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Mini Review

Aberrant phosphorylation in the pathogenesis of Alzheimer's disease

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The modification of proteins by reversible phosphorylation is a key mechanism in the regulation of various physiological functions. Abnormal protein kinase or phosphatase activity can cause disease by altering the phosphorylation of critical proteins in normal cellular and disease processes. Alzheimer's disease (AD), typically occurring in the elderly, is an irreversible, progressive brain disorder characterized by memory loss and cognitive decline. Accumulating evidence suggests that protein kinase and phosphatase activity are altered in the brain tissue of AD patients. Tau is a highly recognized phosphoprotein that undergoes hyperphosphorylation to form neurofibrillary tangles, a neuropathlogical hallmark with amyloid plaques in AD brains. This study is a brief overview of the altered protein phosphorylation pathways found in AD. Understanding the molecular mechanisms by which the activities of protein kinases and phosphatases are altered as well as the phosphorylation events in AD can potentially reveal novel insights into the role aberrant phosphorylation plays in the pathogenesis of AD, providing support for protein phosphorylation as a potential treatment strategy for AD. [BMB reports 2009; 42(8): 467-474]

INTRODUCTION

Alzheimer's disease (AD) is an irreversible, progressive brain disorder characterized by learning and memory impairment. The risk of developing AD increases with age, creating a rapidly increasing burden on patients' families and society as a whole. Although numerous studies have focused on the pathogenic mechanism of AD, progress towards the development of effective treatments has been slow. The brains of AD patients share specific pathological hallmarks, namely amyloid plaques and neurofibrillary tangles, which are insoluble deposits made of Aβ proteins and hyperphosphorylated Tau, respectively. The generation of Aβ peptides from amyloid precursor protein (APP) is initiated by the β-secretase BACE1. Subsequently, Aβ proteins are formed upon cleavage by a γ-secretase complex consisting of Presenilin (PS), Nicastrin, anterior pharynx- defective phenotype 1 (Aph-1) and PS enhancer 2 (Pen-2). The importance of Aβ in AD pathogenesis is best demonstrated in rare familial AD cases in which mutations in the APP or Presenilin genes produce more Aβ. Since $A\beta_{42}$ is more fibrillogenic, the ratio of $A\beta_{42}$ to $A\beta_{40}$ could be associated with disease states despite $A\beta_{42}$ being less common than $A\beta_{40}$. However, the mechanisms underlying sporadic AD, the most common type of AD, are not well understood.

The level of protein phosphorylation is controlled by the opposing actions of protein kinases and phosphatases. Accumulating evidence reveals the alteration of protein kinase and phosphatase activity in the brains of AD patients (Table 1). Protein kinases such as Glycogen synthase kinase 3β (GSK3β), P25/Cyclin-dependent kinase 5 (Cdk5), Dual-specific tyrosine (Y) Regulated Kinase 1A (Dyrk1A) and Mitogen-activated pro-

Table 1. Summary of altered expression and/or activity of protein kinases and protein phosphatases in AD

Protein	Expression and/or activity in AD	References
Protein kinases		
GSK3β	Increased (?)	5, 6, 7, 8
P25/Cdk5	Increased (?)	8, 13, 14
Dyrk1A	Increased	24, 25, 26
ERK1/2	Increased	8, 32, 33
JNK	Increased	8, 34, 35
p38	Increased	8, 36
CKI	Increased	43
Akt/PKB	Increased	47, 48, 49
PKA	Decreased	50, 51
PKC	Decreased	52, 53
Protein phosphatases		
PP1	Decreased	59
PP2A	Decreased	57, 59, 60
PP5	Decreased	57, 58
PP2B (calcineurin)	Increased	57, 61
Cdc25A	Increased	66
Cdc25B	Increased	65
PTEN	Decreased	49, 67

(?), controversial.

