Targeting G protein-coupled receptor for pain management

Hongyan Li¹,²,³, Rong Wang², Yinying Lu², Xuehua Xu⁴, Jiaxiang Ni¹

Abstract:

Pain is an unpleasant sensory and emotional experience associated with actual or potential tissue damage or described in terms of such damage. Great progress has been made in understanding the important roles of various G protein-coupled receptors in the regulation of pain transmission. However, many important questions remain uncertain about the precise signal transduction mechanisms. This review focuses opioid receptor and CXC receptor 4 on the effects and mechanisms of pain. Taken together, chemokines and their receptors are potential targets for the development of novel pain management and therapy.

Keywords: Chemokine receptors, chemokines, CXC receptor 4, opioid receptor, pain

Introduction

The heterotrimeric G protein-coupled receptors (GPCRs) are the largest family of cell surface receptors. In spite of their diverse functions, all GPCRs consist of seven transmembrane domains linked by intracellular and extracellular loops.¹ The binding of ligands to their receptors induces the conformation change of the receptor and allows to interact to the specific heterotrimeric G proteins with their intracellular domains.² This, in turn, leads to coupling to and signaling through activation of one or more G proteins inside the cell.

GPCRs can block pain upon targeting opioid, cannabinoid, α2-adrenergic, muscarinic acetylcholine, gamma-aminobutyric acid⁴ (GABA⁴), Group II and III metabotropic glutamate, and somatostatin receptors. Therefore, we focus GPCRs, especially opioid receptor and CXC receptor 4 (CXCR4), on the mechanisms and targets of pain management.

The heterotrimeric GPCRs are the largest, most diverse receptor families in the mammalian cells. The G proteins consist of three subunits: Gα, Gβ, and Gγ. It has been demonstrated that 5 genes encode the β subunits, 12 genes encode the γ subunits, and 17 genes encode the α subunits.³ GPCRs interact with heterotrimeric G proteins composed of α, β and γ subunits that are GDP bound in the resting state. Agonist binding triggers a conformational change in the receptor, which catalyses the dissociation of GDP from the α subunit followed by GTP-binding to Gα and the dissociation of Gα from Gγ subunits. Gβγ subunits activate a diverse array of effectors, such as enzymes and ion channels.⁴ Moreover, Gα subunits have a key role in determining the receptor coupling specificity and influencing the efficiency of ion channel modulated by Gβγ subunits.⁵ Gα subunits can be broadly classified into four major subfamilies: Gαs-, Gαi/o-, Gαq/11-, and Gα12/13-coupled receptors.⁶

GPCRs regulate and are involved in diverse diseases, including cancer, kidney, inflammatory, central nervous system (CNS), and chronic diseases. GPCRs
play an important role in pain transduction, especially cancer pain and chronic pain. They regulate the pathways and mechanisms during pain progress. Almost all GPCR agonists that have an analgesic action are coupled to Gi/o proteins. Therefore, they become a new target in blocking pain. Here, we focus opioid receptor and CXCR4 on the effects and mechanisms of pain.

Opioid Receptors and Pain

Opioid receptors are members of the Gi protein-linked GPCRs. These receptors, as well as the chemokine and opioid peptide ligands, are widely distributed in the brain tissue and the periphery. Four major opioid receptors have been cloned: µ-, δ-, κ-, and nociceptin/orphanin FQ receptors (opioid receptor-like 1 [ORL1]).[6] Each of the opioid receptor genes expressed in brain tissue and immune cells has been cloned and sequenced.[7-12] Stimulation of opioid receptors promotes Ca²⁺ release of intracellular Ca²⁺ stores through activation of phospholipase C.[13] The expression level of µ-, δ-, and κ-opioid receptors is correlated to the pain conditions. In a diabetic neuropathy rat model, the expression of µ-opioid receptor is attenuated in the spinal dorsal horn.[14,15] µ-opioid receptor has been reduced by injury in the spinal dorsal horn.[16] On the other hand, an increased expression of δ-opioid receptor of dorsal root ganglion (DRG) neurons is detected in chronic inflammatory pain rat model.[17] Consistent with above, the level of κ-opioid receptor is upregulated in the DRG neurons of mice following nerve injury.[18]

