



Evolutionary Role of Epigenetics in Ischemic Stroke: A Review

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ABSTRACT

Stroke is a Central Nervous System (CNS) disorder which occurs due to the obstruction in the brain blood flow. Stroke is mainly of two types, such as ischemic and hemorrhagic stroke. Ischemic stroke (80%) caused due to obstruction of blood flow through Middle Cerebral Artery (MCA) and characterized by a decreased supply of oxygen and glucose to CNS. In comparison, Hemorrhagic stroke (20%) mainly occurs due to the rupturing of blood vessels. Epidemiologically, it is the common reason of death after cancer and affecting millions of global population. There are many risk factors such as hypertension; hypercholesterolemia etc. which can exaggerate the condition of stroke. Various conventional therapies like Antiplatelets, Thrombolytic are available, but, there is a need to obtain a therapeutic approach that can provide prevention as well as a cure for the stroke. So the present review is primarily focused on epigenetic approach for ischemic stroke by Endogenous Transplantation of Neural Stem/Progenitor Cells (NSPCs). This, in turn, will decrease the level of REST protein at the genetic level and enhance the activity of Na⁺-Ca⁺ exchanger activity and lowers the excitotoxicity.

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INTRODUCTION

Stroke is a debilitating neurological condition and defined as a sudden loss of functions of neurons including either loss of supply of glucose and oxy-

gen, i.e. Cerebral Blood Occlusion (Ischemic Stroke) Figure 1 or inter cerebral bleeding (Haemorrhagic Stroke) (Moretti *et al.*, 2015). It shows symptom like limb weakness, bilateral or unilateral immobility, and inability to speak and become a reason of death and disability worldwide. It generally involves 1 in every 18 deaths and requires long-term care due to their functional and cognitive disabilities. There is a marked decrease in the survival rates of the victims suffering from ischemic stroke (68%) as compared to Haemorrhagic stroke (28%) (Krishnamurthi *et al.*, 2013). This is due to the formation of thrombus in the cerebral arteries, which leads to the obstruction of blood flow through the cerebral blood vessels, which cause irreversible cell injury. The present article is aimed to introduce the evolutionary epigenetic perspective for the prevention and treatment of ischemic stroke by endogenous

transplantation of Neuronal Stem/ Progenitor Cells (NSPCs) to enhance the activity of $\text{Na}^+ \text{Ca}^+$ exchanger to reduce the excitotoxicity and decrease the activity of REST (RE1-silencing transcription factor) and Co-REST. This will ultimately lead to the treatment of neuronal cell death (Paciaroni *et al.*, 2009).

Pathophysiology of Ischemic Stroke

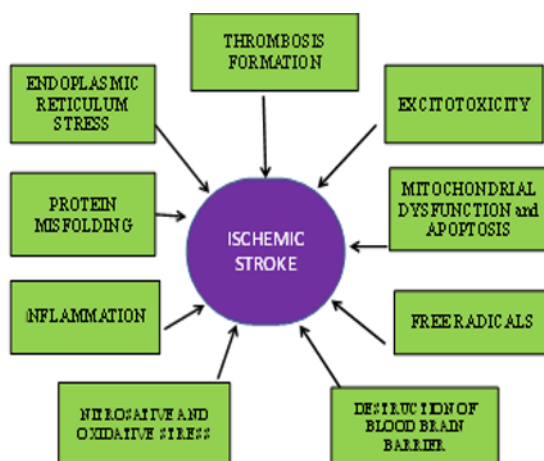


Figure 1: Pathophysiology of Ischemic stroke.

Thrombosis Formation

Blockage of the cerebral artery occurs due to thrombus formation, which can cause an ischemic lesion. If this blockage continues and flow of blood becomes less than 12 ml/min for a prolonged time, it results in necrotic death of tissues of neurons. This leads to stoppage of supply of oxygen and results in the expiration of Neurons, Astrocytes and oligodendrocytes in this infarcted region (Dirnagl *et al.*, 1999).

Excitotoxicity

Excitotoxicity tends to decrease the level of oxygen and glucose and cause neuronal death. This may be due to the impairment of ion exchange pumps that results in the reversed release of extracellular glutamate by various neurotransmitter transporters. This glutamate acts on its receptor present on the post-synaptic neuronal membrane. It results in the entry of calcium ions, loss of mitochondrial functions, depletion of the energy levels, exhaustion, and finally apoptosis (Dirnagl *et al.*, 1999).

