Advancement of Epigenetics in Stroke Research

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Abstract

With the advancement of epigenetic tools and technologies associated with intervention medicine, stroke research has entered into a new fertile, dynamic era of epigenetic studies, a wide plethora of intervention procedure, administration of tissue plasminogen activator, the introduction of mechanical thrombectomy, clinical studies, and drug developments over the last decennium. Against this vivid background of newly emerging pieces of knowledge, there is little to none advancement in the overall outcome of the disease. The stroke involves an overabundance of inflammatory responses arising in part due to the body's immune response to brain injury. Neuroinflammation contributes to significant neuronal cell death and the development of functional impairment and death in stroke patients. Recent studies demonstrated epigenetic plays a key role in the overall outcome of the disease. In this review, we summarize the progress of epigenetics which provides an overview of recent advancements on the emerging key role of epigenetics over the last decade contributing to the regulation of neuroinflammation in stroke, potential epigenetic targets that might be key factors in the development of stroke therapies and their relation in respect to clinical practice.

Keywords Stroke, Neuroinflammation, Oxidative stress, Apoptosis, miRNAs, Epigenetics.

Stroke, including ischemic stroke and intracerebral hemorrhage, results in loss of neuronal function and brain tissues leading to sensory-motor function deficit and disability among diseased. Stroke has been theorized to be the second most common cause of death worldwide next to ischemic heart disease [1,2,6] and is suspected to remain the same until 2030[2,3]. Additionally, patients surviving stroke may suffer from a disability which might require temporary or lifelong assistance, resulting in an extensive burden to the family, and economic costs are reflected over countries economy. Corroboration suggesting socioeconomic deprivation not only corresponds to stroke and its risk factors but also intensively cohesive to the severity of the disease [4], mortality [5], and prevalence among the relatively younger generation [4]. So, understanding the stroke at the molecular level will help us, researchers, to produce key modulator alteration strategies to minimize post-stroke neuroinflammation, oxidative stress, pathological apoptosis, and promotion of neuroprotection thus reducing GBD (global burden of disease) and DALYs (disability-adjusted life year) associate with stroke. The acknowledgment of the role of epigenetics is the next step in better understanding of the disease.

Etiology

Stroke or cerebrovascular accidents as mentioned earlier are broadly classified into two categories; ischemic stroke and hemorrhagic stroke. The mechanism and pathophysiology involved among this type are quite different but involve some overlapping. Two major mechanisms considered responsible for ischemic stroke are thromboembolism and hemodynamic failure. Embolism, more precisely cardio-embolism has been demonstrated to produce 20% to 30% of all ischemic stroke.[7,8] The risk factor associated with cardio-embolism is Atrial Fibrillation [9-11], Systolic Heart Failure [12-15], Acute Myocardial Infarction [16,17], Patent Foramen Ovale [18-20], Aortic Arch Atheroma [21-23], Prosthetic Heart Valves [24-26], Infective Endocarditis [27-29], and others [30]. Large Vessel Atherosclerosis (LVA) is another main contributor to ischemic stroke. LVA accounts for nearly 15% to 20% of all ischemic stroke [31,32]. Similarly, 25% of all reported ischemic stroke cases have been shown to have an association with Small Vessel occlusion [33,34]. Hemorrhagic stroke on the other hand has a well-established relationship with Traumatic Brain Injury [35,36], Cerebral Aneurysm [37,38], Anti-thrombolytic therapy [39,40], Hypertension (high blood pressure) [41-43], Arteriovenous Malformation [44-46].

Pathophysiology

The pathophysiology involving stroke is quite complex and involves various cascade processes, which include: loss of cellular homeostasis, energy failure, metabolic acidosis, significantly increased in intracellular Ca2+ levels, free-radical mediated toxicity, metabolic acidosis, generation of arachidonic acid products, cytokine-mediated cytotoxicity, complement activation, disruption of Blood-Brain Barrier (BBB), activation of glial cells and infiltration of leukocytes. The mechanism involved in both ischemic and hemorrhagic stroke produces a significant decrease in cerebral blood flow (CBF) leading to oxygen (O_2) deprivation causing an increase in anaerobic metabolism and eventually, lactic acidosis which in turns sequentially causes astrocyte demise and an increase in neuroinflammatory cytokines thus promoting neuroinflammation. Neuroinflammation has been recognized as one of the main culprits in promoting further insults in post-stroke condition, however, they also have been reported to play a beneficial role in promoting recovery. Similarly, a decrease in CBF can also produce malfunction of the ionic pump causing potassium ions (*K+*) efflux and sodium and calcium (*Na+ and Ca2+ respectively*) influx into the neuronal cells causing excitotoxicity, oxidative stress, and eventually necrosis. (Fig. 1)

