Dysfunctional gene splicing in glucose metabolism may contribute to Alzheimer's disease

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Abstract

The glucose metabolism is crucial for sustained brain activity as it provides energy and is a carbon source for multiple biomacromolecules; glucose metabolism decreases dramatically in Alzheimer's disease (AD) and may be a fundamental cause for its development. Recent studies reveal that the alternative splicing events of certain genes effectively regulate several processes in glucose metabolism including insulin receptor, insulin-degrading enzyme, pyruvate kinase M, receptor for advanced glycation endproducts, and others, thereby, influencing glucose uptake, glycolysis, and advanced glycation end-products-mediated signaling pathways. Indeed, the discovery of aberrant alternative splicing that changes the proteomic diversity and protein activity in glucose metabolism has been pivotal in our understanding of AD development. In this review, we summarize the alternative splicing events of the glucose metabolism-related genes in AD pathology and highlight the crucial regulatory roles of splicing factors in the alternative splicing process. We also discuss the emerging therapeutic approaches for targeting splicing factors for AD treatment.
Keywords: Alzheimer's disease; Alternative splicing; Glucose metabolism; Splicing factors

Introduction

Alzheimer's Disease (AD) is a progressive neurodegenerative disorder, which is closely associated with aging. With an increase in the proportion of aging population, AD has become a phenomenon of worldwide concern; with at least 55 million people living with AD or other dementias, this number is estimated to grow to 139 million by 2050 according to the World Alzheimer Report 2021. Neuropathologically, AD brains can be broadly divided into two based on their typical clinicopathological features, namely, extracellular senile plaques and intracellular neurofibrillary tangles. However, AD is a very complex disease involving many factors, and multiple hypotheses for AD development have been proposed, including amyloid β (Aβ), tau, abnormal glucose metabolism, cholinergic neuron damage, oxidative stress, inflammation, and aberrant alternative splicing mechanisms.[1-4] With the development of transcriptomic sequencing techniques, research on alternative splicing in AD has drawn great attention in recent years.

Alternative splicing involves post-transcriptional modification of genes. It produces different messenger RNA (mRNA) splicing isoforms from a precursor mRNA and is an important mechanism for the generation of proteomic diversity. In conventional splicing, the spliceosome joins exons at the 5′ splice (donor) site with the downstream 3′ site, producing a linear RNA. Previous studies on linear alternative splicing show that there are several ways of alternative splicing and at least five basic modes are generally recognized, including exon skipping, mutually exclusive exons, alternative 3′ acceptor sites, alternative 5′ donor sites, and intron retention.[5] Under normal physiological conditions, the occurrence of alternative splicing of genes greatly increases the protein diversity and complexity of gene expression, thus contributing to the maintenance of the corresponding physiological activities of cells and tissues. Similarly, several specific gene alternative splicing processes also occur under pathological conditions, which are of great significance for the prevention, detection, and treatment of these diseases.[6] As aging is a critical risk factor of occurrence of AD, Deschenes *et al*,[7] in their review on aging-related alternative splicing, suggest an indispensable role of abnormal alternative splicing in the development of AD. Exon array testing performed by Lai *et al*[8] shows many

Access this article online

Quick Response Code:

Website: www.cmj.org

DOI: 10.1097/CM9.0000000000002214

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Chinese Medical Journal 2023;136(6)

Received: 14-12-2022; **Online:** 09-03-2023 **Edited by:** Yanjie Yin and Xiuyuan Hao

abnormal alternative splicing processes of specific genes in AD, apart from the Aβ protein precursor (*APP*) and *tau* genes. Thus, the abnormal alternative splicing events for key genes have gained traction as a novel hypothesis for explaining AD development.[7,9]

Glucose is essential as an energy substrate and is a carbon source for the synthesis of several important biomolecules required to sustain neuronal activity. It includes several processes, such as glucose uptake, glycolysis, oxidative phosphorylation in Krebs Cycle, and advanced glycation end-products (AGEs) production. Impaired cerebral glucose metabolism is regarded as an invariant pathological feature underlying AD.[2] AD is also considered as type 3 diabetes, owing to the similar molecular and cellular characteristics of glucose metabolism impairment as in type 1 diabetes and type 2 diabetes.[10] Epidemiological investigations indicate the link between AD and diabetes, in particular, type 2 diabetes. They demonstrate that the prevalence of AD in diabetic rats is much higher than that in control individuals.[11] Recently a genome-wide association study based on the large-scale genome-wide cross-trait analysis by Liang *et al*[12] demonstrates the connection between AD and disorders of glucose metabolism. Notably, Croteau *et al*[13] show that in mild cognitive impairment as compared to cognitively healthy older adult controls, the glucose hypometabolism rate is ~7%. This evidence suggests that abnormal glucose metabolism is a potential risk factor underlying AD development. Insulin signaling pathway, glycolysis regulation, oxidative phosphorylation regulation in the Krebs Cycle, and AGEs regulation are crucial steps in glucose metabolism. Increasing evidence shows that alternative splicing events of specific genes, including insulin receptor (*INSR*),[14] insulin-degrading enzyme (*IDE*),[15] pyruvate kinase M (*PKM*),[16] 6-phosphofructo-2-kinase/fructose-2,6-biphosphatase 2,[17] exocyst complex component 3 like 4,[18] receptor for advanced glycation endproducts (*RAGE*),[19] and others, significantly influence these steps and subsequently, impair the glucose metabolism in AD. In this review, aberrant gene alternative splicing events involved in glucose metabolism have been described and alternative splicing-related therapeutic implications for AD are discussed.

