Review Article

Life Conflux

Research Progress of Epigenetic Regulation in Stroke Treatment

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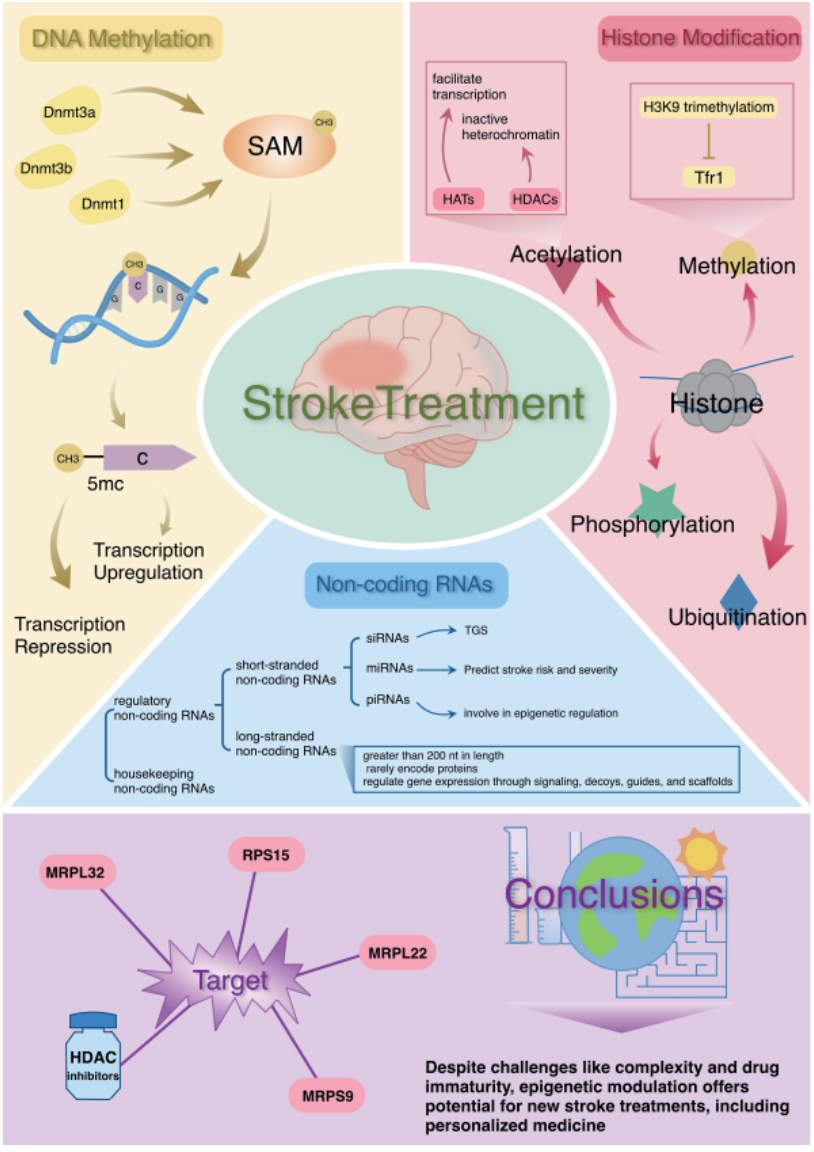
Graphical Abstract



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Research Progress of Epigenetic Regulation in Stroke Treatment

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Abstract

Cerebrovascular diseases are a serious threat to human health, among which stroke is extremely harmful. In recent years, more and more studies have shown that epigenetic regulation is crucial in the development of cerebrovascular diseases. In this review, we focus on the research progress of epigenetic regulation in stroke treatment, detailing the regulatory mechanisms of DNA methylation, histone modification and non-coding RNAs, and analyzing their roles in the pathophysiology of stroke. It was found that ischemic stroke causes dynamic changes in DNA methylation, which affects gene expression and alters the process of injury and recovery; histone modification levels are also altered after stroke, which affects chromatin status and gene transcription; and noncoding RNAs, such as miRNAs, siRNAs, piRNAs, and lncRNAs, which play a key role in gene expression regulation, are associated with the risk of stroke, severity and symptom onset. In addition, therapeutic strategies targeting epigenetic regulation are also discussed. Although facing challenges such as complex mechanisms, susceptibility to environmental influences, and the early stage of drug development, epigenetic regulation is still very promising in the treatment of stroke, and it is expected to provide a new theoretical basis and research direction for the prevention and treatment of stroke in the future.