Epigenetics in research frontline

Epigenetic is defined as the branch of biology which studies the causal interactions between genes and their products which bring the phenotype into being. Recent epigenetic studies have been demonstrated to play a key role in post-stroke condition leading to inflammatory responses and alteration of the microenvironment within the ischemic foci. Current understanding and development of epigenetic tools have given the researchers a more reliable method of competitive differentiation of normal versus diseased conditions at the molecular level. Contemporary studies in the

field of epigenetics involve Histone modification, DNA-methylation, RNA modifications, and non-coding RNA and their association concerning both pre and post-stroke conditions. (Fig. 2)

DNA methylation

DNA methylation has been one of the most extensively studied epigenetic modifications, exclusively occurring at CpG dinucleotides in mammals and always symmetrical to maintain the methylation during the cell division process. DNA methylation is carried out by *de novo methyltransferases (DNMT); precisely* DNMT3a and DNMT3b in mammals. CpGs are clustered into CpG islands, often at the promotor site of the gene. CpG island tends to be protected from methylation. Methylation observed at CpG island is entirely associated with the silencing of gene expression and carried out either by the formation of repressive chromatin structure or inhibiting transcription factor binding and alteration of gene expression.

LINE-1, which is a class I transposable element in the DNA and a member of long interspersed nuclear elements (LINEs) has been a center of many study discussions after their discovery concerning the association in predicting increase risk of ischemic stroke and cardiovascular events. Hypomethylation of LINE-1 is associated with an increase in the risk of developing ischemic stroke [47-50]. However, a single sex-specific analytic study has demonstrated that LINE-1 hypomethylation is suggestive of advanced atherosclerotic lesions, which leads to global hypomethylation and has more in association in determining the risk of development of ischemic stroke in men as compared to that of women [47]. Association of hypomethylation of LINE-1 was further investigated concerning that of circulating vascular cell adhesion molecule-1 (VCAM-1), intercellular adhesion molecule-1 (ICAM-1), and C-reactive protein (CRP) which displayed the co-relation between hypomethylation of LINE-1 and increase the level of circulating VCAM-1 but no association with ICAM-1 or CRP [51]. A cross-sectional study was conducted in the Japanese population aiming to determine the relationship between methylation of LINE-1 in leukocyte and that of dyslipidemia. Hypermethylation of LINE-1 in leukocytes was showcased to have a higher odds ratio in individuals with dyslipidemia [62]. Thus, the methylation status of LINE-1 can be a key risk factor predictor. Similarly, Hypomethylation of TNF receptor associated factor 3 (TRAF3) and hypermethylation of thrombospondin-1 (THBS1) has also been illustrated to be a crucial predictor of stoke related outcomes [52-55]. DNMT, especially DNMT1 and DNMT3a has also been identified as pivotal enzymes regulating methylation of various genes [56-58], of which DNMT1 dependent DNA methylation has been pinpointed as a mediator of chronic inflammation and development of atherosclerotic disease *via* the PPAR-γ pathway [59]. On the other hand, DNMT3a has also been identified to promote ischemic brain damage [60,61].

MMP-2 (Matrix metalloproteinase-2) is one of the most studied enzymes concerning their changes in peripheral blood concentration both in acute and chronic phases of post-stroke symptoms [66-68]. However, various studies have produced not identical data, creating confusion within the research field. A study conducted over a sample size (*n*) of 556 participants (298 with ischemic stroke versus 258 control) successfully showcased lower concentration of MMP-2 methylation level in peripheral blood exclusively in male small-vessel occlusion participants [69]. Thus, narrowing the use of MMP-2 serum concentration as an effective marker in post-ischemic stroke. Apart from the common methylation at the fifth position of the pyrimidine ring of cytosine (5mC), other forms of modifications are also noted at a similar position namely, 5-hydroxymethyl (5hmC), 5-formal (5fC), and 5-carboxyl (5caC). Various studies have successfully showcased 5-hmC to regulate several cellular processes which include neuronal development as well. A neoteric study was conducted in murine specie (*mice*), demonstrating the use of ascorbate (*mineral salt of ascorbic acid; vitamin C)* in post-stroke reperfusion led to Ten-eleven translocation 3 (TET3) dependent conversion of 5mC to 5hmC, promoting up-regulation of neuroprotective genes and functional recovery in mice [63].

5-aza-2'-deoxycytidine which is a DNA methyltransferase inhibitor (DNA methylation inhibitor) has been illustrated to significantly reduce the infarct volume in treated versus vehicle.[64] Mice pre-treated with 5-aza-2'-deoxycytidine showed a reduction in infarct volume as compared to that of the vehicle. Likewise, another study using zebularine which is also a DNA methylation inhibitor has demonstrated dose-dependent (500 μg and 100 μg) reduction in infarct volume [65].