Gene alternative splicing in insulin signaling

The insulin signaling pathway regulates glucose homeostasis and also exerts a profound influence on learning and memory.[20] Insulin signaling activates the phosphatidylinositol 3 kinase/Akt serine/threonine kinase 1 (PI3K/Akt) pathway to suppress glycogen synthase kinase 3 (GSK3), with two highly conserved homologous forms, α, and β. Akt phosphorylates GSK3β at Ser9, consequently inactivating it and subsequently suppressing the GSK3β-induced tau hyperphosphorylation to alleviate the levels of intracellular neurofibrillary tangles,[21] and inhibit GSK3α activation enhancing beta-secretase 1 and γ-secretase activity for efficient Aβ degradation.[22,23] Estrogen not only activates the insulin signaling pathway to regulate glucose metabolism in the brain but also phosphorylates GSK3β at Ser9 through the mitogen-activated protein kinase (MAPK) pathway to prevent AD pathology[24,25] [Figure 1]. Estrogen deficiency impairs glucose homeostasis mediated by insulin resistance, increasing AD incidence in older women; those who had undergone estrogen replacement therapy showed improved glucose metabolism and this may be useful for preventing or delaying the onset of dementia to some extent.[26-28] These observations indicate that impairment

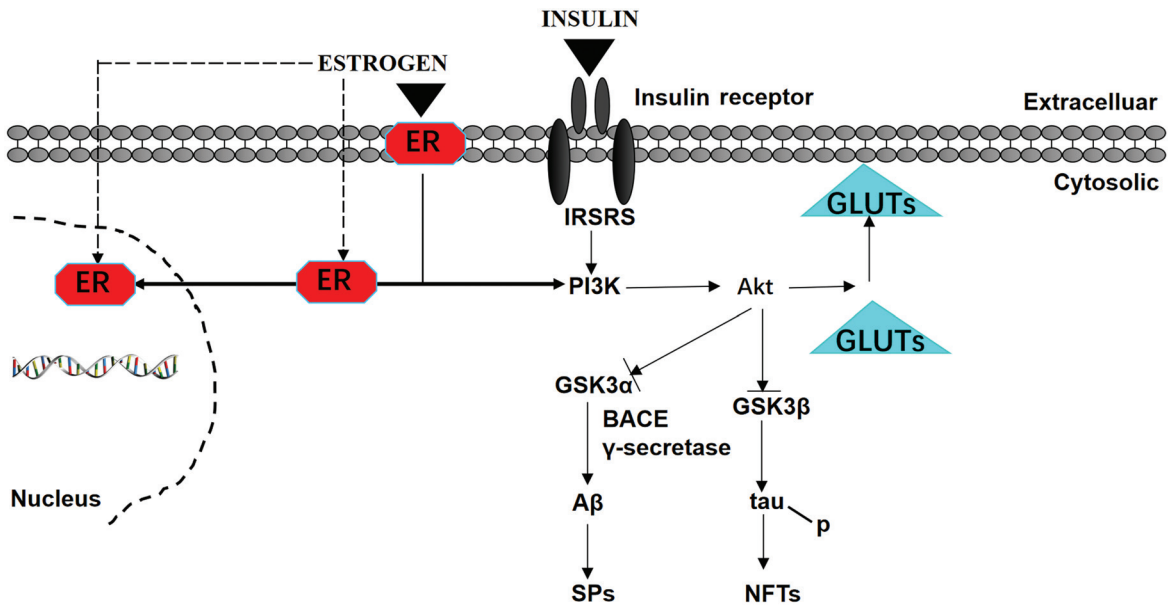

Figure 1: Insulin signaling suppresses AD neuropathology. Insulin acts on INSRs, thereby activating the PI3K/Akt pathway and inhibiting glycogen synthase kinase 3 (GSK3). GSK3 contains two highly conserved homologous forms, namely, GSK3α and GSK3β. GSK3α increases the activity of BACE γ secretase, leading to the degradation of amyloid β and senile plaques, while GSK3β induces hyperphosphorylation of tau and reduces intracellular neurofibrillary tangles. Moreover, estrogen can activate the insulin signaling pathway to reduce the formation of senile plaques and neurofibrillary tangles (NFTs) by inhibiting GSK3α/3β, respectively. AD: Alzheimer's disease; Aβ: Amyloid β; BACE: Beta-secretase 1; ER: Estrogen receptor; GLUTs: Glucose transporters; GSK3: Glycogen synthase kinase 3; INSR: Insulin receptor; NFTs: Neurofibrillary tangles; PI3K/Akt: Phosphatidylinositol 3 kinase/Akt serine/threonine kinase 1; SPs: Senile plaques.

of glucose metabolism regulated by insulin signaling results in AD development. Insulin signaling is activated upon insulin binding to the INSR, and degradation of insulin molecule by IDE, whereas the aberrant alternative splicing events of *INSR* and *IDE* are implicated in AD.

Alternative splicing of INSR

INSR is bound by insulin leading to the activation of downstream signaling cascades, including the PI3K/AKT and MAPK pathways using the INSR as a substrate. INSR contains two subunits, namely the α-subunit and β-subunit, resulting from the cleavage of a single polypeptide encoded by the *INSR* gene. The α-subunit is the extracellular domain that binds insulin. Alternative splicing of the *INSR* gene at exon 11 generates two isoforms of the α-subunit, namely, INSR-A and INSR-B. Skipping of exon 11 occurs in INSR-A and distributes in the embryo and adult neurons, while INSR-B having the exon 11 is mainly localized to insulin-sensitive organs, including adipocytes, liver, kidney, and muscles. Both INSR-A and INSR-B isoforms are expressed in human astrocytes and microglia, however, only INSR-A is detected in immortalized human neurons *in vitro* by real-time quantitative polymerase chain reaction (qPCR) analysis.[29] Furthermore, in a recent study by Spencer *et al*[30] on human brain samples, INSR-A and INSR-B isoforms were visualized in neurons by qPCR *in situ* and fluorescence in situ hybridization assay *in vivo*. These observations indicated that both INSR-A and INSR-B isoforms are present in the brain.

