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The Role of the MAPK Signaling Pathway in LTP Reduction Associated with Alzheimer's Disease

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ABSTRACT

The MAPK signaling pathway plays a key role in contributing to a major symptom of Alzheimer's Disease: memory loss. Memory power can be measured by LTP (long-term potentiation) levels, as LTP is the process that strengthens synapses, allowing them to store memories upon repeated stimulation. The primary mechanism behind the MAPK signaling pathway is a large phosphorylation cascade between several proteins, including Ras, Raf, MEK, ERK, NF-kB, and other cytoplasmic and nuclear proteins. Under normal circumstances, the MAPK pathway results in an increase in LTP by boosting glutamate receptor concentration on a postsynaptic neuron. However, the overstimulation of this pathway results in excitotoxicity, causing cytoskeletal collapse and neuronal death, a phenomenon that commonly occurs due to Alzheimer's Disease. Treatments for Alzheimer's Disease can target stages of the MAPK signal transduction pathway or change the expression of genes that cause excitotoxicity. Understanding the MAPK signaling pathway is crucial for the development of memory improving Alzheimer's treatments, remedies that would greatly better the lives of Alzheimer's patients.

Introduction

Alzheimer's Disease is a neurodegenerative disease that results in drastic physiological changes throughout the brain, such as a significant loss of brain mass and the accumulation of abnormal proteins. Symptoms include memory problems, personality changes, and difficulty performing complex tasks, and as time progresses, the brain deteriorates further, causing the symptoms of Alzheimer's to worsen. One hallmark of Alzheimer's Disease is the reduction of long-term potentiation (LTP). LTP is the process of strengthening synapses, critical for the formation of long-term memories. During LTP, the number of glutamate receptors on the postsynaptic neuron of a synapse increases because of a signal transduction pathway (Society For Neuroscience, 2012).

Glutamate is the most common neurotransmitter in the nervous system, and the two classes of glutamate receptors, NMDA and AMPA, are ion channels that stimulate changes in the postsynaptic neuron upon binding to glutamate. Specifically, the NMDA receptor allows calcium ions to flow into the postsynaptic neuron when bound to glutamate, and the AMPA receptor allows sodium ions to flow into the postsynaptic neuron when bound to glutamate. These ions act as second messengers, activating kinases that increase the synapse's efficiency of carrying electrical impulses. In addition, the ions also activate cAMP molecules, which amplify the signal and activate enzymes that increase the number of glutamate receptors on the postsynaptic neuron. The extended release of cAMP molecules activates the cAMP-response element binding protein (CREB). Activated CREB can switch on nuclear genes that code for neurotrophins, which are proteins that promote synaptic growth and intensify synaptic responses to neurotransmitters (Society For Neuroscience, 2012).

The MAPK signal transduction pathway plays a crucial role in the development of Alzheimer's, since its overstimulation hinders LTP and results in memory problems witnessed by most Alzheimer's patients. This pathway is characterized by mitogen-activated protein kinases (MAPKs) that facilitate a phosphorylation cascade, eventually altering gene expression and degrading LTP in Alzheimer's victims. Mitogens are extracellular signals that stimulate

growth and cell division (Hazra et al., 2017). In humans, growth factors are a common example of mitogens. As suggested by its name, the MAPK pathway is activated by a mitogen ligand, which binds to an epidermal growth factor receptor (EGFR) on a cell membrane (Guo et al., 2020).

The Role of the Ras Protein in the MAPK Pathway

Ras is a G protein that transmits signals as part of the MAPK pathway. When bound to GTP (guanosine triphosphate), Ras is activated, but when bound to GDP, Ras is deactivated. In the MAPK pathway, the replacement of GDP with GTP in Ras is facilitated by an assortment of proteins, resulting in the activation of the Ras protein (Guo et al., 2020).

EGFR is one of the proteins that stimulates Ras activation. EGFR is a receptor tyrosine kinase (RTK), a special class of receptors with intracellular tails consisting of tyrosine subunits. When a mitogen activates EGFR by binding to it, EGFR undergoes dimerization, in which two activated EGFR subunits combine to form an activated RTK complex. Within a EGFR dimer, each kinase monomer phosphorylates the tyrosines on the other monomer, in a process known as transphosphorylation. Then, the SH2 domain of GRB2 (growth factor receptor-binding protein 2) can bind to the phosphate group on a tyrosine subunit. GRB2 is an adaptor protein, meaning that it acts as a scaffold to position other proteins into their ideal binding orientation (Guo et al., 2020). Attached to the phosphate group of the tyrosine subunit, GRB2 can now bind to the proline sequences at the C-terminus of the SOS (son of sevenless) protein. SOS is a guanine exchange factor (GEF) that replaces the GDP bound to Ras with a GTP molecule (Chatterjee & Ghosh, 2023). To catalyze this reaction, Ras binds to the Cdc25 domain of SOS, which contains its active site. However, for SOS to function, Ras+GTP must attach to the allosteric site of SOS, causing SOS to adhere to the cell membrane. As a result, a positive feedback loop occurs: the product of the reaction catalyzed by SOS acts as an allosteric activator, stimulating SOS to produce more and more Ras+GTP (Bandaru et al., 2018).