DNA modifications have been widely studied over the last decade. However, their contribution to stroke research is still limited and further studies need to be carried in this field to produce a significant clinical outcome.

Histone modification

Histone is the basic proteins found in the nucleus of eukaryotic cells wrapped around by 146 base pairs (bp) of DNA into a compact structure known as a nucleosome [70]. The interaction between histone and DNA is due to the electrical charges between them. Briefly, the histones are positively charged due to the presence of a large amount of positively charged amino acids (*mainly lysine and arginine*). On the other hand, DNA is negatively charged and thus interaction of positive and negative charge maintains the structural integrity of nucleosome. Unlike DNA methylation, histone modification exclusively occurs at the amino-terminal tail protruding out of the histone subunit and is short-term reversible modifications. The amino-terminal tails are subjected to post-translational modification namely methylation, acetylation, phosphorylation, and ubiquitination. Post-translational modification of amino-terminal tails is associated with DNA repair, activation or repression of gene expression, telomere integrity, and the total interaction changes in response to these modifications are determined by 'histone code' [55].

The immune system, especially innate immune cells play a decisive role in producing signal depended on the response in cerebrovascular accidents. The predominant innate immune cell in the central nervous system (CNS) is microglia along with subsidiary infiltrating myeloid cells due to the disruption of the blood-brain barrier (BBB). Microglia even under ramified (resting) condition constantly monitors the surrounding environment and acts promptly per changes within the microenvironment [71]. Activated microglia are subjected to alter their morphology, gene expression, and consequently undertaking their role per the changes in the microenvironment [72]. Microglia, similar to macrophages are characterized as M1 (pro-inflammatory) and M2 (anti-inflammatory). The pro-inflammatory subtype (M1) has been illustrated to up-regulate inflammatory genes namely IL-1α/β, IL-6, IL-12, IL-23, TNF-α, iNOS whereas the M2 subtype has been illustrated to up-regulate neuroprotective genes such as Arg-1, IGF-1, Ym-1, and FIZZ [73-76]. Simultaneous down-regulation of M1 and up-regulation of the M2 phenotype in post-stroke condition can be beneficial in minimizing the post-stroke insults to the CNS. H3KAc (histone 3 lysine acetylation) is up-regulated in microglia around peri-infarct and infarct zone after ischemic stroke. Similar up-regulation in H3KAc was also noted in LPS (lipopolysaccharide) mediated microglial activation. Thus, proving the fact that H3KAc up-regulation is highly associative to the inflammatory cytokines and down-regulation might help to minimize CNS insults. HDAC (histone deacetylase) is a key regulator of H3KAc [77-79]. HDAC inhibition promotes downregulation of pro-inflammatory genes such as TNF-α, iNOS, STAT1, and IL-6 and up-regulation of IL-10 and STAT3 genes in activated microglia both in *vivo* and *vitro*. The up-regulation of anti-inflammatory genes promotes neuronal survival, reduction in brain infarct volume, and suppression of microglia activation (M1) which is indicative of the neuroprotective abilities of HDAC inhibitors [80,81]. SAHA (*Suberoylanilide Hydroxamic Acid; vorinostat*), which is an HDAC inhibitor has been exhibited to up-regulate 70 kilo Dalton heat shock protein (*Hsp70; essential in protein folding and stress-related protection in cells*) and B-cell lymphoma 2 (*Bcl-2; anti-apoptotic*) along with the reduction of pro-inflammatory cytokines IL-1, thus preventing neuronal loss and promoting favorable outcome in post-stroke condition [82-86]. Apart from SAHA, other HDAC inhibitors such as valproic acid, sodium butyrate, trichostatin-A, sodium 4-phenylbutyrate have shown to promote similar neuroprotective abilities by regulation of excitotoxicity, oxidative stress, endoplasmic reticulum stress (ER-stress), apoptosis, inflammation, and BBB breakdown [77]. Reactive oxygen species (ROS) have a well-established association with cerebrovascular accidents [87,88]. Nuclear factor erythroid 2-related factor 2 (Nrf-2) has been identified as a key regulator in ROS dependent oxidative insults to CNS [89,90]. Up-regulation of Nrf-2 using HDAC inhibitor such as valproic acid and trichostatin-A (TSA) has been exemplified to promote neuroprotection against oxidative stress [91,77].