Compared to INSR-B, INSR-A has a higher insulin affinity.[31] INSR-A has a high affinity for insulin-like growth factor-1, while INSR-B shows favorable binding with insulin-like growth factor-1,[32] which implies that different types of INSRs may lead to alternative signaling cascades and corresponding cell fates. Studies on selective insulin signaling through INSR-A and INSR-B show that insulin/INSR signaling stimulates synaptic upregulation in neurons through the MAPK pathway and this activation is most likely mediated by the INSR-A isoform.[33] In another study, INSR-A could preferential activate the PI3K class Ia and p70 s6 kinase (p70s6k) and promote insulin transcription. INSR-B intracellular signaling occurs through the activities of PI3K class II-like and protein kinase B pathways, which activate the transcription of the glucokinase gene.[14] Insulin-like receptor subunit beta, an IRSR-B analog in *Caenorhabditis elegans*, is involved in learning and memory and localizes to the synapses of chemosensory neurons.[34] Thus, both INSR-A and INSR-B may be expressed in the brain and execute distinct functions. Therefore, any alteration in the INSR-A/INSR-B ratio may result in diseases.

Indeed, high INSR-A/INSR-B ratios in muscle result in myotonic dystrophy.[35,36] Moreover, aging induces a shift in the alternative spliced forms of INSR from INSR-B to INSR-A,[37] which may be related to insulin resistance and glucose intolerance with increasing age.[38] INSR is desensitized in late-onset sporadic AD cases as compared to that in normal aging and individuals with early-onset familial AD.[39] Further, alterations in insulin signaling are associated with AD.[40] Thus, any alteration in the INSR-A/INSR-B ratio in the brain may result in age-related pathologies, including AD [Figure 2].

Splicing factors play an important role in regulating alternative splicing of genes; examples include serine/arginine-rich splicing factor 3 (SRSF3),[41] serine/arginine-rich splicing factor 1 (SRSF1),[41,42] heterogeneous nuclear ribonucleoprotein F,[43] and muscleblind like splicing regulator 1 (MBNL1),[44] which promotes exon 11 inclusion in INSR gene, resulting in the production of the INSR-B isoform; heterogeneous nuclear ribonucleoprotein A1 (hnRNP A1)[43] and CUGBP Elav-like family member 1, is a suppressor for exon 11 inclusion.[35,45] The levels of splicing factors in the AD brain are altered. For instance, hnRNP A1 expression decreases in AD brain, and experiments in transgenic mouse models support the hypothesis that hnRNPA1 loss contributes to AD; however, alternative splicing of INSR has not been examined in this context.[46] Intriguingly, estrogen, a potent factor improving glucose metabolism, increases the expression of hnRNP A1 and regulates alternative splicing of APP, thereby, resulting in enhanced expression of APP695 and attenuation of secreted Aβ levels.[47]

