A focus on CXCR4 in Alzheimer’s disease

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Abstract:
Alzheimer’s disease (AD) is one of the most common and devastating aging-related neurodegenerative diseases. Besides the well-known role of chemokines and their receptors in the immune system, they are widely expressed in the nervous system, where they play roles in the regulation of cell migration and neurotransmission. The chemokine CXC motif receptor 4 (CXCR4) is evolutionarily highly conserved seven-transmembrane G-protein-coupled receptors (GPCRs). It has been demonstrated that CXCL12/CXCR4 signaling pathway involved in the pathologic process of AD. In this review, we demonstrated the GPCR family proteins and summarized the relationship between CXCR4 and GPCR, CXCR4 and AD. The review aimed to provide the novel insight of CXCR4 into the early prevention of mild cognitive impairment and in the diagnosis and treatment of AD.

Keywords:
Alzheimer’s disease, CXCL12, CXC motif receptor 4, G-protein-coupled receptor

Introduction

G-protein-coupled receptors (GPCRs) are integral membrane proteins that convert extracellular signals into intracellular responses including responses to hormones and neurotransmitters. GPCRs, also called seven transmembrane (TM)-spanning receptors, represent the largest family of cell surface receptors and are the targets of intense drug discovery efforts. While a number of available drugs on targeting GPCR signaling pathways, overall <20% of GPCRs are targeted. However, despite structural similarities, GPCRs have unique combinations of signal-transduction activities involving G-protein-dependent signaling pathways, as well as G-protein-independent signaling pathways and complicated regulatory processes. Therefore, the development of new therapeutic targets on GPCRs could be a promising method to maintain the effect and control the side effects of inhibitors based on biased ligands or allosteric modulators.

The chemokine CXC motif receptor 4 (CXCR4) is an evolutionarily highly conserved GPCR family member. CXCR4 belongs to seven-TM GPCRs. CXCL12/CXCR4 pathway has been known to be involved and regulated inflammatory response. Interestingly, ADs pathological changes also include a variety of inflammatory mechanisms. However, whether and how CXCL12/CXCR4 molecular transduction plays a role in AD, especially inflammatory process is to be investigated.

The main purpose of our review is to provide an overview of the involvement of CXCR4 in AD.
CXCR4 in AD. We summarized the signaling pathways and the current findings on the regulatory roles of CXCR4 in AD. It is expected that CXCR4 may serve as a novel target for treatment of AD.

**G-protein-coupled receptor family**

GPCR is one of the largest family of cell surface receptors. A GPCR is basically composed of three parts: the extracellular region, the TM region, and the intracellular region. The extracellular region contains N-terminus and three extracellular loops (ECL1–ECL3); the TM region contains seven TMα-helices (TM1–TM7); the intracellular region contains three intracellular loops (ICL1–ICL3); and an intracellular amphipathic short α-helix (H8) lying perpendicular to the membrane plane and the C terminus.[9,10] Although their molecules and functions are diverse, they consist of seven TM domains linked by intracellular and ECL.[11] Ligand was recognized and bind to extracellular domains, then it induces the conformation change of the receptor coupling to G proteins with intracellular domains.[12] This, in turn, leads to coupling and signaling activation of one or more G-proteins inside the cell.

GPCR is the largest and most diverse protein families in the mammalian cells. The G-proteins consist of three subunits: α, β, and γ. It has been demonstrated that five identified genes encode the β subunit, 12 encode the γ subunit, and 17 encode the α subunit.[13] Activation of G proteins dissociates the Gα subunit from the Gβγ subunits of GPCRs. The Gβγ subunits activate the downstream effectors such as enzymes and ion channels.[14] Moreover, the Gα subunits have a key role in determining the receptor coupling specificity and influencing the efficiency of ion channel modulated by Gβγ subunits.[15] On the basis of their G protein-coupling preference, it can be broadly classified into four subfamilies: Gs, Gq/11, Gi, and G12/13.[16]

All chemokines exert their biological effects through the activation of an extended family of seven TM GPCRs. Approximately 19 chemokine receptors (CKR) have been characterized to date, including six CXC receptors (CXCR1–6), ten CC receptors (from CCR1-10), one lymphotactin receptor (XCR1), and one fractalkine receptor (CX3C3CR1). CKRs are notoriously promiscuous. Chemokines are small proteins consisting of about 100 amino acids. More than 50 different chemokines have been identified in higher vertebrates.[17] Chemokines have been classified into four families: C, CC, CXC, and CX3C according to their conserved N-terminal cysteine residues.[18] These residues can be adjacent (CC) or separated by amino acids (CXC and CX3C). Most chemokines are members of the CC (CC motif and β-chemokine) and CXC (α-chemokine) subfamilies. CC subfamily chemokines contain two contiguous cysteines near the amino terminus of the molecule, whereas a single amino acid separates the two cysteines in members of the CXC subfamily. Chemokines in the CX3C (δ-chemokine) subfamily have three amino acids between the two cysteines. The fourth subfamily comprises chemokines with a single cysteine designated the C (γ-chemokine) subfamily. Each subfamily of chemokines acts on a group of related GPCRs.[19] It has been observed in *in vitro* that a single chemokine can activate more than one receptor; conversely, a single cloned receptor can frequently be activated by more than one chemokine although it is probable that their selectivity is actually higher *in vivo*.[19] There are, however, instances when a CR is activated by a single chemokine, *i.e.*, the CXCR4 receptor has only one known ligand, stromal-derived factor-1 alpha (CXCL12).