Histone methylation has also been extensively explored to determine factors associated with prognostic outcomes in both pre and post-stroke conditions. Aging is one of the principal determinants of functional outcome in cerebrovascular accidents [94,95] and is highly associated with a reduction in brain plasticity [92,93]. A murine study displayed a significant reduction of H3K4me3 (Trimethylation of Histone H3 at lysine 4) in cortical astrocytes with progression in age [96]. H3K9 (Histone 3 lysine 9) has also been identified as a potential target therapy region as inhibition of Histone-lysine N-methyltransferase SUV39H1 and G9a (Euchromatic histone-lysine N-methyltransferase 2) promotes up-regulation of Brain-derived neurotrophic factor (BDNF) in E17 neuronal cells [97]. Another study using dimethyloxalylglycine (DMOG) to inhibit histone lysine demethylase subfamily 4 (KDM4) has been shown to promote neuronal repair via H3K9me2 dependent manner in CD1 mouse [98].

Apart from histone acetylation and methylation, post-translational phosphorylation has also been identified in cerebral ischemic conditions [99-102]. According to one of the studies, an increase in ionotropic glutamate receptor

(NMDA) activity promotes histone phosphorylation (γ-H2A.X) in rat cortical neurons. However, pretreatment with vitamin E and BAPTA-AM (calcium chelator) attenuated γ-H2A.X formation [99]. A study using the *Drosophila* model demonstrated neuronal necrosis is through phosphorylation of histone 3 serine 28 (H3S28Ph) [100]. A list of commonly undertaken histone modification and histone binding module has been enlisted in Fig. 3(A-C).

Non-coding RNA

Non-coding RNA (ncRNA) is the RNA that is not translated into proteins. Their association with a cerebrovascular accident has recently been under the spotlight because of their role as potential biomarkers and as well as target therapy. Functional ncRNA includes transfer RNA (tRNA), ribosomal RNA (rRNA), microRNA (miRNA), smallRNA, small-interfering RNA (siRNA), piwi-interacting RNA (piRNA), small nucleolar RNA (snoRNA), small nuclear RNA (snRNA), extracellular RNA (exRNA), small cajal body-specific RNA (scaRNA) and long-non coding RNA (lncRNA).

lncRNAs

lncRNA are defined as non-coding transcripts greater than 200 base-pairs in length transcribed by RNA Pol II from an independent promoter. lncRNAs such as MALAT1 (metastasis-associate lung adenocarcinoma transcript 1), ANRIL (antisense non-coding RNA in the INK4 locus), N1LR, MEG3 (maternally expressed gene 3), H19, C2dat1 (CaMK2D-associated transcript 1), FosDT (Fos downstream transcript), SNHG14 (small nucleolar RNA host gene 14) and TUG1 (taurine-upregulated gene 1) is up-regulated in cerebral ischemic animal models and/or oxygen-glucose deprivation cells. Gene ontology studies suggested MEG3, H19, and MALAT1 are also associated with angiogenesis [198-200], neurite growth [201], and neuroinflammation through gene regulation.

One of the well-explored lncRNA is MALAT1. MALAT1 has been documented to regulate apoptosis through various signaling pathways. MALAT1 has been successfully showcased as a competing endogenous RNA (ceRNA) for miR-205-3p, regulates apoptosis in PTEN dependent manner in the OGD model [202]. Similarly, MALAT1 has also been demonstrated to be ceRNA for miR-26b and up-regulates expression of ULK2 in OGD/R (oxygen-glucose deprivation/reoxygenation) *vitro* model [205]. MALAT1/MDM2/p53 signaling pathway was documented as a key regulator of apoptosis in the MCAO/R (middle cerebral artery occlusion/ reperfusion) murine model [203]. Down-regulation of MALAT1 in the OGD model (both *vivo* and *vitro*) attenuates neuronal cell death by suppression of Beclin1-dependent autophagy and regulating miR-30a expression [204]. So, it can be concluded that MALAT1 is a key regulator of apoptosis in oxygen-glucose deprivation state (mimicking ischemic stroke) and can be a potential therapeutic target to reduce ischemic insults in post-ischemic stroke patients.

Similar to MALAT1, ANRIL has also been shown to have a close relation with OGD-induced cellular injury and apoptosis. OGD-induced PC-12 cells were used to mimic the ischemic stroke model in *vitro*, demonstrated down-regulation of ANRIL negatively regulates miR-127 expression which in turn further negatively regulates Mcl-1. Over-expression of ANRIL produces a significant reduction in cellular injury, increases cell viability, and decreases apoptosis. Contrastingly, the up-regulation of miR-127 produces a significant increase in OGD-induced PC-12 injury [206]. So, lncRNAs and their association with stroke have showcased, there lies a definite correlation between lncRNAs and miRNAs, and regulation of one or the other produces a significant effect on cellular mechanism.