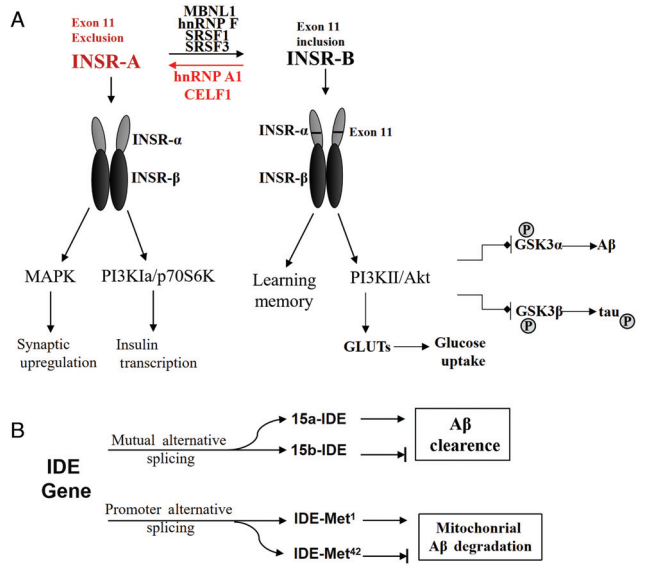

Figure 2: Alternative splicing of INSR and IDE in AD pathology. (A) Alternative splicing of INSR gene at exon 11 generates two isoforms of the α-subunit, namely, INSR-A and INSR-B, with INSR-A skipping exon 11 and INSR-B including the exon. SRSF3, SRSF1, hnRNP F, and MBNL1 promote exon 11 inclusion in the *INSR* gene to generate INSR-B, while hnRNP A1 and CELF1 function as suppressors for exon 11 inclusion. INSR-A shows preferential activation of the PI3K class Ia/p70s6k to promote insulin transcription and activate MAPK signaling, thereby contributing to synaptic upregulation. INSR-B intracellular signaling occurs through PI3K class II-like and (PKB/Akt) activities to stimulate the transcription of the glucokinase gene and inhibit amyloid β deposition and tau hyperphosphorylation. INSR-B signaling is also beneficial for learning and memory. (B) By mutual alternative splicing of the *IDE* gene, two isoforms are produced, namely, 15a-IDE and 15b-IDE. The former can reduce Aβ, while the latter inhibits the clearance of Aβ. In addition, two isoforms are produced through promoter-mediated alternative splicing of the *IDE* gene; IDE-Met^{1} facilitates the degradation of Aβ in mitochondria, while IDE-Met^{42} exerts opposite functions. AD: Alzheimer's disease; GLUTs: Glucose transporters; GSK3: Glycogen synthase kinase 3; hnRNP A1: Heterogeneous nuclear ribonucleoprotein A1; hnRNP F: Heterogeneous nuclear ribonucleoprotein F; IDE: Insulin-degrading enzyme; INSR: Insulin receptor; INSR-A: Insulin receptor A; INSR-B: Insulin receptor B; MAPK: Mitogen-activated protein kinase; MBNL1: Muscleblind like splicing regulator 1; NFTs: Neurofibrillary tangles; PI3K: Phosphatidylinositol 3 kinase; PKB: Protein kinase B; SRSF1: Serine/arginine-rich splicing factor 1; SRSF3: Serine/arginine-rich splicing factor 3.

Together, these findings provide strong evidence for splicing factors being promising therapeutic targets.

Alternative splicing of IDE

As a very important protease degrading insulin, IDE is also closely related to AD pathology. IDE is a zinc metalloprotease, extensively expressed in the liver, testis, muscle, and brain. It can effectively degrade insulin and Aβ.[48] Under normal physiological conditions, IDE is localized to the cytoplasm, cell surface, and mitochondria.[49] *In vitro* observations provide strong evidence that IDE not only cleaves the endogenous Aβ but also degrades the intracellular domain of Aβ precursor protein,[50] leading to its clearance. Genetic deletion of *IDE* in mice,[50] and rats[51] leads to significant elevations in Aβ levels in the brain. Overexpression of IDE in transgenic mice attenuates Aβ levels and plaque formation in the brain.[52] Clinical studies show reduced expression or suppressed activity of IDE in the AD brain,[53] and its inverse correlation with the level of Aβ42.[53,54] Collectively, IDE has an important role in the regulation of Aβ. Furthermore, IDE is significantly upregulated by insulin signaling, consistent with the beneficial function of insulin signaling in AD.[55]

Increasing evidence from genetic data implicates *IDE* in AD; consequently, the alternative splicing of the *IDE* gene is of critical importance. No less than six variants have been identified in the human brain and one novel splice isoform, 15b-IDE, wherein exon 15b replaces the canonical exon 15a, shows reduced ability to degrade insulin and Aβ.[15] Another isoform of IDE, IDE-Met^{1}, (the longer isoform of IDE as compared to IDE-Met^{42}) is generated by alternative splicing of the promoter and localizes to the mitochondria.[49] It is further known to specifically and effectively degrade Aβ in the mitochondrial membrane, unlike IDE-Met^{42}. Although to date, no alterations in 15b-IDE isoform expression have been found in chromosome 10-linked AD families,[54] we believe that further effort for functional characterization of specific-localized isoforms and other IDE isoforms having underlying regulatory mechanisms mediated by gene alternative splicing events will benefit the development of treatment strategies for AD.

Glycolytic regulation

Glucose metabolism is an essential biochemical process that provides energy for the brain.[56] Glucose is taken up by the cells using glucose transporters present downstream in the insulin signaling cascade. One glucose (six carbons) molecule can be converted into two pyruvate molecules (three carbons), releasing two molecules of adenosine 5′-triphosphate (ATP). Pyruvate, in the presence of adequate oxygen, undergoes oxidative-phosphorylation in mitochondria, generating energy ATP for cellular physiological processes. In cases of insufficient oxygen (hypoxic conditions), pyruvate is transformed to lactate, which releases lesser energy; the process of conversion from glucose to lactate is called anaerobic glycolysis and it results in less ATP generation. Though it results in the production of lesser energy, anaerobic glycolysis offers the advantage of speed, as energy generation is faster. Unlike normally differentiated cells, most cancer cells metabolize glucose to lactate, generating less ATP, even in the presence of adequate oxygen, also known as aerobic glycolysis or "the Warburg effect." In the brain, oxidative phosphorylation is the main mode of energy production in neurons, whereas aerobic glycolysis is predominant in astrocytes.[57] Briefly, the quick energy generation through aerobic glycolysis supports the uptake of synaptically released glutamate by the astrocytes, and the lactate produced undergoes oxidative phosphorylation to fuel the neuronal activities; this process is referred to as the astrocyte–neuron lactate shuttle. However, a strong spatial correlation between Aβ deposition and aerobic glycolysis is found in the AD brain by positron emission computed tomography imaging.[58] With aging, an increase in lactate levels in the brain is reported in AD mice and the elevated expression of lactate-producing enzyme is correlated with poorer memory performance.[59] Indeed, higher levels of lactate in the cerebrospinal fluid from AD patients have been reported,[60] suggesting a possible shift in glucose metabolism during AD development.