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tein kinases (MAPKs) were increased in expression and/or activity, whereas decreased activity was observed for protein phosphatases (PPs) such as PP1, PP2A and PP5 in AD brains. Abnormal protein phosphorylation may contribute to the progression of AD by modifying substrates in various functions such as enzymatic activity, subcellular localization, ligand binding, interaction with other proteins, and other properties. Therefore, the regulation of protein phosphorylation by protein kinases/phosphatases could be a promising therapeutic target for the treatment of AD. In this review, I briefly summarize altered protein phosphorylation pathways in the brain tissue of AD patients, focusing on altered protein kinases and phosphatases as well as on the phosphorylation of AD-related proteins.

Dysregulation of protein kinase activity in human AD brains

The role of GSK3 in AD

The protein kinase GSK3, comprised of the highly homologous proteins GSK3α and GSK3β (also called Tau kinase I), is constitutively active in resting cells, unlike most other kinases. GSK3 activity is inhibited via phosphorylation at specific serine residues (Ser 9 and Ser389 for GSK3β and Ser21 for GSK3α) by Akt, also known as Protein kinase B (PKB), and other kinases (1, 2). The activation of GSK3β and GSK3α depends upon the phosphorylation of Tyr216 and Tyr279, respectively, by autophosphorylation or other kinases (1, 3, 4). GSK3β is a multifunctional serine/threonine kinase that plays an important role in many cellular processes, including signaling pathways, metabolic control, apoptosis/cell survival and oncogenesis. In addition, GSK3β has been implicated in a wide range of diseases such as bipolar mood disorder and AD (3). The expression and activity of GSK3β were found to be increased in AD brains compared to non-diseased human brains (5, 6). However, some studies found no change or even a reduction in GSK3 activity (7, 8) due potentially to scientific and technical difficulties in measuring enzymatic activity in postmortem brains. Conditional transgenic mice overexpressing GSK3β displayed hyperphosphorylation of Tau as well as neurodegeneration (9). GSK3β is implicated in the pathogenesis of AD through Aβ-induced neurotoxicity and interaction with Presenilin 1 as well as Tau hyperphosphorylation (4, 9). GSK3α has been shown to regulate APP cleavage, resulting in the increased production of Aβ (10).

The role of Cdk5 in AD

Cdk5 (Tau kinase II) is a proline-directed serine/threonine kinase that, upon activation by the non-cyclin activator p35 or p39, phosphorylates various substrate proteins containing S/T-P-X(X)-K/R/H consensus motifs, including Tau, APP, P35, PAK1, β-catenin, DARPP32, Munc18-1, Amphyphysin1 and Synapsin1 (11). Cdk5 is responsible for the regulation of neurogenesis and cytoarchitecture during neurodevelopment, and plays a role in a variety of cellular functions such as actin dynamics, microtubule stability, axon guidance, membrane transport and dopamine signaling (11). Under neurotoxic conditions, activated calpain cleaves P35 to P25 (12). In contrast to P35, P25 is more stable and remains inside the cytosol due to the absence of a membrane-binding myristoylation signal, thus aberrantly activating Cdk5. In human AD brains, P25 expression and Cdk5 activity are increased compared with age-matched control brains (8, 13), although the increase in P25 amounts remains controversial (14). The debate over P25 expression in AD brain samples likely stems from the short half-life of P25 as well as the difficulty in preparing brain samples. Inducible transgenic mice overexpressing P25 demonstrate Tau hyperphosphorylation, neurofibrillary pathology, increased Aβ levels and neurodegeneration, most likely due to Cdk5-mediated phosphorylation of Tau, APP and Presenilin 1 (15-17).