miRNAs

miRNAs have been most extensively studied over the last decade concerning post-stroke excitotoxicity, oxidative stress, neuroinflammation, and neuronal apoptosis. Neurotransmitters are endogenous chemical substances that are released by neuronal cells and act as a signaling molecule leading to convey of action potential to the adjacent neurons. The main known classes of neurotransmitters include amino acids, peptides, monoamines, gasotransmitters, trace amines, purines, catecholamines, and so on. Glutamate which belongs to the amino acid class of neurotransmitters is the most abundant excitatory neurotransmitter in the nervous system. Glutamate like any other neurotransmitter works via receptors present within the nervous system. Receptors of glutamate are mainly categorized into α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor (AMPA), N-methyl-D-aspartate

receptor (NMDA), and metabotropic glutamate receptors (mGluRs). Hypo or hyper-activity of these receptors is associated with excitotoxicity in post-stroke conditions. Over-expression of miR-107 has been identified to decrease the expression of glutamate transporter-1(GLT-1), thus promoting glutamate accumulation within the neurons and subsequently leading to excitotoxicity in post-ischemic stroke condition [103]. An increase in GLT-1 expression has been identified as a potential therapeutic target to minimize post-ischemic excitotoxicity [104,105]. Various pharmacological reagents have been studied to up-regulate GLT-1 expression of which histamine [112] and Magnesium Lithospermate B [106] are worth mentioning. However further studies and trails need to be conducted to determine their efficacy in clinical settings. Contrariety, over-expression of miR223 has been demonstrated to promote protection against excitotoxicity via the down-regulation of GluR2 and NMDA subunit NR2B [107]. miR-181a has been shown to possess a highly conserved binding site within GluA2 (GRIA2) mRNA, a subunit of AMPA-Rs. Thus, it was concluded that miR-181a is a key regulator of AMPA [108].

Neuroinflammation in post-ischemic stroke has been recognized to be largely modulated by activated microglia within the CNS [109,110]. The up-regulation of miR-155 within microglia was identified in response to pro-inflammatory cytokines and a decrease in a suppressor of cytokine signaling-1 (SOCS-1) expression simultaneously [111]. A study exhibited miR-155 knockdown promoted protection against I/R injury (ischemic/reperfusion) *via* inhibition of pro-inflammatory cytokines (IL-1β, IL-6, and TNF-α) as well as down-regulation of iNOS and COX-2 *via* MafB dependent manner [112]. Similarly, miR-181c down-regulation in BV-2 microglial cells was observed in contrast to the up-regulation of tumor necrosis factor-α (TNF-α) expression under oxygen-glucose deprivation (OGD) condition in *vitro* [113]. Another study illustrated miR-18 suppresses TLR4 (toll-like receptor-4) by directly binding to 3'-untranslated region of TLR4 and thus a potential therapeutic target pathway in ischemic stroke management [114]. Similarly, miR-181a has been demonstrated to promote anti-inflammatory actions *via* the down-regulation of IL1-α [115]. miR-145-5p is a key regulator of Nurr1 (Nuclear receptor related-1 protein). Over-expression of miR-145-5p during the ischemic condition in both *vivo* and *vitro* has been shown to attenuate Nurr1 expression and TNF-α up-regulation simultaneously. Thus, promoting further neuronal injury. However, the administration of anti-miR-145-5p promoted Nurr1 expression and a significant reduction in infarct volume in acute cerebral ischemia [116]. Over the last decennium role of some miRNA has been pinpointed to harmonize the quiescence state of microglia thus minimizing neuroinflammation in post-stroke condition. miR-124, miR-424, and miR-let-7c-5p has shown to be key regulators of the quiescence state of microglia and thus promote neuroprotection by inhibiting microglia activation in both *vivo* and *vitro* [117-119]. Similarly, miR-203 has been shown to negatively regulate activation of microglia *via* MyD88-NF-κβ (*myeloid differentiation primary response 88-NF-κβ*) pathway under ischemic condition [120]. miR-125b has been demonstrated to down-regulate ubiquitin-editing enzyme A20 and up-regulation of NF-κB concurrently in microglia in a P2X7 receptor-dependent manner [121]. Thus, making miR-125b as a key modulator of microglia activation.

To date, a total of 2300 human mature miRNAs have been identified [123] and further researches are been carried out as we speak for the discovery of another few thousand or even more miRNAs. Nuclear factor erythroid 2-related factor 2 (NRF2) is a leucine zipper protein (bZIP) that regulates the expression of antioxidant proteins in response to oxidative stress [124] has been shown to have an association with 85 miRNAs and 7469 transcription factors till date [125]. miR-424 has been showcased to decrease infarct volume, modulate apoptosis, up-regulate expression, and activation of manganese superoxide dismutase (MnSOD), superoxide dismutase (SOD), and NRF2 [126]. Similarly, miR-93 has been identified as pro-inflammatory miRNA. Treatment with miR-93 antagomir significantly reduces infarct volume, cortical neuronal apoptosis, and promotes better neurological scores in C57BL/6J mice [127]. Furthermore, treatment with miR-93 antagomir has been demonstrated to up-regulate NRF2 and heme oxygenase-1 (HO-1) and luciferase reporter assay foresee direct binding of miR-93 at 3'-UTR target sites of NRF2 gene in the same study. Prostaglandin-endoperoxide synthase-2 also known as cyclooxygenase-2 or COX-2 has been identified to produce reactive oxygen species (ROS) under chronic ischemic conditions [128]. miR-146a has been identified to reduce IL-6 and COX-2 expression upon activation by IL-1β [129].

