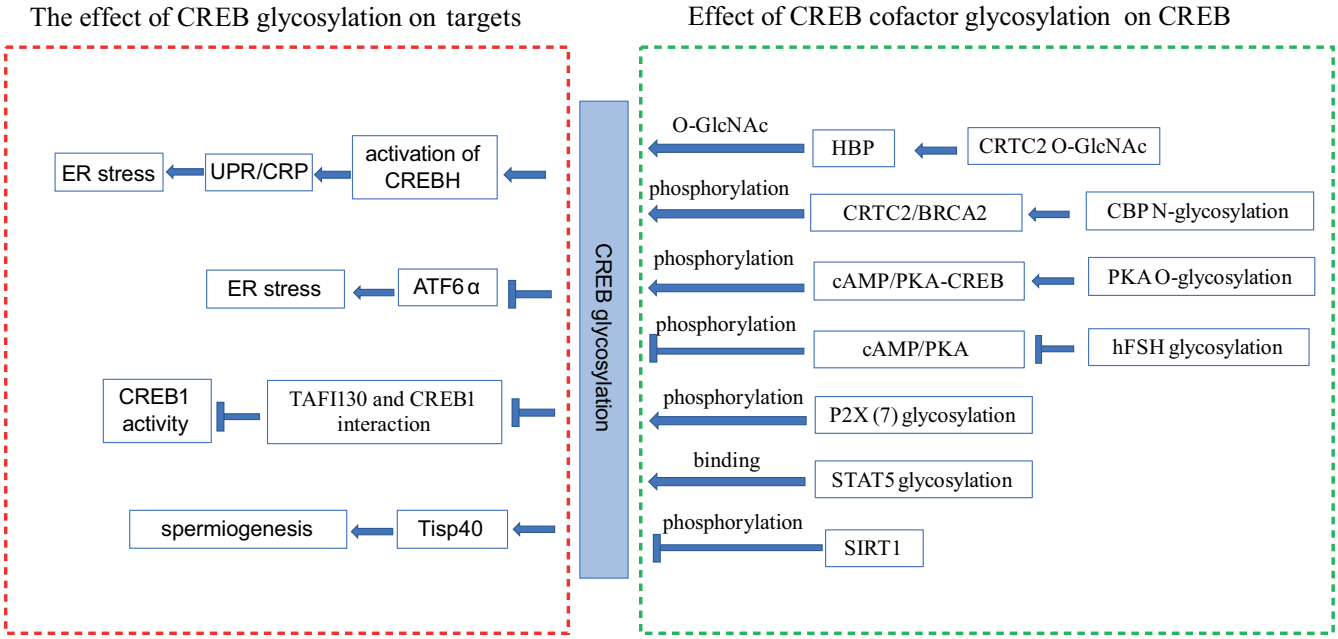

Fig. 2. The regulating network between the glycosylation of cyclic adenosine monophosphate (cAMP) response element-binding protein (CREB) and cofactors. The left panel in the red frame represents the effect of CREB glycosylation on targeted genes. The right panel in the green frame represents the effect of glycosylation on CREB cofactors and CREB post-translational modifications (PTMs)

ER – endoplasmic reticulum; ATF – activating transcription factor; HBP – hexosamine biosynthetic pathway; CRTC – CREB transcription coactivator; CBP – CREB-binding protein; cAMP – cyclic adenosine monophosphate; PKA – protein kinase A; hFSH – human follicle-stimulating hormone; SIRT1 – Sirtuin 1.

as the phosphorylation sites of CREB could be O-glycosylated. Thus, the O-glycosylation of CREB is crucial for bone formation, remodeling and fracture healing, and the effects of glycosylation on protein function and regulation warrant further study.[33] The CREB-binding protein is a transcription cofactor that has been validated to interact with BRCA2 and facilitate the N-linked glycosyl-mediated regulation of BRCA2. The binding of CBP to the N-terminal region of BRCA2 is required for the N-glycosylation of residue 272 on BRCA2 (Table 1). Studies have shown that this CBP-mediated N-glycosylation changes the structure of CBP-related proteins, resulting in the regulation of targeted gene expression, cell growth and differentiation (Fig. 2).[16]

Glycosylation of other factors regulating CREB and its cofactors

The CREB and its cofactors are also regulated by the glycosylation of other factors including kinases, transcription factors, cytokines, and enzymes. Phosphorylation and O-glycosylation are often reciprocal and may affect the subsequent activation and biological function of CREB. Here, we summarize the recent research on this topic (Fig. 2).