The Role of the Raf and MEK Proteins in the MAPK Pathway

Raf is a serine/threonine kinase, meaning that it phosphorylates the hydroxyl groups of serines and threonines on other proteins. Located at its N-terminus, the CR1 region of Raf contains the binding site of Ras+GTP. Ras+GTP binds to this CR1 region as well as to Raf's CRl region, which is composed primarily of cysteine residues (Guo et al., 2020). Then, Ras+GTP phosphorylates numerous tyrosine, serine, and threonine R-groups in the Raf protein (Stokoe & McCormick, 1997). Upon activation by Ras+GTP, Raf can sustain signal transduction using its CR3 region, which is located at the C-terminus of Raf and contains its catalytic domains (Guo et al., 2020).

MEK is a kinase that is localized in the cytoplasm. It contains a nuclear export sequence of leucine residues that prevents it from getting sequestered in the nucleus (Eblen, 2018). The CR3 region of Raf activates MEK by phosphorylating the serine R-group of MEK's VIII subregion (Guo et al., 2020).

The Role of the ERK Protein in the MAPK Pathway

ERK is a serine/threonine kinase, like Raf, and is attached to the cytoplasm by MPK-3 (MAP kinase phosphatase 3). IQGAP1 (IQ Motif Containing GTPase Activating Protein 1) also anchors ERK to the cytoplasm by attaching it to the actin cytoskeleton of the cell (Eblen, 2018). ERK is phosphorylated by MEK on the tyrosine and threonine residues of its N-terminal region (Muta et al., 2019). Once activated, ERK phosphorylates several other cytoplasmic proteins, such as MAP1, MAP2, and MAP4, which are cytoskeletal proteins that redistribute the microtubules of a cell (Guo et al., 2020).

After undergoing dimerization, phosphorylated ERK can also enter the nucleus, with the help of the importin7 protein (Chatterjee & Ghosh, 2023). Importins are proteins that transport cytoplasmic proteins through the nuclear membrane. In this scenario, importin7 recognizes a NLS (nuclear localization signal) sequence on phosphorylated

ERK and forms a ternary complex along with ERK and other importins, allowing ERK to travel into the nucleus (Okada et al., 2008). Once inside the nucleus, ERK is anchored to the nucleus by DUSP5 phosphatase. In addition, leptomycin B inhibits the Crm1 nuclear export protein, hindering ERK from exiting the nucleus (Eblen, 2018). Since phosphorylated ERK is trapped inside the nucleus by these proteins, ERK can phosphorylate nuclear substrates, such as transcription factors, which bind to the DRS (D-site recruitment site) and FRS (F-site recruitment site) regions of ERK (Paul et al., 2020). ERK can also directly act as a transcription factor in the nucleus, altering the expression of various nuclear genes (Eblen, 2018).

When inside the nucleus, ERK increases the expression of IEGs (intermediate early genes), which code for transcription factors that progress the cell cycle. For example, ERK increases the transcription of the *cyclin D1* gene, which codes for the cyclin protein, a crucial "go" signal for the cell cycle. ERK also phosphorylates ETS (E26 transformation-specific) proteins such as ELK-1, which binds to the SRF (serum response factor) protein to increase the transcription of the *c-Fos* gene. The products of the *c-Fos* gene can then increase the expression of other genes that enable cells to pass through the G1 phase of the cell cycle. ERK also decreases the expression of genes that slow the cell cycle, such as *tob1*. The Tob1 protein, produced by the *tob1* gene, is a co-repressor of the *cyclin D1* gene, so decreasing the expression of Tob1 would allow more cyclin to be produced, promoting cell proliferation. Furthermore, ERK phosphorylates the FOXO3a transcription factor, facilitating the binding of FOXO3a to E3 ubiquitin ligase Mdm2, an enzyme that promotes protein degradation. Under normal conditions, FOXO3a represses the *cyclin D* gene, activates p27Kip1, a cyclin-dependent kinase (CDK) inhibitor, and increases the expression of *bim* and *fasL*, genes that stimulate apoptosis. By causing FOXO3a to be degraded, ERK allows cyclin-CDK complexes to accumulate, enabling cells to pass through the G1, S, and G2 phases of the cell cycle (Eblen, 2018).

Increased Expression of NF-kB Stimulated by ERK

ERK also increases the expression of the NF-kB (nuclear factor-kappa B) transcription factor. There are two ways that ERK accomplishes this. ERK can directly phosphorylate p65 homodimers, which activate NF-kB producing genes (Chen & Lin, 2001). The other way that ERK activates these genes is by stimulating the phosphorylation of IkB kinase alpha (Sun et al., 2022).