Following ischemic insult to the CNS; apoptosis, necrosis, and necroptosis are observed from the onset of symptoms and may last for a significant amount of time determining the severity of the insult. Apoptosis which is referred to as the programmed cell death is both physiological and pathological. However, cerebrovascular accidents produce pathological apoptosis. Several miRNAs have been studied to inhibit this pathological apoptosis, thus promoting neuroprotection in post-ischemic stroke. miR-25 has been reported to express in brain ischemic tissues and a key

regulator of I/R induced neuronal apoptosis *via* Fas/FasL down-regulation [130]. miR-200c has also been identified to reduce CD95 mediated apoptosis via FAP-1 (anti-apoptotic gene) dependent manner [131]. miR-99a over-expression in neuro-2a cells promoted neuroprotection against hydrogen peroxide (H2O2) *via* suppression of lactate dehydrogenase (LDH) release and inhibition of H2O2 induced G1/S phase transition in neuro-2a cells along with a significant reduction in cyclin D1 protein levels and down-regulation of CDK6 expression [132]. Thus, the miR-99a overexpression target strategy in post-ischemic conditions might be a potential therapeutic intervention to minimize neuronal apoptosis by re-entry to the cell cycle. miR-106b-5p was also identified as a key modulator of apoptosis in I/R injury. Briefly, miR-106b-5p antagomir significantly reduces malondialdehyde (MDA) levels along with the restoration of SOD, increase expression myeloid cell leukemia-1 (Mcl-1) and B cell lymphoma-2 (Bcl-2), and significant reduction of Bax in male Sprague Dawley rats with middle cerebral artery occlusion (MCAO). In *vitro*, miR-106b-5p antagomir up-regulate Mcl-1 and Bcl-2 levels along with down-regulation LDH and promoting SOD activity in PC12 cells. Thus, it can be concluded that miR-106b-5p antagomir promotes neuronal protection against apoptosis by up-regulating SOD activity both in *vivo* and *vitro* [133]. Another significant miRNA, miR-216a up-regulation has been shown to promote neuroprotection against apoptosis and inflammation *via* negative regulation of janus tyrosine kinase-2 (JAK2)/signal transducer and activator of transcription-3 (STAT3) signaling pathway [134]. A handful of miRNA and their associated genes and pathophysiology have been summarized in Fig. 4(A). Furthermore, miRNAs have also been studied to promote neurogenesis [135-143] and angiogenesis [144-156] in both *vivo* and *vitro*. A compact list of miRNAs involved in neurogenesis and angiogenesis has been listed in Table 1.

circRNAs

A subtype of miRNAs has recently been identified as a key biomarking modality, which is circulating miRNAs (circRNAs). circRNAs has been identified as an acute and chronic phase biomarker in various forms of *cancers* [157-160]. In cerebrovascular accidents, standardized assessment of the incoming patients involves imaging evaluation which includes Non-Contrast CT (NCNT) or magnetic resonance imaging (MRI) to exclude intracerebral hemorrhage (ICH) before IV-tPA. CTA (CT angiography) with CTP (CT perfusion) or MRA (MR angiography) with diffusion-weighted magnetic resonance imaging (DW-MRI) with or without MR perfusion is also recommended [161]. However, cost-effectiveness for the above-mentioned imaging modality still plays a crucial drawback in the effective management of incoming patients. circRNAs are shown to be somewhat low cost-effective and less time-consuming in trials. Early detection circRNAs associated with cerebrovascular insults can be a game-changer in the effective and precise management of the disease. A study conducted on 48 patients (24 acute-ischemic strokes versus 24 vascular risk factor control) demonstrated that level of miR-122, miR-148a, Let-7i, miR-19a, miR-320d, miR-4429 were significantly reduced as compared to elevated levels of miR-363, miR-487b in the blood plasma of acute-ischemic stroke patients [162]. miR-124-3p, miR-125b-5p, and miR-192-5p were also demonstrated to be potential biomarkers in determining prognostic outcomes following IV-tPA [163]. miR-422a and miR-125b-2-3p were showcased as potential biomarkers for ischemic stroke [164]. A list of circRNAs illustrating association with cerebrovascular accidents and their respective changes in post-ischemic stroke plasma levels are enlisted in Fig. 4(B) [162-173].