Mitochondrial dysfunction associated apoptosis

Mitochondria of the cell act as a powerhouse and always maintain the energy levels but, when ATP synthesis and energy balance gets destructed, which leads to ischemic cell death. Glutamate acts on ionotropic receptor N-methyl-D-aspartate (NMDA) and gives rise to an increased amount of intracellular Ca^{2+} influx. This shows the activation of Ca^{2+} -dependent enzymes comprising proteases, calpain, and caspases dependent cellular death pathways.

This pathway contains caspase-12, caspase-9, and caspase-3 after the liberation of cytochrome C and resulting finally into the mitochondrial pathway of necrosis and cell death. These give rise to swelling and crumble of mitochondrial membranes and eventually cause apoptotic cell death (Iadecola and Anrather, 2011).

Free radicals, nitrosative/oxidative stress and destruction of BBB

The entry of Ca^{2+} ions is also responsible for the generation of Nitric Monoxide (NO) due to the enzyme Nitric Oxide Synthase (NOS). This results in the release of oxygen free radicals and peroxynitrite and shows the action of the fireman of necrosis. Some of the destruction of the brain following ischemia is due to the release of oxygen free radicals, nitric oxide and also contributes to the further damage to the neuronal tissues (Iadecola, 1997).

Inflammation

After the cerebral injury, Blood-Brain Barrier permits entry of the immune cells like mononuclear phagocytes, T-cells or helper cells, large granular lymphocytes, neutrophils basophils and eosinophils. After a neuronal injury, these immune cells then generate and secrete the cytokines which are responsible for neurological inflammation and gliosis. Within a few hours of cerebral ischemia, there is a dysregulation of pro-inflammatory mediators such as chemokines and cytokines with enhanced expression of cell adhesion molecule (CAM) and following the transendothelial movement of inflammatory cells. Most importantly, interleukin-1 (IL-1), tumour necrosis factor- α (TNF- α) and Toll-like receptors (TLRs) act as a chief part in neuro-inflammation caused due to ischemic stroke. The astrocytes and microglia play a predominant role in the neuro-inflammation due to ischemic stroke, mainly in the penumbra region (Kleinig and Vink, 2009).

Endoplasmic Reticulum (ER) stress and protein misfolding and others

ER is the biggest storehouse of calcium which helps in the regulation of synthesis of proteins and also responses when there is protein refolding but, in ischemic damage, these process of ER gets affected. The sarcoplasmic/ER calcium ATPase (SERCA) pump can't function because of the decrease in the levels of energy, and this contributes to necrotic cell death due to excitotoxicity. The enhanced aggregation of misfolded proteins also triggers the protein kinase-like ER kinase (PERK) pathway regulating eIF2 α kinase activation, which stops new protein formation (Althausen *et al.*,

2001).

Neuron RE1 Silencing Transcription Factor (NRSF/REST) antibody and its association with Ischemic stroke

REST is a zinc finger DNA binding protein which is low in the normal individual brain cell but exceeds in the various pathological conditions such as epilepsy, ageing and ischemic necrosis. It also plays a critical role in the last stage of neuronal differentiation, stages of embryogenesis to have stable neuronal gene repression for the normal neuronal fate of synapse formation to maintain its synaptic plasticity in both foetal and mature neural progenitor cells. The neuronal REST is expressed at lower levels and decreases as the differentiation progresses ([Baldelli and Meldolesi, 2015](#)).

REST and Its location

REST is involved in both neuronal and non-neuronal cells and is relatively low during our normal brain development and can be seen in the various parts of the brain ([Palm et al., 1998](#)). But, there are the increased levels of this transcriptional factor under the influence of various nervous disorders, including the necrotic cell death such as stroke. The changes in the levels of the REST occur as the age increase, and hence enhanced levels of REST is observed in geriatric patients as compared to young individuals. These levels are also observed in cerebral disorders such as epilepsy, gliomas, ischemic insults etc. in the hippocampal area of the central nervous system of the human being ([McClelland et al., 2014](#)). These occur due to the exaggerated release of excitatory neurotransmitter glutamate which further increases the level of formation of REST in hippocampal and cortical neurons in addition to the various severe disorders such as epilepsy ([Palm et al., 1998](#)).