Keywords: Epigenetic regulation; Stroke; DNA methylation; Histone modification; Non-coding RNA; Therapeutic targets

Introduction

In 2021, stroke maintained its status as the second most prevalent cause of death globally, surpassed only by ischaemic heart dise ase, and occupied the third spot in terms of Disability-Adjusted Life Years lost due to noncommunicable diseases , trailing behind ischaemic heart disease and neonatal disorders[11].

Cerebrovascular diseases are lesions of blood vessels in the brain, including cerebral infarction, cerebral hemorrhage, and subarachnoid hemorrhage, which will lead to stroke[72]. In recent years, epigenetic regulation has played an important role in stroke pathogenesis and stroke repair as a higher-order regulatory mechanism for tissue repair. Epigenetic regulation refers to mechanisms that regulate gene expression through non-DNA sequence changes (DNA methylation, histone modifications, and non-coding RNAs)[44] .These changes can affect multiple aspects of gene transcription, splicing, RNA stability and translation without altering the DNA sequence[29]. Epigenetic regulation is a key post-transcriptional regulation of gene expression and plays an important regulatory role in organogenesis, homeostasis and pathology[67].

In cerebrovascular diseases, alterations in epigenetic regulation may lead to neuronal damage and abnormal responses of vascular endothelial cells. Therefore, investigating the role of epigenetic regulation in cerebrovascular diseases can help us better understand the pathogenesis of these diseases and find new therapeutic strategies.

Epigenetic regulation

DNA methylation

DNA methylation is the process by which methyl groups are added to the DNA molecule. This modification can occur at different locations in the genome and plays an important role in gene regulation. Several enzymes are involved in DNA methylation, including DNA methyltransferases (dnmt) such as Dnmt3a, Dnmt3b, and Dnmt1. DNMTs catalyze the transfer of methyl groups from S-adenosylmethionine (SAM) to cytosine residues in DNA to form 5-methylcytosine (5mC). However, post-mitotic neurons in the mature mammalian brain still express a large number of Dnmts, raising the possibility

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that Dnmts and DNA methylation may play new roles in the brain[18, 24].

DNA methylation can influence the pathogenesis of stroke by modulating genetic or environmental risk factors. DNA methylation regulation is shaped by two opposing processes, the addition and removal of the fifth methyl group of cytosine in DNA[31, 33, 36, 37]. It causes transcriptional repression or transcriptional upregulation by preventing RNA polymerase from recognizing specific DNA regions or by eliminating "DNA methylation breaks", respectively. The effect of DNA methylation on the expression of transcripts depends on their location in the gene region (promoter and or gene body), and transcriptional changes are usually dependent on methylation at multiple locations in a given gene[36, 37].DNA methylation can be a biomarker for stroke[40].

Zhan, L. et al. demonstrated that the Piwil2/piRNAs pathway plays a role in the epigenetic regulation of CREB2 expression and neuroprotection of tGCI by HPC in rats. Specifically, HPC-induced downregulation of Piwil2 in rat CA1 inhibited CREB2 hypermethylation and promoted CREB2 transcription by retraining DNMT3A to induce promoter site CpG methylation, leading to an increase in CREB2 after tGCI[75].