The role of Dyrk1A in AD

The Dyrk1A gene is localized on human chromosome 21 and encodes a proline-directed protein kinase that may be responsible for mental retardation in Down syndrome (18, 19). The *DYRK1A* gene is a mammalian ortholog of the *Drosophila minibrain* gene, which is essential for normal neurogenesis in flies (20). Knockout mice that lack the Dyrk1A protein show a general delay in fetal development and are embryonic lethal, which strengthens the notion that Dyrk1A has vital and non-redundant biological functions (21). Upon autophosphorylation of Tyr321, Dyrk1A phosphorylates numerous substrate proteins such as transcription factors (NFAT, STAT3, FOXO1a, CREB, Gli1), eukaryotic protein synthesis initiation factor 2Bε (eIF2Bε), Dynamin 1, cyclin L2, and Huntingtin interacting protein 1 (22, 23). The mRNA and protein levels of DYRK1A have been shown to be increased in DS and AD brains (24-26). Transgenic mice that overexpress Dyrk1A show learning and memory deficits, Tau hyperphosphorylation, and elevated Aβ levels (19, 27-29), all suggesting that Dyrk1A plays a role in the pathogenesis of AD.

The role of MAPK in AD

MAPKs are proline-directed serine/threonine kinases activated by phosphorylation on threonine and tyrosine residues in response to extracellular stimuli. MAPK cascades are composed of 3 protein kinases: MAPKs, MAPK kinases (MAPKKs), and MAPKK kinases (MAPKKKs). MAPKs regulate many fundamental cellular processes including gene expression, cell proliferation and cell death (30, 31). MAPK pathways, such as the extracellular signal regulated MAP kinase (ERK) 1/2 pathway (8, 32, 33), the c-Jun-N-terminal kinase (JNK) pathway (8, 34, 35) and the p38 (8, 36) pathway, are activated in the brains of AD patients. JKK1 and MKK6, upstream activators of JNK and p38, respectively, are also activated in AD individuals (37, 38). All three MAPK pathways mentioned above phosphorylate Tau protein *in vitro* (39-41) while the activation of JNK and p38, specifically, was associated with age-dependent amyloid pla-

que deposition in a Tg2576/PS1 double transgenic AD mouse model (42). Taken together, these findings indicate the importance of MAPK pathways in the pathogenesis of AD.

The role of other protein kinases in AD

In AD brains, kinases such as casein kinase 1 (CK1) and Ak/PKB become activated, whereas protein kinase A (PKA) and protein kinase C (PKC) are reduced in activity. The level of CK1 δ mRNA is increased in the brain tissues of AD patients (43). Overexpression of constitutively active CK1ε caused an increase in Aβ amount, suggesting that Aβ-related proteins (APP, BACE1, PS1, PS2, Aph-1, Pen-2 and Nicastrin) are potential targets of CK1 (44, 45). Ak/PKB, another serine/threonine kinase, has been shown to phosphorylate Tau at Ser214 and Thr212 (46). Akt is downstream of phosphatidylinositol-3 kinase (PI3K) and inhibits the activity of GSK3β by phosphorylation at the Ser9 residue. The amount of activated Akt is increased in AD brains compared to normal aged controls (47-49). This finding suggests that GSK3β activity in AD brains is finely tuned by the opposing actions of several inhibiting and activating kinases. Along with the enzyme activity of PKA, the levels of various subunits/isoforms are decreased in the brains of AD patients due to overactivation of calpain (50, 51). PKC signaling pathways regulate the α and β-secretase-mediated cleavage of APP, resulting in a reduction of Aβ peptides (52, 53). In AD brain tissue, there is considerable evidence demonstrating reduced activity of PKC (52, 53), which may be responsible for the increased level of Aβ peptides observed in AD.

Dysregulation of protein phosphatases in human AD brains

Protein phosphases are classified into three classes according to the structure of the catalytic site: serine/threonine protein phosphatases, phosphoprotein phosphatase (PPP) and protein phosphatase metal-dependent (PPM) families, and the protein tyrosine phosphatase (PTP) superfamily. The PPP family includes PP1, PP2A, PP2B (also called calcineurin), PP4 and PP5, whereas the PPM family comprises Mg^{2+}-dependent enzymes such as PP2C and mitochondrial protein phosphatases. Members of the PTP superfamily are categorized as either classical PTPs or dual specificity protein phosphatases (DSPases). DSPases dephosphorylate the serines, threonines and tyrosines of a particular substrate and consist of cdc25 and phosphatase and tensin homolog (PTEN).