PKM alternative splicing

Glucose metabolism is strictly regulated and restricted to some RNAs and their corresponding key proteins maintaining glucose homeostasis.[61] Pyruvate kinase is a rate-limiting enzyme in the process of glucose metabolism and it catalyzes the process of conversion of phosphoenolpyruvate to pyruvate and transfer of the phosphate group to ADP to produce a molecule of ATP.[62] Pyruvate kinase also affects the subsequent steps of pyruvate reduction to lactate or oxidation to acetyl-CoA.[43] Elevated levels of pyruvate kinase and lactate dehydrogenase A are found in the frontal and temporal cortex of the brain in AD patients.[63] PKM is one of the isozymes of the two pyruvate kinase, namely, pyruvate kinase M and pyruvate kinase L-R protein, which is found to be significantly elevated in cerebrospinal fluid from AD patients through quantitative proteomic profiling. *PKM* gene has two alternative splice variants, PKM1 and PKM2. PKM1 is exclusively expressed in neurons and PKM2 in astrocytes.[62] PKM1 promotes the oxidation of pyruvate into acetyl-CoA through pyruvate dehydrogenase and feeds into the Krebs cycle in neurons.[64] Pyruvate dehydrogenase kinase phosphorylates (PDH), resulting in its inactivation in the astrocytes, owing to much higher levels of pyruvate dehydrogenase kinase 4 (>30-fold) as compared to that in neurons.[11] Owing to the diminished PDH activity in astrocytes, PKM2 promotes the conversion of glucose to lactate-by-lactate dehydrogenase.[65] PKM2 is elevated in AD mice and cells treated with Aβ,[16] resulting in a shift of glucose metabolism and consequently is consistent with the observation of higher lactate levels in cerebrospinal fluid from AD patients.[66] However, aerobic glycolysis increases in Aβ-resistant neurons and this shift in cell metabolism processes may promote neuronal survival in response to Aβ exposure.[67] Although aerobic glycolysis exerts a protective effect against Aβ toxicity, the shift in metabolism from oxidative phosphorylation to aerobic glycolysis may be beneficial only in the short term for providing ATP to the brain and maintaining cell viability, whereas, in the long term, consequences probably weaken the brain function and

result in neurodegenerative diseases. This may be attributed to the following: (1) aerobic glycolysis is a quick but inefficient means of energy production and insufficient energy leads to impairments in ATP-requiring processes, including Aβ clearance, synaptic dysfunction, and neuronal survival;[68,69] (2) Besides providing energy, glucose is also a source of neurotransmitters, such as acetylcholine, glutamate, gamma-aminobutyric acid, glycine, and lipids, and feeds in through glycolysis and tricarboxylic acid cycle. The brain metabolizes glucose through the pentose phosphate pathway (also known as the hexose monophosphate shunt), providing ribose for the synthesis of DNA, RNA, and nicotinamide adenine dinucleotide phosphate for removing excessive reactive oxygen species;[2] (3) An increase in lactate levels may result in a decrease in the level of the antioxidant glutathione, aggravating oxidative stress, which may be involved in the development of AD, owing to the increased Aβ deposition, tau hyperphosphorylation, and consequent loss of synapses and neurons.[70,71] Therefore, the precise regulation of alternative splicing of the *PKM* gene is important.

The *PKM* gene consists of 12 exons and two variants, PKM1 and PKM2, are generated by alternative splicing of mutually exclusive exons 9 and 10, respectively [Figure 3]. PKM1 specifically contains exon 9 and PKM2 includes exon 10 encoding 22 different amino acids out of a total of 531 residues.[72] hnRNP A1, polypyrimidine tract binding protein 1 (PTBP1), also referred to as the polypyrimidine tract binding protein, heterogeneous nuclear ribonucleoprotein A2, SRSF3, RNA binding motif protein 4 (RBM4), and insulin-like growth factor 2 mRNA binding protein 3, are the six splicing factors that are known to regulate the *PKM* splicing process.[73,74] The binding of hnRNPA1, hnRNPA2, and PTBP1 to exonic splicing silencer cis-action elements in exon 9 promotes exon skipping, while SRSF3 binding to exon 10 promotes exon inclusion, resulting in PKM2 generation. Insulin-like growth factor 2 mRNA binding protein 3 also contributes to the generation of the PKM2 isoform, however, the underlying mechanism remains unknown.[74] RBM4 binding to exon 8 suppresses the interaction of PTBP1 with exon 9, thereby, favoring PKM1 formation. Thus, the expression of different splicing factors may be crucial for regulating the splicing of the *PKM* gene in AD.

Regulation of advanced glycation end products in AD

The consequence of dysregulation of glucose metabolism is hyperglycemia, further leading to protein glycation. Glycation is a non-enzymatic reaction between sugar and protein or lipid resulting in the formation of AGEs.[75] Several metabolic diseases, including diabetes, cause the accumulation of AGEs in tissues.[76] AGEs are implicated in the AD pathology, owing to their deposition in astrocytes and co-localization with Aβ plaques and tau protein neurofibrillary tangles.[77,78] Some studies show that AGEs can modify tau and Aβ levels, leading to their glycation, thereby, enhancing the formation of Aβ aggregates and tau helical filaments.[79,80] The downstream signaling components of AGEs can regulate APP processing and tau phosphorylation in primary cortical neurons, thereby, providing a mechanistic link between AGEs and AD pathology.[75]

AGEs can induce toxic effects through their interactions with receptors (AGEs receptor and AGEs connexin, scavenger receptor on the surface of macrophages) involved in the pathogenesis of AD. RAGE is the main receptor of AGEs *in vivo* and belongs to the immunoglobulin superfamily; it is an intracellular signaling transduction factor and inflammatory factor. The interaction of AGEs with cell surface RAGE can activate downstream signaling transduction pathways, resulting in inflammation, oxidative stress, autophagy, and apoptosis.[81,82] Increased RAGE is also found in the AD brain and most likely participates in the transport of Aβ peptides, finally leading to mitochondrial damage and neuronal dysfunction.[83] Furthermore, glycated Aβ is a more suitable ligand of RAGE than unmodified Aβ, thereby, exacerbating the neuronal toxicity.[84] However, further understanding of the isoforms of RAGE in AD etiology and progression is necessary for its development as a therapeutic target.[85]