The µ-opioid agonists are still the gold standard for the treatment of moderate and severe pain. µ-opioid receptor is probably coupled to different signaling pathways and heterogeneously expressed in different phenotypes of DRG neurons.[19] Intrathecal injection of µ-opioid receptor antagonists abolishes the inhibitory effect on dorsal horn neurons and the analgesic action produced by µ-opioids administered systemically,[20,21] indicating that µ-opioid receptor in the DRG and spinal cord is involved in pain transmission. However, the heterodimerization of the κ-and δ-opioid receptors synergistically increases the binding of their selective agonists. On the contrary, co-expression of µ- and δ-opioid receptors decreases the binding of their selective agonists.[22] In contrast to the individually expressed µ- and δ-opioid receptors, the co-expressed receptors are insensitive to pertussis toxin in COS cells.[22] Therefore, co-activation of both Gq and Gi/o may be required for this opioid excitatory effect. However, the functional outcome of this effect is not clear, but it may play a role in opioid-induced hyperalgesia reducing pain. The µ-, δ-, κ-, and ORL1-opioid receptor agonists inhibit neuronal activity through (1) inhibition of voltage-gated calcium channels in the DRG neurons[23-25] and (2) suppression of neuronal excitability through activation of GIRK channels in the postsynaptic neurons in the spinal cord.[26] Above all, opioid receptor novel inhibitors will be a potential treatment target for pain management.

CXC Receptor 4 and Pain Neurotransmission

Chemokines are responsible for the recruitment of leukocytes during inflammation and disease. Chemokines, based on the position of conserved cysteines, have been classified into four families: C, CC, CXC, and CX3C.[27] Besides their well-known role in the immune system, they are highly expressed in the nervous system, indicating that they might play roles in the regulation of stem cell migration and neurotransmission. Chemokine signaling is also of key importance in the regulation of neuroinflammatory responses. Many chemokines and their receptors may play a distinct role in chronic pain syndromes.[28] Although it is uncertain of the exact mechanism by which chemokines and their receptors act in these pain states, pain strategies aimed at limiting the actions of chemokines may result in an important new direction of therapies on pain.

GPCR also involved the chemotaxis and inflammatory pathway signaling. A common response of all nonexcitable cells by chemokine stimulation is chemotaxis. The presence of chemokine receptors on neurons often triggers downstream signaling cascades through dissociation of G proteins which induce the phosphoinositide 3-kinase pathway or activates phospholipase C resulting in Ca²⁺ influx and protein kinase C activation.[29] It is important to note that most responses toward chemokines are blocked with pertussis toxin, indicating that many chemokine receptors are Gi/o coupled. Recent functional characterizations of chemokine receptors suggest that these proteins form dimers that could further regulate their signaling.[29,30] In addition, chemokines may activate mitogen-activated protein kinase by either Ga or G-protein independent signaling.[29,31]

The chemokine CXC motif receptor 4 (CXCR4) is a major GPCR for CXCL12. CXCL12/CXCR4 chemokine signaling plays a critical role in modulating various nervous system developmental processes and in regulating synaptic plasticity. CXCR4 is highly expressed in the peripheral nervous system (PNS) and CNS and exerts functions as modulation of neurotransmission, synaptic plasticity, and neuroglial interactions.[32] In pain processing, CXCR4 is overexpressed on primary sensory neurons, satellite cells, Schwann cells, and endothelial cells in the peripheral nociceptive structure.[33-39] Besides functions in CNS, CXCR4 is involved in the CNS pain signaling. In a central neuropathic pain model, CXCL12/CXCR4 was upregulated in neurons, astrocytes,
microglia/macrophages, and leukocytes in the lumbar spinal cord.[40] However, the role of CXCR4 in pain transduction remains largely unknown. A few studies evaluate the effects of pharmacological inhibition of CXCR4 on central pain signal processing. Increased signaling by stromal-derived factor-1 (SDF-1/CXCL12) and its receptor, CXCR4, has been shown to contribute to chronic pain behavior.[35] Specific chemokine receptor antagonists for CXCR4 successfully may reverse nociceptive pain behaviors.[37]