Histone modifications

Histone modifications include several types, each with a specific function. Histone methylation can occur on the Lys and Arg residues of histones and has the effect of reducing the positive charge carried by histones and directly or indirectly affecting the transcriptional activity of genes. Histone methylation is usually associated with gene activation or gene silencing[5]. Histone acetylation occurs predominantly and diversely on core histones. The degree of acetylation is higher in the structural domains of DNA containing active genes. Acetylation/deacetylation modifications can regulate the level of gene transcription, participate in DNA repair, splicing and replication, chromosome assembly, and cellular signaling[61]. Histone phosphorylation is involved in gene transcription, DNA repair, apoptosis and chromosome condensation[27]. Histone ubiquitination is involved in X chromosome inactivation and affects histone methylation and gene transcription[70].

Histone acetyltransferases (HATs) acetylate lysine residues in histone tails. This promotes DNA unfolding and chromatin deconcentration, which facilitates transcription and protein synthesis. Histone deacetylases remove acetyl groups from histones. This leads to the formation of transcriptionally inactive heterochromatin in which gene expression is blocked. To maintain the transcriptionally active state of chromatin, HATs and hdac need to work in concert[30, 58, 69].

H3K9 trimethylation is a specific methylation modification of the 9th amino acid lysine of histone 3 that inhibits the key transferrin receptor (Tfr1), which determines whether or not a neuron will experience iron death. By decreasing H3K9 trimethylation protects neurons from iron death, whereas increasing it leads to neuronal iron death[42].

non-coding RNA

Non-coding RNAs that are not translated into proteins can be categorized into housekeeping non-coding RNAs and regulatory non-coding RNAs. RNAs with regulatory roles are divided into two main categories according to their size: short-stranded non-coding RNAs (including siRNAs, miRNAs and piRNAs) and long-stranded non-coding RNAs (LncRNAs)[63, 74].In recent years, a large number of studies have shown that non-coding RNAs play an important role in epigenetic modifications, which can regulate expression at the gene level and chromosome level and control cell differentiation[2, 12, 23, 73].

siRNA is derived from long double-stranded RNA molecules (including RNA produced by viral replication, transposon activity, or gene transcription) that can be cleaved by Dicer's enzyme into 19-24 nt (nt: nucleotide) RNA fragments, which are then loaded onto the Argonaute (AGO) protein to perform their function[34, 52].

miRNAs are single-stranded RNAs of approximately 19-24 nt, 50% of which are located in chromosomal regions prone to structural changes[57].Initially, it was thought that there were two main differences between siRNAs and miRNAs as a class of regulatory RNAs. One is that miRNAs are endogenous, being the products of biological gene expression, whereas siRNAs are exogenous, originating from viral infections, gene transfer points, or gene targets. The other difference is that miRNAs consist of incomplete hairpin-shaped double-stranded RNAs processed by Drosha and Dicer, whereas siRNAs are products of fully complementary long double-stranded RNAs processed by Dicer[9].Despite these differences, it has been hypothesized that miRNAs and siRNAs have similar mechanisms of action in mediating transcriptional gene silencing because miRNAs and siRNAs are closely related, e.g., the two fragments are similar in size. Recently, nearly 1,800 putative miRNAs have been identified in the human genome, and the number of miRNAs continues to increase rapidly due to the development of high-throughput sequencing technologies[26, 41, 47].

piRNAs are a class of RNA molecules approximately 26-31 nt in length. Its name piRNA (Piwi-interacting RNA) reflects the fact that piRNA binds to Piwi proteins under physiological conditions.The role of Piwi proteins as epigenetic regulators is reflected by their silencing of the homeobox gene by binding to PcGs (polycistronic ribosomal histones) to genomic PcG response elements. Thus, it has been hypothesized that piRNAs associated with Piwi proteins should also play an important role in epigenetic regulation[45].

LncRNAs are another class of non-coding regulatory RNAs. lncRNAs are generally greater than 200 nt in length, are located in the nucleus or cytoplasm, and rarely encode proteins.[8, 51].