Alternative splicing of RAGE

The human *RAGE* gene is located at the major histocompatibility complex, class III region of chromosome 6. It consists of 11 exons, 10 introns, and a 3′-untranslated regions region. Full-length RAGE contains 404 amino acids and its molecular weight is 42,803 Da. Human RAGE protein has an extracellular domain, a transmembrane domain, and a cytoplasmic tail, wherein the extracellular domain includes two Ig-like C-type regions and an Ig-like V-type region; the latter contains at least one ligand-binding region.[86] However, the *RAGE* gene can alternatively be spliced into different isoforms and individually or cooperatively perform multiple functions in the brain.[87]

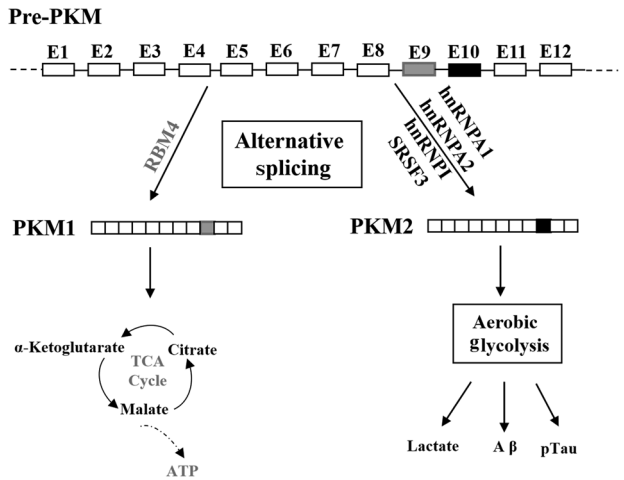

Figure 3: Schematic representation of the human *PKM* gene and its alternative splicing products. *PKM* gene is alternatively spliced into PKM1, containing exon 9 and lacking exon 10. It is modulated by RBM4, thereby contributing to ATP production in the tricarboxylic acid cycle (TCA) cycle of neurons. Likewise, hnRNP A1, hnRNP A2, hnRNP 1, and SRSF3 are engaged in the production of PKM2 from alternative splicing of PKM gene, which includes exon 10 but lacks exon 9. PKM2 leads to conversion of aerobic oxidation to aerobic glycolysis, which leads to higher lactate levels and has a strong spatial correlation with Aβ deposition as well as tau hyperphosphorylation. ATP: Adenosine 5′-triphosphate; hnRNP A1: Heterogeneous nuclear ribonucleoprotein A1; hnRNP A2: Heterogeneous nuclear ribonucleoprotein A2; INSR-A: Insulin receptor A; PKM: Pyruvate kinase M; PKM1: Pyruvate kinase M1; PKM2: Pyruvate kinase M2; RBM4: RNA binding motif protein 4; SRSF1: Serine/arginine-rich Splicing Factor 1; SRSF3: Serine/arginine-rich Splicing Factor 3.

Approximately 20 RAGE isoforms are reported in mice[88] and humans,[86] and three major isoforms among them are highly expressed in the human brain[87] and human vascular endothelial cells,[89] namely the full-length RAGE receptor (flRAGE), endogenous secretory RAGE (esRAGE), and N-truncated RAGE (NtRAGE). esRAGE contains a part of intron 9 and subsequently a stop codon, thus lacking exon 10 [Figure 4]. NtRAGE has a retained intron 1, also introducing a stop codon, thereby, resulting in the loss of exons 1 and 2, which encode the V-type immunoglobulin domain. The V-type immunoglobulin domain endows the receptor with the property to bind to the corresponding RAGE ligand.[89] esRAGE is a well-characterized isoform of the *RAGE* gene, that is present in human brain astrocytes and peripheral blood mononuclear cells.[90] esRAGE level is approximately four times that of flRAGE or NtRAGE in the hippocampus of the human brain.[87] flRAGE is mainly anchored to the cell membrane and participates in signaling pathways and material transport, especially in mediating the neurotoxic effects of Aβ and the pathophysiological processes related to AGEs. esRAGE, containing a ligand recognition region but no transmembrane region, is localized to plasma rather than being anchored on the cell membrane and acts as a decoy receptor resulting in decreased binding of AGEs or Aβ to flRAGE.[85] esRAGE can attenuate the cytotoxicity induced by the interactions of flRAGE with its ligands, indicating its neuroprotective properties.[91] esRAGE expression is low in the hippocampus and inferior parietal lobule of the AD brain and is closely associated with the development of AD.[34,92]

The esRAGE isoform is generated by the alternative splicing of RAGE, which is regulated by splicing factors [Figure 4]. Studies show that the splicing factor, heterogeneous nuclear ribonucleoprotein H2, promotes the generation of esRAGE by binding to the G-rich cis-element of exon 9B in the *RAGE* gene.[93] Other splicing factors, such as hnRNP A1 and transformer 2 beta homolog (Tra2b-1), are also involved in the formation of esRAGE. hnRNP A1 can increase the mRNA level ratio of flRAGE/esRAGE, while Tra2b-1 exerts an opposite effect. Glucose deprivation increases flRAGE expression by upregulation of hnRNP A1 and downregulation of Tra2b-1. A decrease in esRAGE level, increases the interaction of flRAGE with its ligands, such as Aβ in the brain, resulting in increased neurotoxicity,[19] which provides further evidence for AD caused by dysfunction in glucose metabolism. However, the regulatory mechanism underlying alternative splicing of RAGE into NtRAGE remains unknown and further studies are required to elucidate the effects of NtRAGE, lacking the V-type immunoglobulin domain.