The involvement of chemokine and their receptors in neuropathic pain processing has recently been established in animal models. It has been shown that the injection of SDF1α/CXCL12 into the un-inflamed adult rat hind paw produces dose-dependent tactile allodynia, designed regulated on activation, normal T-cell expressed, and secreted (RANTES/CCL5) or macrophage inflammatory protein-1α (MIP1α/CCL3).[38] These behavioral studies in combination with reverse-transcription polymerase chain reaction, calcium imaging, and immunohistochemistry confirmed the presence and functionality of the respective chemokine receptors, CXCR4, CCR5, and CCR4 in rodent DRG sensory neurons.[34] CXCR4-knockout mice show abnormalities in the development of several neuronal structures, such as the dentate gyrus of the hippocampus, the cerebellum, and the DRG.[40,42] These phenotypes result from deficits in the chemokine-mediated migration of neural stem cells. Chemokines are involved in the regulation of neuronal excitability, neurotransmitter release, and neuronal survival.[41] These possibilities are supported by the extensive expression patterns of some chemokines and their receptors throughout the developed brain[34,41–43] and by the reported actions of chemokines on phenomena such as neuronal excitability and transmitter release, and neuronal survival.[34,41] Interestingly, although many chemokines are not commonly expressed at high levels in the brain, they can be dramatically upregulated due to neuroinflammatory responses. Increased signaling by SDF-1/CXCL12 and its receptor, CXCR4, has been shown to contribute to chronic pain behaviors. The use of specific chemokine receptor antagonists for CXCR4 successfully reverses nociceptive pain behavior. Taking all this evidence into consideration, drugs that inhibit chemokine receptor function would be predicted to be useful in treating painful neuropathies. In the spinal cord injury-induced central neuropathic pain model, it is demonstrated that SDF1 and CXCR4 expression was continuously increased at the spinal cord level.[53] Moreover, by mapping the cellular and subcellular localization of SDF1 and CXCR4, Reaux-Le et al. reported that SDF1/CXCR4 system was closely related to the nociceptive pathway, especially in the primary nociceptive neurons, and they also found that activating the CXCR4 by intrathecal SDF1 injection could induce mechanical allodynia, which could be prevented by the CXCR4-neutralizing antibody.[54] However, the underlying mechanisms of SDF1/CXCR4 involvement in the chronic and persistent pain remain unclear. Recently, this chemokine signaling has attracted much attention because of its emerging involvement in nociceptive signal regulation.

In summary, chemokines and their receptors could potentially be important for the development and maintenance of pain. Chemokines can be synthesized by nociceptive neurons and by other cells in response to injury. These chemokines activate receptors on macrophages and microglia, resulting in their migration and enhancing their activation. Importantly, the chemokines, as RANTES, SDF1α, MCP1, and fractalkine,[34,55] can act directly on nociceptive neurons to produce excitation and pain.[34,55] Opioid receptors are members of the Gi protein-linked GPCRs. The µ-opioid agonists are still the gold standard for the treatment of moderate and severe pain. Furthermore, specific chemokines/receptors are upregulated following peripheral nerve injury and appear to participate in neural signal processing, leading to chronic pain states.[56] CXCL12/CXCR4 signaling is now proven to be a potential analgesic target for pain management.

**Conclusion**

Tissue damage, inflammation, or injury of the nervous system may lead to chronic neuropathic pain characterized by hyperalgesia, allodynia, and spontaneous pain.[57,58] Significant progress has been made in understanding the important roles of various GPCRs in the regulation of pain transmission. However, intracellular signaling is complex and diverse process, and many important questions remain to be answered, especially in the precise signal transduction mechanism that underlies the diverse effects of individual GPCR agonists on ion channels and transmission during the pain. Further studies on the signal transduction pathways and molecular interactions between GPCRs are essential for a better understanding of drugs’ action through GPCRs. Drug development by targeting each GPCR will improve the efficacy of traditional GPCR analgesics used to treat acute and chronic pain. The opioid receptor agonists are still the gold standard for the treatment of chronic pain. Furthermore, CXCR4 as a new therapeutic target has the pivotal role for pain managements. GPCRs function, their downstream effectors, and signaling pathways in pain processing need to be further illustrated. Taken together, chemokines and their receptors are potential targets for the development of novel pain management and therapy.

**Financial support and sponsorship**

This study was supported by National Natural Science Foundation of China (No. 81502553); Beijing
Municipal Administration of Hospitals Clinical Medicine Development of Special Funding Support (No. ZYLX201507); Capital Characteristic Clinic Project (No. Z141107002514065); National Natural Science Foundation of China (No. 81271556); National Natural Science Foundation of China (No. 81672467); National Natural Science Foundation of China (No. 81470165); the National Science Foundation of Beijing (No. 7172207).

Conflicts of interest

There are no conflicts of interest.