REST and its complex formation for gene silencing

REST links with the element RE1 of target genes and mobilise to the cofactor of C-terminal Co-REST and mSin3A which then links with the Histone Deacetylases (HDACs) 1 and 2 and deacetylate core histone proteins. Along with the recruitment to the histone methyltransferase, enzyme G9a results into dimethylation of histone 3 at lysine 9 (H3K9me2), which may be Co-REST dependant and independent mechanisms ([Ballas et al., 2005](#)). The histone deacetylation is first, and the foremost a symbol of dynamic gene repression, protein histone and DNA methylation are described as in continuing, steady gene subjugation and forms the REST-Sin3a-coREST repressor complex providing the deacetylation of

histone proteins and diminishes gene expression. Besides, REST also controls the arrangements of noncoding RNAs like miRNAs which can be further in future can be used as an innovative therapeutic strategy for ischemic stroke ([Wu and Xie, 2006](#)).

REST and its role in Ischemic stroke

REST at present is anticipated as the most important target for the therapeutics of various neuronal disorders including stroke. It is crucial for neuronal development and its maintenance. It also takes part in the cellular excitation along with neurotransmitter production, but its abnormal activation leads to excitotoxicity. This cause ischemic stroke besides with the increased expression of miR-21 through the calcium dependent mechanism in association with ERK-stimulated induced matrix metalloprotease-9 (MMP9) expression ([Deng et al., 2013](#)). REST/NRSF and its Co-factor Co-REST and miRNA levels are higher in hippocampal CA1 neurons in the cerebral disorders, i.e. ischemic stroke in adults which leads to the down regulation of gene expression which is essential for synaptic plasticity and causes CA1 neuronal deaths which then results into an ischemic stroke. This again leads to aberrant enhancement of neuronal cell death by decreasing the levels of its downstream genes such as NF κ B2, ionotropic glutamate receptor, N-methyl D-aspartate 1 (GRIN1), and alpha-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid selective glutamate receptor complex (GRIA2). The crucial factor in ischemia is GluA2 lacking AMPARs resulting in the toxic levels of Calcium and zinc ions in the affected CA1 neurons, which results into ischemic neuronal apoptosis ([Calderone et al., 2003](#)).

Stem cells therapy: an epigenetic perspective for the development of therapeutics of ischemic stroke

Neural Stem Cell (NSC)/Progenitor Cells: its location, migration and proliferation

Stem cells have the capacity of self-renewing and self-differentiating into many different types of cells. Adult CNS composed of neural stem cells can give two daughters NSCs, which have identical stem cell properties as the parental cell or dissimilar division which may give rise to one identical daughter cell or lineage-committed cell which ultimately forms the basis for the origination of the three dominant cells neurons, oligodendrocytes or astrocytes. There are two regions which involve the continuous process of formation of new neurons, i.e. Subgranular Zone (SGZ) and Subventricular Zone (SVZ) in the dentate gyrus of hippocampus and lateral ventricles respectively. The neurons which are formed in SGZ

are slowly dividing or quiescent neural stem cells (qNSCs) which can be recognized by various markers such as Glial fibrillary acidic protein (GFAP) positive cells (Doetsch, 2003). These cells then provide the ultimate generation of rapidly expanding Neural Stem/Progenitor Cells and formation of neuroblasts developing a chain which then moves through a rostral migratory stream (RMS) to the olfactory cells inside an astrocyte derived tube.

It includes primary as well as other local neurons in the olfactory bulb whose axon terminals get near glomeruli, where it gets ultimately differentiated (Brill *et al.*, 2009). These are procured from radial glia and are believed to hold basis for progenitor function, and these progenitors play a remarkable role in the formation of new neurons (Merkle *et al.*, 2004). NSPCs may relocate from rostral migratory system to the olfactory lobe and other parts of the brain and enters in the injured area of the brain to manage the neurological offend (Goings *et al.*, 2004). They are the parent for both neurons and neuroglia and have the ability to regenerate into the leading cells of nerves. They can be procured from embryonic and foetal human neurons which ultimately discover the three essential nerve cells, astroglia, and oligodendroglia. These newly formed astrocytes help in the maintenance of neuronal agitation, activity of synapse, plasticity, and modulate neural circuitry of information coding. The signals obtained from chemokines, such as vascular permeability factor, recruits the NSPCs to extend to the site of injury from the blood vessels (Araque *et al.*, 2014).