PKA signaling pathway

This pathway is also called the cAMP/PKA-CREB signaling pathway and is associated with the activation of CREB. When PKA is activated by cAMP, it immediately enters the nucleus inducing the inactivation of CREB phosphorylation and thus, its biological activity and regulation of target gene transcription.[34] In particular, the Ser residue at position 133 of CREB plays an important role in the regulation of transcriptional activity. The phosphorylation at the site 133 by PKA increases CREB transcriptional activity 10–20 times.[35] Recent studies have shown that high levels of the PKA O-glycosylation enhance the phosphorylation of CREB, but CRBH inhibits this process; such phenomenon suggests that the O-glycosylation of PKA affects the PKAc-CREB signaling pathway by regulating CREB phosphorylation.[36]

With regard to learning and memory, glucose uptake and the levels of O-glycosylation are decreased in brains of individuals with Alzheimer's disease (AD).[37] It has been found that the catalytic subunit of PKA can be modified by O-linked GIcNAc. Afterwards, the subcellular localization of PKAcα and PKAcβ was shown to change and their kinase activity enhanced. This study suggests that in addition to cAMP and phosphorylation, glycosylation is a novel regulating mechanism in PKA-CREB signal transduction.[38]

Human follicle-stimulating hormone

Jiang et al. purified recombinant human follicle-stimulating hormone (hFSH) into low-glycosylated hFSH and fully-glycosylated hFSH.[39] Granulosa cells were then treated with either a high concentration of complete hFSH or low-glycosylated hFSH for 48 h. Those treated with low-glycosylated hFSH showed a more significant accumulation of cAMP, PKA activation and phosphorylation of the S133 site on CREB. Human follicle-stimulating hormone with low glycosylation also stimulated CREB response element-mediated transcription more effectively than that of hFSH with complete glycosylation. It implies that low-glycosylated hFSH exhibits higher biological activity than fully-glycosylated hFSH. These processes regulate the CREB signaling pathway and thus play a positive role in follicle stimulation in older patients using assisted reproductive technology.

Nucleotide receptor P2X (7)

Nucleotide receptor P2X (7), an immunomodulatory cation channel protein, is expressed in immune cells such as monocytes and macrophages. It is activated by extracellular adenosine 5′-triphosphate (ATP) after tissue injury or infection.[40] Ligand binding to P2X (7) can stimulate the production of ERK1 and transcriptional activation of CREB. It was found that P2X (7) is sensitive to Endo H and PNGase F, and is glycosylated at sites N187, N202, N213, N241, and N284. Mutations at the N187 site result in significantly decreased phosphorylation of ERK and CREB by a P2X (7) agonist, suggesting that residue N187 is essential for receptor transport and function.[41]

Signal transducer and activator of transcription

Signal transducer and activator of transcription (STAT) can bind to specific peptides containing phosphorylated tyrosine. Some studies have demonstrated that O-linked GIcNAc is the second modification necessary for STAT5-induced transcription. This glycosylation was only found in activated STAT5 within the nucleus. The STAT5 binds to CBP only after glycosylation.[42]

Sirtuin 1

The CREB functions show a close relationship with learning and memory, which is also related to AD. In this disease, Sirtuin 1 (SIRT1) has neuroprotective properties and the dysregulation of SIRT1 is associated with an aberrant accumulation of the key AD-associated tau protein. A recent study showed that SIRT1 could deacetylate CREB and decrease p-CREB levels. Then, inactivated CREB could attenuate the O-GlcNAcylation of tau by inhibiting OGT.[43]

Others

Drosophila NOT, human clone hNOT-1/ALG3-1 and yeast *ALG3* gene undergo N-glycosylation modification

and bind with the CREB3 precursor. However, there are no interactions with the CREB3 protein cleavage products in the ER and nucleus. A prerequisite for the interaction between these 2 chaperones is the proteolytic activation of CREB3.[44]