References

1. Pan HL, Wu ZZ, Zhou HY, Chen SR, Zhang HM, Li DP. Modulation of pain transmission by G-protein-coupled receptors. Pharmacol Ther 2008;117:141-61.
2. Lu ZL, Saldanha JW, Hulme EC. Seven-transmembrane receptors: Crystals clarify. Trends Pharmacol Sci 2002;23:140-6.
3. Hur EM, Kim KT. G protein-coupled receptor signalling and cross-talk: Achieving rapidity and specificity. Cell Signal 2002;14:397-405.
4. Neves SR, Ram PT, Iyengar R. G protein pathways. Science 2002;296:1636-9.
5. Jeong SW, Ikeda SR. Effect of G protein heterotrimer composition on coupling of neurotransmitter receptors to N-type Ca(2+) channel modulation in sympathetic neurons. Proc Natl Acad Sci U S A 2000;97:907-12.
6. Evans CJ, Keith DE Jr., Morrison H, Magendzo K, Edwards RH. Cloning of a delta opioid receptor by functional expression. Science 1992;258:1952-5.
7. Chen Y, Mestek A, Liu J, Yu L. Molecular cloning of a rat kappa opioid receptor reveals sequence similarities to the mu and delta opioid receptors. Biochem J 1993;295(Pt 3):625-8.
8. Li S, Zhu J, Chen C, Chen YW, Deriel JK, Ashby B, et al. Molecular cloning and expression of a rat kappa opioid receptor. Biochem J 1993;295(Pt 3):629-33.
9. Evans CJ, Keith D Jr, Morrison H, Magendzo K, Edwards RH. Cloning of a delta opioid receptor by functional expression. Science 1992;258:1952-6.
10. Chuang LF, Chuang TK, Killam KF Jr., Chuang AJ, Kung HF, Yu L, et al. Delta opioid receptor gene expression in lymphocytes. Biochem Biophys Res Commun 1994;202:1291-9.
11. Belkowski SM, Zhu J, Liu-Chen LY, Eisenstein TK, Adler MW, Rogers TJ. Sequence of kappa-opioid receptor cDNA in the R1.1 thymoma cell line. J Neuroimmunol 1995;62:113-7.
12. Sedq M, Roy S, Ramakrishnan S, Elde R, Loh HH. Complementary DNA cloning of a mu-opioid receptor from rat peritoneal macrophages. Biochem Biophys Res Commun 1995;209:563-74.
13. Spencer RJ, Jin W, Thayer SA, Chakrabarti S, Law PY, Loh HH. Mobilization of Ca2+ from intracellular stores in transfected neuro2a cells by activation of multiple opioid receptor subtypes. Biochem Pharmacol 1997;54:809-18.
14. Chen SR, Pan HL. Antinoceptive effect of morphine, but not mu opioid receptor number, is attenuated in the spinal cord of diabetic rats. Anesthesiology 2003;99:1409-14.
15. Chen SR, Pan HL. Spinal GABAB receptors mediate antinoceptive actions of cholinergic agents in normal and diabetic rats. Brain Res 2003;985:67-74.
16. Porreca F, Tang QB, Bian D, Riedl M, Elde R, Lai J. Spinal opioid mu receptor expression in lumbar spinal cord of rats following nerve injury. Brain Res 1998;795:197-203.
17. Morinville A, Cahill CM, Aibak H, Rymar VV, Pradhan A, Hoffert C, et al. Morphine-induced changes in delta opioid receptor trafficking are linked to somatosensory processing in the rat spinal cord. J Neurosci 2004;24:5549-59.
18. Sung B, Loh HH, Wei L. Association of kappa opioid receptor mRNA upregulation in dorsal root ganglia with mechanical allodynia in mice following nerve injury. Neurosci Lett 2002;291:163-6.
19. Wu ZZ, Chen SR, Pan HL. Differential sensitivity of N- and P/Q-type Ca2+ channel currents to a mu opioid in isoleucin B4-positive and -negative dorsal root ganglion neurons. J Pharmacol Exp Ther 2004;311:939-47.