LncRNAs are another class of non-coding regulatory RNAs. lncRNAs are generally greater than 200 nt in length, are located in the nucleus or cytoplasm, and rarely encode proteins[55]. However, these five major categories mainly involve only four typical mechanisms by which lncRNAs regulate gene expression: signaling, decoys, guides, and scaffolds[65].

Non-coding rna (ncRNAs) are key regulators of gene expression, and the most studied ncRNAs are long-stranded non-coding rna (lncRNAs), micro rna (miRNAs), and circular rna (circRNAs)[66].For ischemic stroke, the search for biomarkers is crucial for early diagnosis and guiding treatment. Changes in ncRNA expression profiles during ischemic stroke have gradually attracted attention[10, 22]. The relative stability, specificity, and reproducibility of ncRNAs make them promising biomarkers for early disease recognition[46].

Table 1. Comparison of Epigenetic Regulation Types and Mechanisms

Regulation Type	Specific Modification	Mechanism	Impact on Gene Expression
DNA Methylation	Methyl group addition	By DNMTs	Promoter: often represses; Gene body: varies
Histone Modification	Methylation	Alters chromatin	Activation or silencing
	Acetylation	HATs add, HDACs remove	HATs promote, HDACs inhibit
Non - coding RNA	siRNA	With AGO, TGS	Silences genes
	miRNA	Bind mRNA	Negatively regulates
	lncRNA	4 mechanisms	Multi - level regulation

Epigenetic regulation and stroke

Association of Epigenetic Modulation with Stroke

Ischemic Stroke Leads to Dynamic Changes in DNA Methylation and Regulates Widespread Differential Gene Expression, Altering Injury and Recovery Processes[3, 13, 60].

Whole blood whole genome methylation levels were higher in adults with ischemic smog disease than in healthy individuals. A total of 759 methylation probes were significantly different between cases and controls. The hypermethylated regions were mainly concentrated in gene spacer regions. Among the genes with the highest degree of differential expression, KCNMA1 and GALNT2 were upregulated and SOX6 and RBM33 were downregulated[28].

Hypomethylation of TNF Receptor-Related Factor 3 (TRAF3), Hypermethylation of thrombospondin-1 (THBS1), and Increased DNMT3A Activity Also Predict Stroke Outcome and the Extent of Ischemic Injury[13, 20].To date, it has been reported that within the BBB, hypermethylation of the TIMP2 promoter or hypomethylation of the VCAM-1 promoter controls BBB permeability and leukocyte recruitment[54, 60].

Ischemic stroke typically reduces overall gene expression levels by inhibiting acetylation of histones H3 and H4[32, 43]. Ischemic stroke overall reduced histone acetylation levels in the ischemic core, penumbral regions, and hemispheres of the affected brain. Acetylation of histone H3 lysine 9 (H3K9Ac) in the ischemic penumbral region of rat cerebral cortex decreased 2-fold at 1 hour and more than 4-fold at 4 and 24 hours after photothrombotic stroke[16]. In addition, Demyanenko's study showed that acetylation levels of histone H4 in the ipsilateral cortical penumbra were either increased or unaffected 7 days after ischemic stroke. Interestingly, the increased acetylation levels returned to control levels 14 days after ischemic stroke[14, 15]. Histone H3 and H4 acetylation levels were reduced in oligodendrocytes of cerebral white matter 7 days after ischemic stroke. The acetylation level of histone H4 in the ipsilateral cortex was not altered at 7 or 14 days after ischemic stroke[68].

It has been shown that ICH significantly reduced the acetylation level of histone H4 in the ipsilateral cortex, and that sodium butyrate (NaB), an HDAC inhibitor, increased the acetylation level and eliminated the decrease in acetylation level caused by ICH. In addition, HDAC inhibitors slightly ameliorated dyskinesia after ICH, and exercise increased the acetylation levels of histones (H3 and H4) in bilateral cortex; interestingly, no synergistic effect of exercise and NaB administration on histone acetylation was observed[48].