**CXC motif receptor 4 and G-protein-coupled receptor**

Besides the well-known role of chemokines and their receptors in the immune system, they are widely expressed in the nervous system, where they play roles in the regulation of cell migration and neurotransmission. Meanwhile, chemokine signaling is also important in the regulation of neuroinflammatory responses. Chemokines are small chemoattractant cytokines that are expressed in discrete anatomical locations. Chemokines are responsible for specific recruitment of leukocytes during inflammation.[10] Chemokines act on CR, members of the seven-TM domain GPCR superfamily. Classically, one of the CR ICLs interacts with heterotrimeric, pertussis toxin-sensitive G proteins called Goi, initiating a cascade of signal transduction events in response to ligand binding.[18]

CXCR4, encoded on chromosome 2q21, is an evolutionarily highly conserved GPCR expressed on monocytes, B-cells, and naïve T-cells in the peripheral blood. Human CXCR4 was originally identified as a receptor for CXCL12 by screening CR orphan genes for their ability to induce intracellular Ca²⁺ in response to human CXCL12. Its ligand, CXCL12, is a homeostatic chemokine, which controls hematopoietic cell trafficking, adhesion, immune surveillance, and development. The amino-terminal domain of CXCL12 binds the second ECL of CXCR4 and activates downstream signaling pathways. The ICL3 of CXCR4 is necessary for Goi-dependent signaling, and ICL as well as the C-terminus of CXCR4 are required for chemotaxis.[10,20,21]

CXCL12 binding to CXCR4 triggers multiple signal transduction pathways that are able to regulate intracellular calcium flux, chemotaxis, transcription, and cell survival.[22] CXCL12 binding promotes CXCR4 conformation changing Goi protein dissociation into...
α and βγ subunits. In turn, different subtypes of the α subunit impart different signals: Gαi subunits inhibit cyclic adenosine monophosphate formation through inhibition of adenyl cyclase activity, and αq subunits activate phospholipase C-β, generating diacylglycerol and inositol 1,4,5 trisphosphate, controlling the release of intracellular Ca2+. While inhibiting adenyl cyclase, the Gαi subunits activate the nuclear factor-kappa B, Janus-activated kinase-signal transducers and activators of transcription (STAT), and phosphatidylinositol 3-kinase-AKT pathways as well as mammalian target of rapamycin, and the Jun N-terminal kinase/p38 MAPKs, regulating cell survival, proliferation, and chemotaxis.

CXCR4 is a major type of receptor for CXCL12. CXCL12/CXCR4 chemokine signaling plays a critical role in modulating various nervous system developmental processes and in regulating synaptic plasticity. CXCR4 is widely expressed in the peripheral and central nervous system (CNS) and exerts functions as modulation of neurotransmission, synaptic plasticity, and neuroglial interactions. In a central neuropathic diseases model, CXCL12/CXCR4 were upregulated in neurons, astrocytes, microglia/macrophages, and leukocytes in the lumbar spinal cord. Unlike the α subunits, βγ dimer subunits promote RAS-mediated MAPK signaling, thereby regulating cell proliferation and chemotaxis. Finally, in addition to these classic signaling pathways, CXCR4 triggers Bruton tyrosine kinase (BTK) phosphorylation and downstream MAPK in mantle cell lymphoma and primary acute myeloid leukemia blasts, suggesting a potential interaction of CXCR4 on BTK and a potential for concomitant CXCR4 and BTK inhibition, as the treatment targets.

**CXCR4 and Alzheimer’s disease**

**Alzheimer’s disease and aging**

Since older individuals (those over 65 years of age) will double between 2000 and 2050, the population is aging. Aging is a natural physiological process, a progressive deterioration of the overall homeostatic brain mechanisms, accompanied by cognitive decline. A consequence of normal aging is a greater susceptibility to learning and memory impairments generally attributed to a decrease in neuronal plasticity of the cortex and hippocampus. Cognitive processes mediated by hippocampus and prefrontal cortex are most vulnerable to aging process. Both brain regions suffer cellular and synaptic changes during aging that can be directly related to the decline of cognitive performance. Considering that the life expectancy of the population has increased, the senescence has been the primary risk factor for the development of aging-related diseases such as AD. Cognitive deficits are the most common consequences of aging process and AD. Both aging process and AD are characterized by a progressive deterioration of learning and memory. This strong relationship between aging and AD is important to investigate the pathophysiological mechanism in each event such as the involvement of the neurotrophic factors in these processes.