20. Chen SR, Pan HL. Activation of muscarinic receptors inhibits spinal dorsal horn projection neurons: Role of GABAB receptors. Neuroscience 2004;125:141-8.
21. Chen SR, Pan HL. Blocking mu opioid receptors in the spinal cord prevents the analgesic action by subsequent systemic opioids. Brain Res 2006;1081:119-25.
22. George SR, Fan T, Xie Z, Tse R, Tam V, Varghese G, et al. Oligomerization of mu- and delta-opioid receptors. Generation of novel functional properties. J Biol Chem 2000;275:26128-35.
23. Acosta CG, López HS. delta opioid receptor modulation of several voltage-dependent Ca(2+) currents in rat sensory neurons. J Neurosci 1999;19:8337-48.
24. Beedle AM, McKory JE, Poirot O, Doering CJ, Altier C, Barriere C, et al. Agonist-independent modulation of N-type calcium channels by ORL1 receptors. Nat Neurosci 2004;7:118-25.
25. Moises HC, Rusin KI, Macdonald RL. Mu- and kappa-opioid receptors selectively reduce the same transient components of high-threshold calcium current in rat dorsal root ganglion sensory neurons. J Neurosci 1994;14:5903-16.
26. Marker CL, Luján R, Colón J, Wickman K. Distinct populations of spinal cord lamina II interneurons expressing G-protein-gated potassium channels. J Neurosci 2006;26:12251-9.
27. White FA, Wilson NM. Chemokines as pain mediators and modulators. Curr Opin Anaesthesiol 2008;21:580-5.
28. Murphy PM. The molecular biology of leukocyte chemotactant receptors. Annu Rev Immunol 1994;12:593-633.
29. Rodríguez-Frade JM, Mellado M, Martínez AC. Chemokine receptor dimerization: Two are better than one. Trends Immunol 2001;22:612-7.
30. Mellado M, Rodríguez-Frade JM, Vila-Coro AJ, Fernández S, Martín de Ana A, Jones DR, et al. Chemokine receptor homo- or heterodimerization activates distinct signaling pathways. EMBO J 2001;20:2497-507.
31. Galjø RK, Brubaker SA, Meyer J, Dutt P, Yang Y, Qin S, et al. The alpha-chemokine, stromal cell-derived factor-1alpha, binds to the transmembrane G-protein-coupled CXCR4 receptor and activates multiple signal transduction pathways. J Biol Chem 1998;273:23169-75.
32. Li M, Ransohoff RM. Multiple roles of chemokine CXCL12 in the central nervous system: A migration from immunology to neurobiology. Prog Neurobiol 2008;84:116-31.
33. Dubovsky P, Klusáková I, Svrčeková K, Brázdová V. Spatio-temporal changes of SDF1 and its CXCR4 receptor in the dorsal root ganglia following unilateral sciatic nerve injury as a model of neuropathic pain. Histochem Cell Biol 2010;133:323-37.
34. Oh SB, Tran PB, Gillard SE, Hurley RW, Hammond DL, Miller RJ. Chemokines and glycoprotein 120 produce pain hypersensitivity by directly exciting primary nociceptive neurons. J Neurosci 2001;21:5027-35.
35. Bhangoo S, Ren D, Miller RJ, Henry K, Lineswala J, Hamdouchi C, et al. Delayed functional expression of neuronal chemokine receptor following focal nerve demyelination in the rat: A mechanism for the development of chronic sensitization of peripheral nociceptors. Mol Pain 2007;3:38.
36. Bhangoo SK, Ren D, Miller RJ, Chan DM, Ripsch MS, Weiss C, et al. CXCR4 chemokine receptor signaling mediates pain hypersensitivity in association with antiretroviral toxic neuropathy. Brain Behav Immun 2007;21:581-9.
37. Bhangoo SK, Ripsch MS, Buchanan DJ, Miller RJ, White FA. Increased chemokine signaling in a model of HIV1-associated peripheral neuropathy. Mol Pain 2009;5:48.