RNA modifications are an important part of epigenetic modifications. Currently, more than 160 chemical modifications have been identified in RNA, which are involved in regulating the structural properties of RNA or altering the affinity of mRNA for ribosomes[76].miRNAs can predict the risk and severity of stroke and the onset of cerebral infarction symptoms, and up- or down-regulation of multiple miRNAs, rather than a single miRNA, occurs during stroke[1, 62].

Clinical studies and experimental results

Recent studies have shown that siRNAs can lead to cellular transcriptional gene silencing (TGS) through DNA methylation and histone modification[7, 39, 53]. Zhou et al. demonstrated

Table 2. Epigenetic Changes Related to Stroke and Their Associations

Stroke Type	Epigenetic Changes	Key Genes/Molecules	Main Impact
Ischemic Stroke	DNA meth. change; histone acetylation↓; ncRNA expression change	KCNMA1, SOX6; H3K9Ac; miRNAs	Affects gene expression, recovery, BBB
ICH	Histone H4 acetylation↓ in ipsilateral cortex	Histone H4	Affects neuronal survival

that siRNA silencing of EZH2 reversed cisplatin resistance in human non-small cell lung cancer and gastric cancer cells[77]. EZH2 acts as a histone methyltransferase that causes methylation of H3K27, which then serves as an anchor for CpG methylation, leading to the formation of silent chromatin and ultimately TGS[64].Chromatin immunoprecipitation experiments showed that the binding of EZH2-inhibited DNMTs to specific genes depends on the presence of EZH2. Bisulfite sequencing results also demonstrated that EZH2 is required for methylation of target promoters repressed by EZH2, suggesting that EZH2 is involved in DNA methylation by recruiting DNA methyltransferases[71].

Dysregulation of histone methylation has been linked to a variety of diseases, including cancer, neurodegenerative diseases and genetic disorders. Histone methylation marks are also thought to be inherited through mitosis and meiosis, which provides a potential mechanism for transgenerational transmission of epigenetic information[6].

Many histone methyltransferases and demethyltransferases have been identified that catalyze the addition and removal of methyl groups from histone tails. These enzymes are key regulators of histone methylation dynamics, which in turn control the transcription of specific genes and gene networks[17].Histone methyltransferases commonly act as oncogenes in cancer, promoting cell proliferation and survival[50],and Histone Demethylases Are Associated with Neurodegenerative Diseases and Age-Related Cognitive Decline[49].

The exploration of epigenetic mechanisms in Parkinson's disease (PD) has witnessed significant advancements, with a particular focus on the role of DNA methylation in the context of PD. A study on a Sudanese cohort has provided valuable insights into this complex relationship.In this clinical investigation, researchers gathered a cohort of 172 Sudanese individuals, with 90 diagnosed with PD and 82 serving as healthy controls. An interesting aspect was the relatively young age of onset in the PD patients, averaging 40.6 ± 22.4 years, and a notable 64 patients had a family history of the disease.The study's findings regarding the methylation of the SNCA gene were quite revealing. Among the analyzed CpG sites, CpGs 16 - 23 exhibited significant hypomethylation in PD patients when compared to the control group. The P - values, ranging from 0.023 to 0.003, indicated a strong association. When sporadic cases were excluded from the analysis, the significance of these results was further enhanced. This suggests that the hypomethylation of these specific CpG sites might be a crucial epigenetic factor contributing to the development of PD, especially in familial cases[4].

Aberrant histone methylation has been reported in a variety of cancers, with global loss and gain of specific histone methylation marks observed. These changes are thought to promote dysregulation of gene expression programs for cancer development by driving cellular transformation and metastasis. Histone methylation is also involved in DNA repair, where histone H3K36 methylation is a key signal for mismatch repair. Defects associated with histone methylation and DNA repair have been linked to hereditary diseases such as hereditary nonpolyposis colorectal cancer and xeroderma pigmentosum[35, 56].