Endogenous transplantation of NSC/Progenitor Cells

Endogenous NSPCs helps in the promotion of neurogenesis and gliogenesis which can be useful in the treatment of ischemic cell death, after brain injuries these may lead to excessive changes in the micro environs surrounding the NSPCs, i.e. astrocytes, endothelial cells, and microglia. These stem cells have the amplitude to replace the damaged neuronal cells (Arvidsson *et al.*, 2002). There are two criteria for the stem cell therapy: 1) Implant of various exogenous cells including embryonic tissue, foetal and matured brain, bone marrow, or preserved cell line; 2) Revitalisation of endogenous NSC with cytokines and growth factors. The various studies have reviewed that NSPCs pools expand after ischemic cell death and can move in the damaged area where they discriminate in the various types of cells in addition to neurons (Burns *et al.*, 2009). The role of NSPCs, their capability to fight with damage and to restore neural tissue, is managed by stem cell and works through signals

obtained from local and long-distance sources. In addition to blood and cerebrospinal fluid borne factors, the endothelial cells, microglia, astrocytes, and local or distal axons regulate self-renewal, differentiation and maintenance of NSPCs (Ohab *et al.*, 2006).

Epigenetic mechanism of NSC/ progenitor cells (NSPCs) and REST

The RE1 silencing transcription factor antibody acts as a principle epigenetic modulator that restructures neuronal gene declaration, outcome and the functions of NSPCs (Qureshi and Mehler, 2009). REST and Co-REST its co-factor are incriminated in the alimentionation of NSPCs, regional neuronal and glial sub types and progressive stages of oligodendrocyte lineage maturation, including myelination (Abrajano *et al.*, 2009a,b). The link with genomic regulatory sequences with neuronal genes provides a medium for enrolling different factors that attribute DNA methylation and chromatin remodeling (Qureshi and Mehler, 2009). Hence, REST and Co-REST manage neural cell type and stages of evolution of specific gene alteration and silencing. These improper regulations of REST act in ischemia-induced cell death, which then plays a significant role in the pathogenesis of ischemic stroke. This monitoring directly connects the epigenesis operation associated with NSPC functions with the molecular mechanisms (Calderone *et al.*, 2003). Attempts should be made to identify and design new molecules which can alter the functioning of REST and other epigenetic factors for treating ischemia-induced neurological ailments.

CONCLUSIONS

Conventional therapies provide the symptomatic and diagnosing parameter associated treatment of stroke. Still, the epigenetic approach, including REST modulation by NSPC and other underlying epigenetic components, may be proved as beneficial in the prevention and management of the ischemic stroke.

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Conflict of Interest

The authors declare that they have no conflict of interest for this study.

REFERENCES

Abrajano, J. J., Qureshi, I. A., Gokhan, S., Zheng, D., Bergman, A., Mehler, M. F. 2009a. Differential