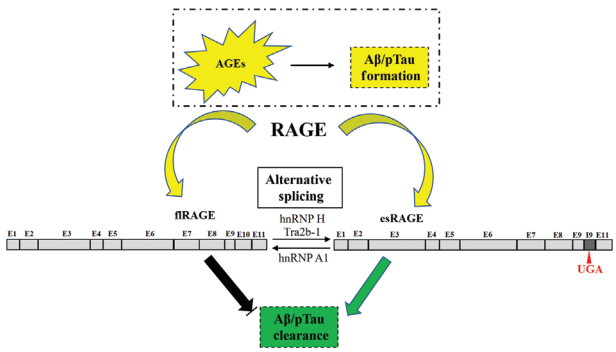

Figure 4: Regulation and effects of RAGE isoforms in AD pathology. The AGEs enhance the formation of Aβ aggregation and tau helical filaments. RAGE gene can be alternatively spliced into several variants, including flRAGE and esRAGE. Compared to the flRAGE, the esRAGE contains a part of intron 9 which introduces a termination codon ZUGA. flRAGE mediates the neurotoxic effects of Aβ and the AD pathophysiological processes related to AGEs, while esRAGE functions as a decoy receptor decreasing AGEs, exerting neuroprotective role by Aβ clearance and inhibition of tau phosphorylation. The splicing factors, hnRNP H and Tra2b-1 promote the generation of esRAGE and hnRNP A1, which can further increase the expression of flR. Aβ: Amyloid β; AD: Alzheimer's disease; AGEs: Advanced glycation end-products; esRAGE: Endogenous secretory RAGE; flRAGE: Full-length RAGE receptor; hnRNP A1: Heterogeneous nuclear ribonucleoprotein A1; hnRNP H: Heterogeneous nuclear ribonucleoprotein H; RAGE: Receptor for advanced glycation endproducts; Tra2b-1: Transformer2b-1.

Splicing factors regulate glucose metabolism in AD

In general, *cis*-acting elements and *trans*-acting factors are crucial in regulating the pre-mRNA alternative splicing processes. *Cis*-acting elements can be found in both exons exonic splicing enhance/silencer and introns intronic splicing enhancer/silencer, and these can function as activators (splicing enhancers) or inhibitors (splicing silencers) of the alternative exons by binding of *trans*-acting factors.[97] *Trans*-acting splicing factors comprise two major classes, namely serine-arginine (SR) proteins and hnRNPs.[98] SR proteins usually promote exon recognition, thereby stabilizing the spliceosomal components at exon-intron boundaries or antagonizing splicing repressors by binding to the splicing enhancers. hnRNPs prevent the association of SR proteins or spliceosomes to alternative exons resulting in exon skipping owing to the binding of splicing silencers.

The expression of splicing factors in neurodegenerative diseases has been studied by genomic sequencing and abnormal changes in the expressions of SR and hnRNP splicing factors, including serine and arginine rich splicing factor 2 and hnRNP K, have been reported in the brain of AD patients.[99] The loss of hnRNP-A/B in AD mice disrupts the splicing process in the cerebral cortex and aggravates their cognitive impairment.[46] These results suggest that alterations in the levels of splicing factors are crucial for maintaining the balance between genes involved in alternative splicing and play important roles in the occurrence of AD. Therefore, if the expression of splicing factors can be regulated, it can, in theory, regulate the alternative splicing processes of its downstream genes, thereby, improving, and aiding treatment of AD.

Moreover, alternative splicing of a single gene is regulated by several splicing factors and one splicing factor can be involved in the regulation of alternative splicing of several AD-related genes. For example, hnRNP A1

can regulate the alternative splicing processes for *INSR*,[43] *PKM*,[100] *RAGE*,[19] *APP*,[47] and *microtubule associated protein tau* genes,[101] having reduced expressions in the AD brain, as reported previously. Therefore, the discovery and functional characterization of specific splicing factors in the brain will lead to novel designs for AD treatment. As accumulating evidence supports the notion that modulation of RNA binding proteins (RBPs) or splicing factors determines the cell fate,[102] Sharp and Jangi[103] has proposed the concept of master splicing regulators, which are expressed in a tissue-specific manner and required for the proper differentiation, thereby, playing critical roles in the cell state transitions. Thus, the identification of master splicing regulators and their underlying mechanisms will benefit AD treatment designs.