Many cellular mechanisms in AD including insulin signal pathway, MAPK signaling, extracellular-signal-regulated kinase pathway have been well explored. Nowadays, chemokine/CKR offers a novel navigation in AD managements and mechanisms. It is well known that chemokine/CKR pathway involved the inflammation. Inflammatory phenomenon also occurs in AD. It might not be a surprise that chemokine/CKRs may affect ADs pathological progress. However, the exact molecular mechanisms are unknown and need to be further determined.

**Chemokines and chemokine receptors in Alzheimer’s disease**

AD is one of the most common and devastating aging-related neurodegenerative diseases. The disease poses a great threat to older individuals and their families, becoming a serious social problem with increasing longevity. The clinical manifestation of disease occurs usually after the age of 65. This illness is characterized by massive neuronal loss, cognitive dysfunction, and loss of memory. The incidence and prevalence continuously increase with advancing age.

AD is the most common cause of dementia in the elderly. AD is characterized by pathological findings in the brain: SFs and NFTs. The former are extracellular aggregates composed of amyloid β peptides, while the latter are intracellular aggregates composed of hyperphosphorylated Tau protein. Its pathological changes also include a variety of “inflammatory” phenomenon such as activation of microglia and astrocytes. The pathological significance of inflammatory responses elicited by resident CNS cells has drawn considerable attention in recent years. Chemokines belong to a rapidly expanding family of cytokines, the primary function of which is control of the correct positioning of cells in tissues and recruitment of leukocytes to the site of inflammation. Study of this very important class of inflammatory cytokines may greatly help our understanding of inflammation in the progress of AD, as well as other neurodegenerative diseases. So far, a number of chemokines and CKR have been demonstrated in resident cells of the CNS, and upregulation of some chemokines and receptors is found associated with AD pathological changes. The expressions of chemokines and their receptors in the CNS are significantly different under physiological and pathological conditions.
CXCL12/CXC motif receptor 4 axis signaling as a novel target of Alzheimer’s disease management

Chemokines are small chemoattractant molecules playing a key role in inflammation and immunity.\(^{[36,37]}\) In addition to their role in neuroinflammation, chemokines, such as CXCL12, have been shown to participate in neuronal signaling.\(^{[38,39]}\) Chemokine CXCL12 and its receptor CXCR4 have been previously shown to modulate neuronal firing and neuron/glia communication.\(^{[40]}\) Moreover, it has been previously shown that inflammatory responses in AD correlated with cognitive decline.\(^{[41,42]}\) Decreased mRNA and protein levels of CXCL12, a chemokine involved in neuron-glia communication, may affect memory through altered communication in the CNS. CXCR4, the receptor for CXCL12, is concurrently decreased at the protein level in transgenic mice compared to age-matched nontransgenic controls. Importantly, it is demonstrated that CXCL12 and CXCR4 levels are decreased in AD patients as compared to nondemented controls, supporting a role for this chemokine in cognitive functioning. The decreased levels of CXCL12 might interfere with proper neuronal signaling and may therefore negatively affect memory. The results of young nontransgenic mice treated with a CXCL12 receptor antagonist AMD3100 show deficits in learning and memory.\(^{[43]}\)

In addition, the lack of CXCL12/CXCR4 impaired memory provides further evidence supporting a role of this chemokine and its receptor in learning and memory. In conclusion, a potential new pathway is in part responsible for aspects of cognitive functioning and can thus represent a novel target in disorders affecting learning and memory. Hence, it has been identified a novel pathway mediated through CXCL12 that directly affects learning and memory and may be responsible for the dementia component of AD.

**Conclusion**

The pathologic changes in dendritic spines and synapses play key roles in cognitive dysfunction. Downregulated CXCR4, as a traditional GPCR, inhibits learning and memory. Therefore, the signaling CXCL12/CXCR4 might be involved in the pathological dysfunction of AD. The prevalence of AD is increasing as the population ages, posing serious threats to the health and lives of elderly people and creating significant socioeconomic burdens for families and the society. Effectively preventing and managing AD has become an important aim. However, the exact mechanisms of CXCL12/CXCR4 axis regulating downstream molecular in AD still need to be further determined. Therefore, targeting on the mechanisms of action, regulatory molecules, and signaling pathways of CXCR4 will be helpful for a comprehensive understanding of the pathogenic mechanisms of AD, particularly early stages. In addition, such studies will provide evidence for early clinical interventions for learning, memory, and for the discovery effective AD diagnosis and therapy.

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**Conflicts of interest**

There are no conflicts of interest.

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