RNA modification

Similar to DNA modifications, RNA modifications have also been shown to be a regulator of gene expression [174-182]. To date, RNA modifications include m^6A (N^6-methyladenosine), m^6Am (N6,2'-O-dimethyladenosine), m^1A (N1-methyladenosine), m^5C(5-methylcytosine), hm^5C (5-hydroxymethylcytosine), ac^4C (N4-acetylcytidine), ψ (rotation isomerization of uridine/ pseudouridine) and m^7G (7-Methylguanosine) (Fig. 5). m^6A (N^6-methyladenosine) is one of the most commonly observed mRNA modifications [183] and was identified in the 1970s [184-186], however their association with small nuclear RNA (snRNA), mRNA, and long non-coding RNA have recently understood [187]. Mapping of m^6A over human and murine RNA has identified over 18,000 m^6A sites in 7,000 human genes with a consensus sequence of [G/A/U][G>A] m^6A[U>A/C] [195-197]. m^6A has also been shown to be highly down-regulated during embryonic brain development in murine species and up-regulated in adulthood [195]. Furthermore, the silencing of m^6A methyltransferase affects gene expression and modulate p53(TRP53) signaling pathway and apoptosis [196]. Likewise, m^6Am, m^1A, m^5C, hm^5C, $ac^{4,}$ C, ψ, and m^7G are somewhat understood in the context of cancer and as potential biomarkers. For example, m^1A was identified as a

modulator of PI3K/AKT/mTOR and the ErbB pathway in gastrointestinal cancer [178] and m^6A were showcased to regulate the brain functions, development of synaptic plasticity, and their association in neuropsychiatric disorders [188]. However, RNA modification and their association with cerebrovascular accidents are yet to be determined.

Discussion

Advancements in stroke research have significantly decreased the death rate due to interventions in the hyperacute stage of the disease. However, long-term disability and institutionalization of the post-stroke remain unchanged. Stroke being a complex, multi-factorial disease in which a wide plethora of pathological processes are simultaneously set in motion, and modulation of a single molecular factor is unlikely to be sufficient to attenuate or reverse the progression of stroke pathology. However, epigenetic alterations such as DNA methylation, Histone modifications, miRNAs, and RNA modifications are potent modulators of gene regulation, and an accumulating body of evidence suggests that they play a pivotal role in regulating brain remodeling after stroke. As a result, efforts are being made to identify key molecular signatures and development of combination therapy strategy similar to cancer [189].

DNA methylation has been one of the heavily researched topics over the last decade and their association regarding risk factor prediction has been well documented. For example, DNA methylation of CDKN2B has been showcased to promote increase risk of arterial calcification in ischemic stroke patients [190,191]. Similarly, histone modifications have been illustrated to be a regulator of gene expression [80,81,97,99]. However, miRNAs have been demonstrated to be both key modulators of gene expression and potential biomarkers. For this reason, miRNAs have acquired a significant interest in target therapies as pharmaceutical intervention because a single miRNA can influence networks of neuronal and/ or nonneuronal genes [192]. Another fascinating aspect of miRNAs is their ability as peripheral biomarkers. miRNAs as biomarkers have been identified in several forms of cancers and a study has showcased miRNAs abilities to determine the clinical outcome in breast cancer patients [193]. Studies have illustrated an increase in associate miRNA level increase in blood plasma within a few hours of myocardial infarction [194]. miRNAs as potential biomarkers in stroke have already been enlisted in our review and several other potential miRNAs are currently undergoing evaluations for clinical practice. Similarly, studies have concluded that their lies a definite co-relationship between lncRNAs and the miRNAs. lncRNAs similar to protein-coding genes, their genomic locations are heavily marked by enrichment of H3K4 trimethylation at the transcriptional site and H3K36 trimethylation throughout the gene body. Studies in the field of cancer research have demonstrated that lncRNAs can be a double-edged sword i.e. they can act as a tumor suppressor as well as oncogenic functions [207]. With the advancement in epigenetic research, lncRNAs have shown to be inhibited by a newly designed approach known as Antisense Oligonucleotide (ASO) technology, which causes degradation of lncRNAs in RNase H-dependent manner [208]. For example, ASO-dependent depletion of lncRNA MALAT1, have been shown to impact growth and metastasis of lung and breast cancer cells in murine species [209,210]. As mentioned earlier, MALAT1 has also been highly associated with stroke. A similar approach to depletion of MALAT1 to regulate post-stroke apoptosis can be a key therapeutic approach.

Studies undertaken to date have successfully demonstrated that stroke leads to epigenetic dysregulation which in turn triggers a series of cascade changes that cause neuroinflammation, oxidative stress, apoptosis, and so on. Thus, regulation of these key triggers such as cytokines, genes, miRNAs will be beneficial to produce the desired outcome in post-stroke conditions.