IkB alpha is a protein that binds to p65/p50 heterodimers, which hides the PEST domain of IkB alpha. Since the PEST domain of IkB alpha must be recognized to initiate its degradation, the binding of IkB alpha to p65/p50 heterodimers prevents its degradation and allows IkB alpha to accumulate. However, when phosphorylated by IkB kinase alpha, IkB alpha releases its p65/p50 heterodimers, allowing the ubiquitin protein to mark IkB alpha for degradation (Mathes et al., 2008). Simultaneously, the released p65/p50 heterodimers enter the nucleus and activate NF-kB producing genes. This entire process is kickstarted by ERK's stimulation of IkB kinase alpha phosphorylation, allowing IkB kinase alpha to phosphorylate IkB alpha and resulting in greater NF-kB expression (Sun et al., 2022).

The Role of NF-kB in LTP Reduction

NF-kB reduces LTP, contributing to memory loss in Alzheimer's Disease, by increasing the expression of various proteins (Laboratories, 2021). For instance, NF-kB increases transcription of the mGluR5 (metabotropic glutamate receptor 5) gene, which codes for the metabotropic glutamate receptor protein (*GRM5 Glutamate Metabotropic Receptor 5 [Homo Sapiens (Human)] - Gene - NCBI*, n.d.). The overexpression of this gene can lead to excitotoxicity, the overstimulation of neurons, which can be very damaging. It can lead to the collapse of neurons due to factors such as microtubule destabilization, and this widespread neuronal death results in fewer occurrences of LTP (Sun et al., 2022). NF-kB also increases the transcription of the TNF (tumor necrosis factor) gene, which codes for the tumor necrosis factor protein (Laboratories, 2021). TNF decreases LTP in a similar manner to that of the metabotropic

glutamate receptor, but the exact mechanism is still unknown (Ren et al., 2021). Like ERK, TNF also phosphorylates IkB kinase alpha, thereby increasing the expression of NF-kB and resulting in a positive feedback loop (Sun et al., 2022).

Conclusion

The reduction of LTP in Alzheimer's patients can cause their lives to become very challenging and can have life-changing effects, such as the loss of recognition of family members (Society For Neuroscience, 2012). For this reason, it is very important to find treatments that deal with memory loss in Alzheimer's patients. One existing treatment that has already been approved is the Memantine drug. Memantine inhibits NMDA receptors, reducing excitotoxicity by lowering the incidence of glutamate binding to neuronal receptors (Sun et al., 2022).

A few new ideas for Alzheimer's Disease treatments can be explored, primarily involving the culturing of altered stem cells. In these stem cells, scientists could insert methyl groups and DNA methyltransferases to the major histocompatibility complex class III region of chromosome 6, which contains the TNF gene. As a result, the TNF gene would become methylated, reducing transcription of the tumor necrosis factor and minimizing the LTP reduction in Alzheimer's Disease. Stem cells with a methylated TNF gene can then be inserted into the brain, resulting in an overall lower expression of the tumor necrosis factor, lowering excitotoxicity, and allowing LTP to remain at a more stable level. The same process can be done with the NF-kB gene or the mGluR5 gene to further stabilize LTP levels.

Scientists can also insert a repressor of the TNF gene into stem cells, preventing RNA polymerase from binding to the TNF gene to initiate transcription. These stem cells can be inserted into the brain, increasing LTP in Alzheimer's patients by lowering the expression of tumor necrosis factor. The same process can be done with the NF-kB gene or the mGluR5 gene to increase the inhibitory effects.

In addition, scientists could sequence the TNF gene and find its complementary mRNA sequence. In this way, they can synthesize miRNA (microRNA) molecules that are complementary to the mRNA sequence of the TNF gene. Then, using these complementary miRNA molecules, scientists can synthetically produce miRNA-protein complexes and add them into stem cells. After these stem cells are inserted into the brain, the miRNA-protein complexes can block the translation of TNF mRNAs or even degrade TNF mRNAs, hindering tumor necrosis factor production and stabilizing LTP levels. The same process can be done with the NF-kB gene or the mGluR5 gene, fine-tuning LTP levels based on the severity of a patient's Alzheimer's symptoms.

Alzheimer's Disease is a very life-changing disorder that drastically impacts the mental state of patients. Fortunately, research targeting the MAPK signaling pathway has shown some promise for the development of memory improving Alzheimer's Disease treatments. There are numerous proteins involved in the pathway of memory deterioration in Alzheimer's Disease, including EGFR, Ras, Raf, ERK, NF-kB, and TNF, so regulation of the signal transduction pathway can occur at several possible stages, potentially allowing a wide variety of treatments to be developed. Moreover, changes in gene expression can be employed to impact neuronal sensitivity and increase LTP levels in Alzheimer's patients. In the future, clinical trials can be conducted on these treatments, enabling researchers to uncover the most effective option and providing Alzheimer's patients with a more positive prognosis.

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