Increasing evidence suggests that dephosphorylation of AD-related phosphoproteins by phosphatases plays a role in the pathogenesis of AD (54). The activity of serine/threonine protein phosphatases (PPs) such as PP1, PP2A and PP5 are decreased in AD brains (54-60), although the activity of PP2B is upregulated in the brains of AD patients (61). The downregulation of PP2A activity, a major Tau phosphatase, may underlie the hyperphosphorylation of Tau in the brains of AD patients. This could partially be due to the upregulation of two endogenous PP2A inhibitors, I1 (PP2A) and I2 (PP2A) (57, 62, 63). DSPases have also been implicated in AD (64). Cdc25A and Cdc25B are activated in the degenerating postmitotic neurons of AD patients (65, 66). PTEN, a major negative regulator of the PI3k/Akt pathway, was found to be significantly less expressed in AD neurons, as well as being altered in distribution (49, 67). However the underlying mechanism governing altered phosphatase activity remains to be investigated.

Phosphorylation of AD-related proteins

Hyperphosphorylation of Tau

The regulation of protein phosphorylation could be a target for the treatment of AD. Neurofibrillary tangles, a pathological hallmark of AD, comprise paired helical filaments (PHFs) composed mainly of hyperphosphorylated Tau protein (68). The hyperphosphorylation of Tau is probably the most extensively studied area in the field of neurodegenerative disease. To date, more than 39 phosphorylation sites have been detected in PHF-Tau, including multiple proline-directed serine/threonine residues (69, 70). The level of Tau phosphorylation (7-10 phosphorylations per mole of Tau) is regulated dynamically by several kinases and phosphatases. Therefore, any imbalance between the activities of kinases and phosphatases could cause Tau hyperphosphorylation in AD brains. Although Tau protein is phosphorylated by numerous kinases *in vitro*, it is GSK3β, Cdk5, Dyrk1A, PKA and microtubule-affinity- regulating kinase (MARK) that are known to modulate the proline-rich regions of Tau *in vivo*, either directly or indirectly (27, 71). Phosphorylation of Tau at different sites can have diverse effects on both its biological function and pathogenic role, such as the conformational changes Tau undergoes from its less-phosphorylated, microtubule-bound form to its hyperphosphorylated, self-aggregated form (72, 73). Among the protein phosphatases involved in Tau dephosphorylation, PP2A has been shown to be the most important and major Tau phosphatase (57). Several recent reviews have appeared covering the Tau pathology and the underlying dysregulation of the phosphorylation system in AD (62, 73-76).

Phosphorylation of APP

Sequential cleavage of APP by β-secretase and γ-secretase complex releases Aβ, a key trigger for AD. The short cytoplasmic C-terminal domain appears to play a central role in the structure and physiological function of APP. Interaction of the APP C-terminal domain with proteins such as X11/Mint and Fe65 affects APP processing either by stabilizing APP or by regulating Aβ production (77). The C-terminal domain of APP contains several residues shown to be phosphorylated by different protein kinases. Specifically, phosphorylation of APP by PKC at residues Thr654/Ser655 may protect the brain from AD by favoring the non-amyloidogenic processing of APP (78).

Reduced PKC activity in the brains of AD patients may lead to amyloidogenic processing of APP, resulting in increased Aβ production. The Thr668 residue of APP is phosphorylated by various proline-directed protein kinases upregulated in AD, including Cdk5 (79), GSK3β, JNK3 (80-82) and Dyrk1A (26). According to nuclear magnetic resonance studies, Thr668 phosphorylation seemed to cause a dramatic conformational change in the APP backbone (83) such that the interaction of APP with its binding partners is affected. Indeed, phospho-Thr668-APP is elevated in AD brains, suggesting that Thr668 phosphorylation of APP might be functionally linked to the increased amounts of Aβ peptide in the brains of AD patients (79). Mutation of Thr668 to Ala results in a significant reduction in Aβ production in primary neuronal cultures. However the brains of APP T668A knock-in mice did not show changes in either the subcellular distribution of APP or the level of Aβ compared with those observed in wild-type mice (84, 85). Further studies into the precise mechanism of the increased Aβ during the processing of APP are still necessary.