Conclusion and perspectives

The risk of AD is higher in the aging population, as well as for individuals with diabetes, indicating that dysregulation in glucose metabolism may be a crucial causal factor for AD occurrence. In this review, we emphasize the regulation of glucose metabolism in AD by alternative splicing processes of genes and discuss the different and even opposite functions of the gene-spliced variants [Figure 5]. The insulin signaling pathway is important in promoting glucose uptake modulated by INSR and IDE. The two isoforms, INSR-A and INSR-B, show differential affinities for insulin and insulin-like growth factors. The *IDE* gene is alternatively spliced into four major variants, 15a-IDE, 15b-IDE, IDE-Met[1], and IDE-Met[42], exhibiting different insulin-degrading efficiencies. Alteration in ratios of INSR and IDE variants may impede the insulin signaling pathway, which is a risk factor for AD development. PKM has two splice variants, PKM1 and PKM2, that influence the process of glycolysis. PKM2 is elevated in the AD brain and leads to a shift in glucose metabolism towards lesser energy production and higher lactate levels. Dysregulation in glucose homeostasis results in the production of AGEs, which induce toxic effects through their interactions with the RAGE, involved in the pathogenesis of AD. The *RAGE* gene also undergoes alternative splicing, resulting in the generation of several variants, including two featured variants, flRAGE, and esRAGE. flRAGE expression is elevated in the AD brain and mediates neurotoxic effects of Aβ, mitochondrial damage, and neuronal dysfunction. While esRAGE, downregulated in the AD brain, functions as a decoy receptor, decreasing AGEs and Aβ binding to flRAGE. In the regulation of alternative splicing processes of these genes, splicing factors play a pivotal role, including the two major classes, namely, the SR proteins and hnRNPs.

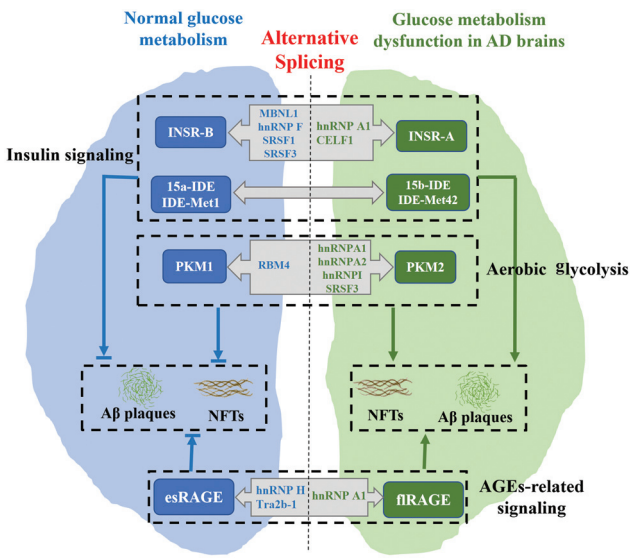

Figure 5: Genes regulating glucose metabolism by alternative splicing are involved in the pathogenesis of AD. INSR, IDE, PKM, and RAGE are related to glucose uptake, glycolysis, oxidative phosphorylation in Krebs Cycle, and AGEs production. Due to the appearance of abnormal isoforms, energy metabolism disorders occur, leading to ATP-dependent Aβ clearance, microtubule formation, and other aberrant processes, subsequently leading to the occurrence of AD. Aβ: Amyloid β; AD: Alzheimer's disease; AGEs: Advanced glycation end-products; ATP: Adenosine 5′-triphosphate; esRAGE: Endogenous secretory RAGE; flRAGE: Full-length RAGE receptor; hnRNP: Heterogeneous nuclear ribonucleoprotein; IDE: Insulin-degrading enzyme; INSR: Insulin receptor; MBNL1: Muscleblind like splicing regulator 1; NFTs: Neurofibrillary tangles; PKM: Pyruvate kinase M; PKM1: Pyruvate kinase M1; PKM2: Pyruvate kinase M2; RAGE: Receptor for advanced glycation endproducts; SRSF: Serine/arginine-rich splicing factor.

Currently, based on the regulation of abnormal alternative splicing processes mediated by *cis*-acting elements and *trans*-acting factors, several therapeutic approaches have been developed for diseases. Antisense short oligonucleotides (ASOs) are uniformly modified oligonucleotides, having a very high affinity towards single-stranded RNAs, thereby targeting specific regulatory regions and having potential utility against the *cis*-acting elements. For example, a specific ASOs, Spinraza was designed to target the cis-RNA silencer flanking the 5′ splice site of the survival of motor neuron 2 gene to promote exon 7 inclusion, thereby producing more functional SMN isoforms. This is a promising therapeutic strategy to treat spinal muscular atrophy.[104] A potent ASOs targeting an enhancer in exon 10 to switch the splicing of *PKM*, thereby suppressing PKM2 expression is being used to treat cancer.[105] To target the splicing factors, small molecules have been developed, including MBNL1 inhibitor[106] and splicing factor 3b subunit 2 inhibitor.[107] Recently, a new protein-degradation technique has been reported, namely, proteolysis-targeting chimeric molecules (PROTACs), which comprises E3 ligase recruiting elements, ligands of target proteins, and linkers. PROTACs can degrade target proteins by hijacking the ubiquitin proteasomal degradation system.[108] RNA-PROTACs are introduced to selectively target RBPs, including splicing factors, whereupon the ligands of target proteins use the small RNA mimics to dock into the RNA-binding site of RBPs. Moreover, the splicing factor, RNA binding fox-1 homolog 1, is efficiently degraded by RNA-PROTAC in cancer cells.[108] Given the role of splicing factors in AD, methods that inhibit or degrade splicing factors are promising avenues for research. The future research focus should target alternative splicing processes for the specific regulation of AD-related genes. Further, the discovery of the master splicing regulators and their underlying mechanisms of action will benefit AD treatment design.[42]

Funding

The work was granted by the joint foundation of Luzhou Government and Southwest Medical University (No. 2018LZXNYD-ZK05), and the Talent Development Project of the Affiliated Hospital of Southwest Medical University.

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How to cite this article: Deng S, Yi P, Xu M, Yi Q, Feng J. Dysfunctional gene splicing in glucose metabolism may contribute to Alzheimer's disease. Chin Med J 2023;136:666–675. doi: 10.1097/CM9.0000000000002214