- Deployment of REST and CoREST Promotes Glial Subtype Specification and Oligodendrocyte Lineage Maturation. *PLoS ONE*, 4(11):e7665–e7665.
- Abrajano, J. J., Qureshi, I. A., Gokhan, S., Zheng, D., Bergman, A., Mehler, M. F. 2009b. REST and CoREST Modulate Neuronal Subtype Specification, Maturation and Maintenance. *PLoS ONE*, 4(12):e7936–e7936.
- Althausen, S., Mengesdorf, T., Mies, G., Oláh, L. 2001. Changes in the phosphorylation of initiation factor eIF-2 α , elongation factor eEF-2 and p70 S6 kinase after transient focal cerebral ischaemia in mice. *Journal of Neurochemistry*, 78(4):779–787.
- Araque, A., Carmignoto, G., Haydon, P. G., Oliet, S. H., et al. 2014. Gliotransmitters Travel in Time and Space. *Neuron*, 81(4):728–739.
- Arvidsson, A., Collin, T., Kirik, D., Kokaia, Z., Lindvall, O. 2002. Neuronal replacement from endogenous precursors in the adult brain after stroke. *Nature Medicine*, 8(9):963–970.
- Baldelli, P., Meldolesi, J. 2015. The Transcription Repressor REST in Adult Neurons: Physiology, Pathology, and Diseases. *eneuro*, 2(4):1–15.
- Ballas, N., Grunseich, C., Lu, D. D., Speh, J. C., Mandel, G. 2005. REST and Its Corepressors Mediate Plasticity of Neuronal Gene Chromatin throughout Neurogenesis. *Cell*, 121(4):645–657.
- Brill, M. S., Ninkovic, J., Winpenny, E., Hodge, R. D., et al. 2009. Adult generation of glutamatergic olfactory bulb interneurons. *Nature Neuroscience*, 12(12):1524–1533.
- Burns, T. C., Verfaillie, C. M., Low, W. C. 2009. Stem cells for ischemic brain injury: A critical review. *Journal of Comparative Neurology*, 515(1):125–144.
- Calderone, A., Jover, T., Min Noh, K., Tanaka, H., et al. 2003. Ischemic Insults Derepress the Gene Silencer REST in Neurons Destined to Die. *The Journal of Neuroscience*, 23(6):2112–2121.
- Deng, X. H., Zhong, Y., Gu, L., Shen, W., Guo, J. 2013. MiR-21 involve in ERK-mediated upregulation of MMP9 in the rat hippocampus following cerebral ischemia. *Brain Research Bulletin*, 94:56–62.
- Dirnagl, U., Iadecola, C., Moskowitz, M. A. 1999. Pathobiology of ischaemic stroke: an integrated view. *Trends in Neurosciences*, 22(9):391–397.
- Doetsch, F. 2003. The glial identity of neural stem cells. *Nature Neuroscience*, 6(11):1127–1134.
- Goings, G. E., Sahni, V., Szele, F. G. 2004. Migration patterns of subventricular zone cells in adult mice change after cerebral cortex injury. *Brain Research*, 996(2):213–226.
- Iadecola, C. 1997. Bright and dark sides of nitric oxide in ischemic brain injury. *Trends in Neurosciences*, 20(3):132–139.
- Iadecola, C., Anrather, J. 2011. The immunology of stroke: from mechanisms to translation. *Nature Medicine*, 17(7):796–808.
- Kleinig, T. J., Vink, R. 2009. Suppression of inflammation in ischemic and hemorrhagic stroke: therapeutic options. *Current Opinion in Neurology*, 22(3):294–301.
- Krishnamurthi, R. V., Feigin, V. L., Forouzanfar, M. H., Mensah, G. A., Connor, M., et al. 2013. The global and regional burden of first-ever ischaemic and haemorrhagic stroke during 1990-2010: findings from the Global Burden of Disease Study. *The Lancet Global Health*, 1(5):259–281.
- McClelland, S., Brennan, G. P., Dubé, C., Rajpara, S., et al. 2014. The transcription factor NRSF contributes to epileptogenesis by selective repression of a subset of target genes. *eLife*, 3:e01267–e01267.
- Merkle, F. T., Tramontin, A. D., Garcia-Verdugo, J. M., Alvarez-Buylla, A. 2004. Radial glia give rise to adult neural stem cells in the subventricular zone. *Proceedings of the National Academy of Sciences*, 101(50):17528–17532.
- Moretti, A., Ferrari, F., Villa, R. F. 2015. Pharmacological therapy of acute ischaemic stroke: Achievements and problems. *Pharmacology & Therapeutics*, 153:79–89.
- Ohab, J. J., Fleming, S., Blesch, A., Carmichael, S. T. 2006. A Neurovascular Niche for Neurogenesis after Stroke. *Journal of Neuroscience*, 26(50):13007–13016.
- Paciaroni, M., Caso, V., Agnelli, G. 2009. The Concept of Ischemic Penumbra in Acute Stroke and Therapeutic Opportunities. *European Neurology*, 61(6):321–330.
- Palm, K., Belluardo, N., Metsis, M., Timmusk, T. 1998. Neuronal Expression of Zinc Finger Transcription Factor REST/NRSF/XBR Gene. *The Journal of Neuroscience*, 18(4):1280–1296.
- Qureshi, I. A., Mehler, M. F. 2009. Regulation of non-coding RNA networks in the nervous system—What's the REST of the story? *Neuroscience Letters*, 466(2):73–80.
- Wu, J., Xie, X. 2006. Comparative sequence analysis reveals an intricate network among REST, CREB and miRNA in mediating neuronal gene expression. *Genome Biology*, 7(9):R85–R85.