Phosphorylation of Presenilin-containing γ-secretase complex

The γ-secretase complex consists of at least four different proteins: Presenilin (PS), Nicastrin, Aph-1 and Pen-2 proteins. The catalytic activity of the complex is contained in Presenilin, an aspartyl protease. Mutations in the two homologous Presenilin genes, PS1 and PS2, are the most common cause of early-onset familial AD. Both PS1 and PS2 are widely expressed in the brain and peripheral tissues. PS1 is an integral membrane protein localized in the ER and Golgi membrane as well as in the plasma membrane with nine predicted transmembrane domains (86). To form an active γ-secretase complex, Nicastrin, a type I transmembrane glycoprotein, and Aph-1 form a dimeric subcomplex and then full-length PS binds to this subcomplex. Finally, Pen-2 is incorporated into the complex and PS is cleaved into two stable pieces, N-terminal fragment (NTF) and C-terminal fragment (CTF), forming an active γ-secretase (87-89).

PS1 has been shown to be phosphorylated by several protein kinases. Phosphorylation of PS1 at Ser353 and Ser357 by GSK3β may influence binding to β-catenin (90). Phosphorylation of PS1 at Ser397 by GSK3β (91) and at Thr354 by P35/Cdk5 residue (17) regulates C-terminal fragment stability. PKA-mediated phosphorylation strongly inhibits proteolytic processing of PS1 by caspase activity during apoptosis, reducing the progression of apoptosis (92). Recent studies have shown that ERK1/2 is an endogenous negative regulator of γ-secretase, potentially through the direct phosphorylation of Nicastrin (93). Tumor necrosis factor α (TNF-α) stimulates γ-secretase activity through JNK phosphorylation of PS1 and Nicastrin (94). PS1 stimulates PI3K/Akt signaling, thus inhibiting GSK3β activity and Tau hyperphosphorylation (95). The significance of phosphorylation of the PS-containing γ-secretase complex component in the pathogenesis of AD remains to be assessed *in vivo*.

Protein phosphorylation as a therapeutic target in AD

Increasing evidence indicates that protein kinase and phosphatase activity are altered in the brains of AD patients. Therefore, altering the activity of protein kinases/phosphatases is a promising treatment strategy for AD. Targeting phosphorylation has already been verified as successful in the development of cancer-fighting drugs such as Gleevec and Herceptin, which both inhibit protein tyrosine kinases. A promising approaches for the treatment of AD over the past decade has been focused on abnormal Tau hyperphosphorylation by inhibiting the activity of Tau kinases such as GSK3β and Cdk5, or by increasing PP2A activity in the brain (73). Over the past years, numerous review articles on this subject have been published (3, 4, 62, 75, 96, 97).

CONCLUSION

In this study, protein phosphorylation pathways altered in the brain tissue of AD patients were summarized with a focus on altered protein kinases and phosphatases and the abnormal phosphorylation of AD-related proteins. An increasing number of studies support a role of aberrant phosphorylation in both Tau hyperphosphorylation and Aβ production in AD pathogenesis. Although Tau hyperphosphorylation has been a major focus of research and drug development during the past decade, recent studies suggest that other AD-related phosphorylation events may also underlie the pathophysiological state of AD. It is predictable that the list of altered protein kinases, protein phosphatases and AD-related substrates in AD will continue to grow, increasing the importance of abnormal phosphorylation in the pathogenesis of AD. A clearer picture of the phosphorylation pathways of AD will depend on studies focusing on the underlying mechanism governing the altered activities of protein kinases and phosphatases. A better understanding of these phosphorylation pathways will eventually lead to the development of novel therapeutic strategies for AD.

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