Epigenetic regulation as a therapeutic target

Histone methylation is a dynamic epigenetic mark in health, disease, and heredity, and understanding its regulation and function is critical to understanding normal biology and disease etiology[25]. A number of small molecule inhibitors of histone methyltransferases and demethyltransferases have been developed, and these inhibitors are currently being evaluated in preclinical trials for the treatment of cancer and other diseases[50]. Histone acetylation is also a target for epigenetic therapies, and drugs such as histone deacetylase inhibitors show promise in the treatment of neurodegenerative diseases and other disorders caused by aberrant histone acetylation[21].

Fujii et al. showed that daily consumption of large amounts of vegetables reduced ABCA1 gene methylation and lowered cholesterol and atherosclerosis. But interestingly, only women validated this study[19].

Exercise and HDAC inhibitors can alter epigenetic modifications in the motor cortex. These changes include increased DNA methylation, decreased histone deacetylase activity, and increased histone acetylation levels. These modifications can affect gene expression and promote neuronal plasticity and anti-inflammatory responses[48].It has been shown that in an ischemic stroke model, administration of a histone deacetylase inhibitor (HDACi) improves motor and cognitive function after stroke, with concomitant neuroplasticity, neurogenesis, and attenuated inflammation[32, 38, 60].

A researcher conducted a PPI network analysis of differential miRNAs, differential genes, and differentially methylated sites, and the results of the study showed that MRPS9, MRPL22, MRPL32, and RPS15 were identified as potential diagnostic and therapeutic targets for ischemic stroke progression. This implies that these genes may be involved in the process of ischemic stroke onset and progression, and therefore could be potential therapeutic targets[78].

Table 3. Epigenetic Regulation as Therapeutic Targets for Stroke and Related Drug Development

Regulation Type	Therapeutic Target	Interventions	Research Stage
DNA Methylation	Gene methylation sites	Diet - based	Clinical obs.
Histone Modification	Methyl/demethyl - transferases; HDACs	Small - molecule inhibitors; HDAC inhibitors	Pre - clinical; some clinical (HDACi)
Non - coding RNA	Differential miRNA/lncRNA pathways	Antisense oligonucleotides, mimics	Pre - clinical

Hypomethylation and altered expression of the miR-223 gene may lead to its inactivation or reduced degradation at the post-transcriptional level, which increases its inhibitory effect on target genes, and consequently contributes to the development and progression of stroke risk factors such as atherosclerosis[59].

Conclusions

Epigenetic modulation as a therapeutic strategy for stroke also faces several challenges. First, epigenetic mechanisms are complex and involve the interaction of multiple factors. This makes therapeutic strategies targeting specific epigenetic modifications difficult. Second, epigenetic modifications may be influenced by a variety of factors such as environment and lifestyle, which increases the difficulty of implementing effective interventions in stroke patients. In addition, most of the current epigenetic drugs are in their early stages and require further research and clinical trials to validate their safety and efficacy.

Despite the challenges, epigenetic modulation holds great promise in stroke treatment. First, by studying epigenetic mechanisms, we can gain a deeper understanding of stroke onset and progression. This can help discover new therapeutic targets and predictive biomarkers. Second, epigenetic drugs have the potential to improve stroke symptoms and prognosis by regulating specific gene expression. For example, neuronal damage after stroke can be reduced by upregulating the expression of genes with protective effects. Finally, epigenetic therapies can target individual differences for precision medicine and provide more personalized treatment plans for stroke patients.

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Author Contributions

All authors were involved in the conceptualization and design of the study. Data collection, first draft of the manuscript, and translation were done by Tiancai Yang. All authors commented on a previous version of the manuscript. All authors read and approved the final manuscript.

Consent for Publication

Not applicable.

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Competing Interests

The authors declare that they have no existing or potential commercial or financial relationships that could create a conflict of interest at the time of conducting this study.

Data Availability

The data that support the findings of this study are available on request from the corresponding author.

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