Concluding Remarks

Advancement in technology has led to a new era of ‘epigenetics’ and knowledge we gather on stoke over the last decade has led to the revolution of target and combination therapies. Further understanding of the key modulators at the molecular level and their alteration in post-stroke conditions would be the answer to the long-standing problem of GBD (Global Burden of Disease) for stroke.

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Author contributions

Dipritu Ghosh conceived the entire review project, conceptualization, literature search, prepared figures. Dipritu Ghosh and Jianhua Peng wrote the manuscript. Shigang Yin, and Yong Jiang overviewed and provided with significant guidance during the conception of the entire project. All the authors have their contribution to the critical revision of the final manuscript and doesn't have conflict of interest.

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No potential conflict of interest was reported by the authors.

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Figure legends

Fig.1 Pathophysiology and Mechanism involved in Ischemic and Hemorrhagic stroke. Color blue (top and middle right) represents classification; color green represents pathophysiology involved and color red represents mechanism involved in each condition. Briefly, mechanisms involved in both ischemic and hemorrhagic stroke involves decrease in cerebral blood flow (CBF) leading to oxygen (O2) deprivation causing increase in anaerobic metabolism and eventually lactic acidosis which sequentially causes astrocyte demise and increase in neuroinflammatory cytokines thus promoting neuroinflammation. Subsequently, decrease in CBF can also cause malfunction of ionic pump causing potassium ions (K+) efflux and sodium and calcium (Na+ and Ca2+ respectively) influx into the neuronal cells causing excitotoxicity, oxidative stress and eventually necrosis.

Fig. 2 Illustrate common epigenetic modifications in stroke which include DNA-methylation, Histone modification, micro-RNA(miRNA) and RNA modifications. DNA methylation occurring exclusively at the CpG island is associated with gene silencing and are irreversible modifications. Known histone modification occurring at the amino terminal tails are short term reversible modifications. A microRNA (miRNA) is a small non-coding RNA molecule (containing about 22 nucleotides) found in plants, animals and some viruses, that functions in RNA silencing and post-transcriptional regulation of gene expression. RNA modifications are the chemical alteration of the RNA molecules post transcription that alters the expression of RNA.

Fig. 3 Histone Modifications; Fig. 3(A) Illustrate pictorial representation of all known till date post-translational modification of histone amino terminal tail and their location regions. ; Fig. 3(B) provides a list of frequently used histone modified regions, functions and location in DNA sequence which includes H3K4me1, H3K4me3, H3K9me3, H3K27me3, H3K36me3, H3K79me2, H3K9Ac, H3K27Ac, H4K16Ac, H3S10P and Gamma H2A.X. ; Fig. 3(C) portraits frequency used histone marks and their histone binding modules such as BRCT, bromodomain, chromodomain, MBT, PHD, Tudor, WD40 repeats and 14-3-3.

Fig. 4 Illustrate the role of microRNAs in post ischemic stroke condition. Figure 4(A) depicts microRNA(miR) and their associative genes in neuroinflammation, apoptosis and oxidative stress in post-ischemic stroke. Rectangular brown color boxes represents miR in association with their respective genes (purple rectangular boxes) which further has been indicated to their respective pathologies (red, blue and green). Figure 4(B) Illustrate a list of circulating miR and associative levels in the circulation post-ischemic stroke. Post ischemic stroke the levels of miR-363; miR-487b; miR-124; miR-125b-2; miR-27a, miR-422a; miR-488; miR-627; miR-290; hsa-miR-106b-5P; hsa-miR-4306; miR-10a; miR-182; miR-200b; miR-298 significantly increases whereas miR-210; miR-122; miR-148a; Let-7i; miR-19a; miR-320d; miR-4429; miR-30a; miR-126; hsa-miR-320e; hsa-miR-320d; miR-124; miR-9; miR-219 levels decrease in the blood plasma. The changes in various miR levels in the blood plasma has been strongly indicative parameter in distinguishing acute versus chronic stroke presentation and thus further investigation is necessary in determining their use in the clinical settings.

Fig. 5 Illustrate DNA to RNA transcription; followed by possible mRNA (messenger RNA) modifications at different nitrogen bases. N6-methyladenosine: m^6A; Pseudouridine: Ψ; 5-methylcytosine: m^5C; 5-hydroxymethylcytidine: hm^5C; N1- methyladenosine: m1A; 7-Methylguanosine: m^7G; N6,2'-O-dimethyladenosine: m^6Am. (Abbreviations: 3'UTR: Three prime untranslated region; CDS: CoDing Sequence; 5'UTR: Five prime untranslated region).