Regulatory factors of CREB glycosylation

The glycosylation of CREB is associated with several factors. As a result of a variety of regulatory mechanisms, CREB glycosylation levels and deglycosylation levels undergo constant dynamic adjustment. For example, micronutrients found in organisms are of vital importance in metabolic regulation and provide fuel and energy for protein glycosylation.[45] Therefore, dynamic changes in micronutrient levels in vivo may regulate target genes, and affect related protein glycosylation levels and the utilization of GlcNAcylated CREB. A recent study showed that the glycosylation of CREB is affected by the nutrient sensing pathway of iron by leptin gene regulation. In this study, researchers discovered that high iron intake changed the CREB occupancy pattern, increasing the occupancy of p-CREB and decreasing the occupancy of O-GlcNAcylated CREB on the leptin promoter.[46]

CREB-associated glycosylation and disease

The CREB is an important transcription regulator in eukaryotic cells and is involved in the development of multiple types of diseases, such as neurodegenerative diseases, diabetes complications, tumorigenesis, and neurogenesis (Fig. 3).

Alzheimer's disease

Of note, CREB is mostly related to the molecular mechanisms of learning and memory which are dysregulated in the degenerative disease. As mentioned above, the dysregulation of PKA-CREB signaling could lead to memory loss and cognitive decline. The O-GlcNAcylation of PKA promotes PKA kinase activity and might be a novel way of improving learning and memory deficits in patients with AD.[38] In addition, region-specific activation of CRTC1-CREB signaling mediates long-term fear memory.[47]

Metabolic disease

Previous studies have shown that CREB glycosylation levels and utilization rates are regulated by micronutrients. Induced nutrient sensing and glycolipid metabolism regulation play an important role in metabolic diseases such as obesity, diabetes and hyperglycemia.