38. Wilson NM, Jung H, Ripsch MS, Miller RJ, White FA. CXCR4 signaling mediates morphine-induced tactile hyperalgesia. Brain Behav Immun 2011;25:565-73.

39. Reaux-Le Goazigo A, Rivat C, Kitabgi P, Pohl M, Melik Parsadaniantz S. Cellular and subcellular localization of CXCL12 and CXCR4 in rat nociceptive structures: Physiological relevance. Eur J Neurosci 2012;36:2619-31.

40. Knerlich-Lukoschus F, von der Ropp-Brenner B, Lucas R, Mehdorn HM, Held-Feindt J. Spatiotemporal CCR1, CCL3(MIP-1α), CXCR4, CXCL12(SDF-1α) expression patterns in a rat spinal cord injury model of posttraumatic neuropathic pain. J Neurosurg Spine 2011;14:583-97.

41. Zou YR, Kottmann AH, Kuroda M, Taniuchi I, Littman DR. Function of the chemokine receptor CXCR4 in haematopoiesis and in cerebellar development. Nature 1998;393:595-9.

42. Lu M, Grove EA, Miller RJ. Abnormal development of the hippocampal dentate gyrus in mice lacking the CXCR4 chemokine receptor. Proc Natl Acad Sci U S A 2002;99:7090-5.

43. Tran PB, Miller RJ. Chemokine receptors: Signposts to brain development and disease. Nat Rev Neurosci 2003;4:444-55.

44. Stumm RK, Rummel J, Junker V, Culmsee C, Pfeiffer M, Kriegstein J, et al. A dual role for the SDF-1/CXCR4 chemokine receptor system in adult brain: Isoform-selective regulation of SDF-1 expression modulates CXCR4-dependent neuronal plasticity and cerebral leukocyte recruitment after focal ischemia. J Neurosci 2002;22:5865-78.

45. Banisadr G, Skrzydelski D, Kitabgi P, Rostène W, Parsadaniantz SM. Highly regionalized distribution of stromal cell-derived factor-1/CXCL12 in adult rat brain: Constitutive expression in cholinergic, dopaminergic and vasopressinergic neurons. Eur J Neurosci 2003;18:1593-606.

46. Banisadr G, Fontanges P, Haour F, Kitabgi P, Rostène W, Melik Parsadaniantz S. Neuroanatomical distribution of CXCR4 in adult rat brain and its localization in cholinergic and dopaminergic neurons. Eur J Neurosci 2002;16:1661-71.

47. Banisadr G, Quéraud-Lesaux F, Bouterin MC, Pélapat D, Zalc B, Rostène W, et al. Distribution, cellular localization and functional role of CCR2 chemokine receptors in adult rat brain. J Neurochem 2002;81:257-69.

48. Cowell RM, Silverstein FS. Developmental changes in the expression of chemokine receptor CCR1 in the rat cerebellum. J Comp Neurol 2003;457:7-23.

49. Tissier F, Wang CE, Goffinet AM. Expression of the chemokine receptor Cxcr4 mRNA during mouse brain development. Brain Res Dev Brain Res 2004;149:63-71.

50. Ragozzino D. CXC chemokine receptors in the central nervous system: Role in cerebellar neuromodulation and development. J Neurovirol 2002;8:559-72.

51. Nelson TE, Grulo DL. The chemokine CXCL10 modulates excitatory activity and intracellular calcium signaling in cultured hippocampal neurons. J Neuroimmunol 2004;156:74-87.

52. Puma C, Danik M, Quirion R, Ramon F, Williams S. The chemokine interleukin-8 acutely reduces Ca(2+) currents in identified cholinergic septal neurons expressing CXCR1 and CXCR2 receptor mRNAs. J Neurochem 2001;78:860-71.

53. Knerlich-Lukoschus F, von der Ropp-Brenner B, Lucas R, Mehdorn HM, Held-Feindt J. Spatiotemporal CCR1, CCL3(MIP-1α), CXCR4, CXCL12(SDF-1α) expression patterns in a rat spinal cord injury model of posttraumatic neuropathic pain. J Neurosurg Spine 2011;14:583-97.

54. Réaux-Le Goazigo A, Van Steenwinckel J, Rostène W, Melik Parsadaniantz S. Current status of chemokines in the adult CNS. Prog Neurobiol 2013;104:67-92.

55. Milligan ED, Zapata V, Chacur M, Schoeniger D, Biedenkapp J, O’Connor KA, et al. Evidence that exogenous and endogenous fractalkine can induce spinal nociceptive facilitation in rats. Eur J Neurosci 2004;20:2294-302.

56. Ghanekar S, Corey S, Stonesifer C, Lippert T, Diamandis Z, Sokol J, et al. Current challenges in regenerative medicine for central nervous system disorders. Brain Circ 2016;2:105-7.

57. Suárez-Meade P, Carvajal HG, Yasuhara T, Tajiri N, Date I, Borlongan CV, et al. Regenerative medicine for central nervous system disorders: Role of therapeutic molecules in stem cell therapy. Brain Circ 2015;1:125-32.

58. Martini SR, Williams SR, Moretti P, Woo D, Worrall BB. A molecular/genetic approach to cerebral small-vessel disease: Beyond aging and hypertension. Brain Circ 2015;1:79-87.