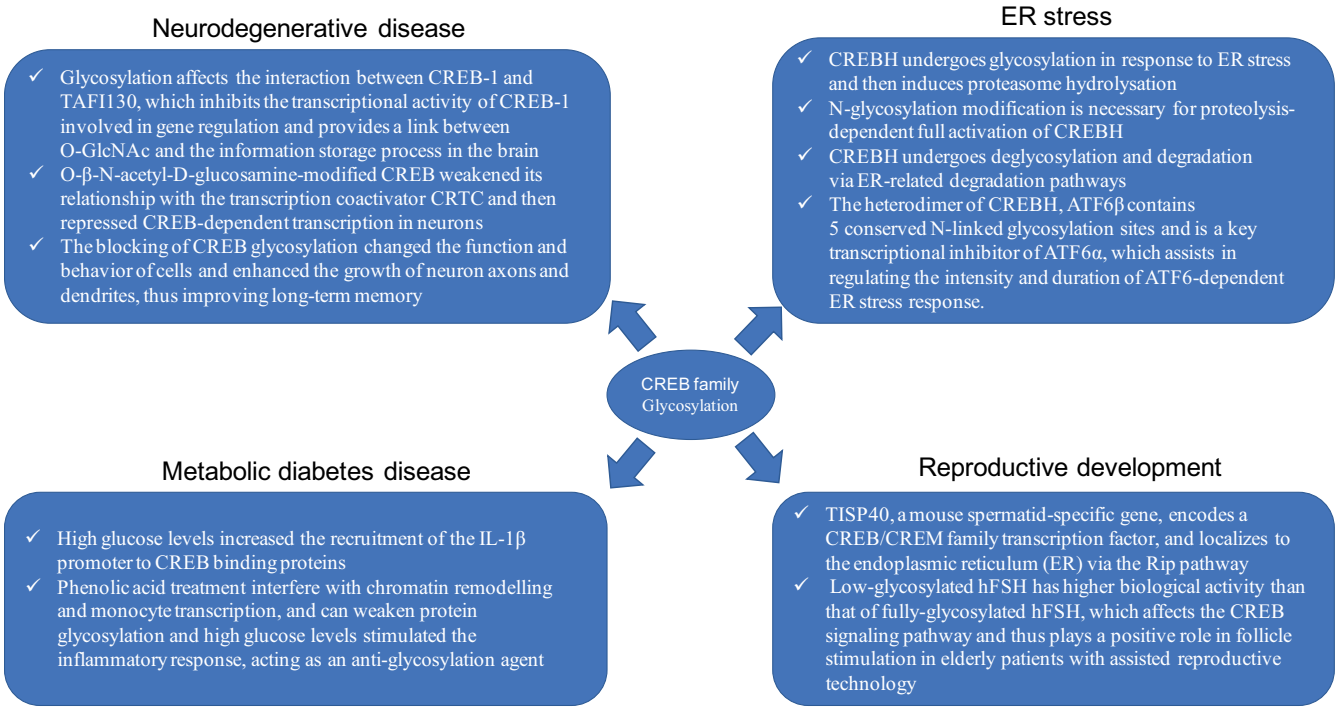

Fig. 3. The regulating mechanism of cyclic adenosine monophosphate (cAMP) response element-binding protein (CREB)-associated glycosylation in disease development, and the involvement of CREB glycosylation in different types of diseases including degenerative diseases, diabetes, reproductive diseases, and endoplasmic reticulum (ER) stress

CRTC – CREB transcription coactivator; IL-1β – interleukin-1β; ATF – activating transcription factor; CREM – cyclic adenosine monophosphate response element modulator; hFSH – human follicle-stimulating hormone.

In addition, CRTC and ATF are both involved in metabolic control. Interestingly, this protein family affects the response of the cell to different external stimuli by sensing extracellular signals such as nutrients, energy state and hormone levels. This results in the changing of the transcriptional activity in order to regulate homeostasis in a variety of important tissues and organs, especially the liver. Disorders of glucose and lipid metabolism can lead to severe metabolic diseases, such as fatty liver disease or hepatocellular carcinoma. It is important to determine how to reverse the disturbance of hepatocyte homeostasis induced by excess nutritional intake. However, protein O-GlcNAc mainly acts through the HBP and, based on its reversible property, might be a promising regulatory mechanism in glucose metabolism.

Reproductive development

Many studies have shown that CREB family genes are related to the development and maturation of germ cells, such as sperm development and follicular cell maturation. In CREB-associated genes, the bZIP-type transcription factors CREB and CREM are reported to play pivotal roles in the events that occur before the morphological changes in spermatogenesis.[48] In particular, CREM is essential for spermatogenesis. The glycosylation process regulates a subsequent signal transduction (such as the RIP pathway) and biological functions (ER degradation).[25]

Regarding female fertility and sex steroid hormone production-related diseases, glycosylation of hFSH changes the bioactivity of hFSH and stimulates the cAMP-PKA-CREB pathway in human granulosa cells. This study suggests that glycosylation of hormones may be the correct direction in the treatment of reproductive and developmental diseases.

Overall, clarifying the relationship between CREB-related glycosylation and diseases is helpful for further applications. By analyzing the glycosylation level of proteins and evaluating the occupancy and heterogeneity of glycans and glycosides, we can further assess the physiological and pathological role of protein glycosylation in diseases. Thus, glycoproteins may be worthy of further study as biomarkers of diseases.[49]

In the future

Glycosylation is one of the most important types of PTMs for CREB functional regulation. The study of the glycosylation of CREB at the proteomic level helps to understand the biological significance of the glycosylation of CREB in life processes. It is also helpful in analyzing the function and significance of CREB from the comprehensive level of genome proteome glycobiology, and enables a more comprehensive understanding of the role of CREB in the pathogenesis of diseases. On the other hand, this research provides scientific guidance for the early diagnosis and treatment of diseases. In conclusion, with the continuous development and improvement of protein glycosylation analysis technology, we will obtain more information on glycoprotein structure and gain a deeper understanding of the biological functions of protein glycosylation.

Admittedly, this review has several limitations. We only discussed CREB-associated glycosylation and its functional regulation, while the noncanonical cellular mechanisms of CREB-associated glycosylation are still unclear. Additionally, CREB-associated glycosylation is involved in a broad number of diseases and only a cursory summary of the most relevant diseases was provided in this study.

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