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Epigenetic mechanisms of neuroplascitic and the implications for stroke recovery

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Abstract

Ischemic stroke is a devastating brain injury and an important cause of neurologic disability worldwide and across the lifespan. Despite the physical, social, and economic burdens of this disease there is only a single approved medicine for the treatment of acute stroke, and its use is unfortunately limited to the small fraction of patients presenting within the narrow therapeutic window. Following stroke, there is a period of plasticity involving cell genesis, axon growth, and synaptic modulation that is essential to spontaneous recovery. Treatments focusing on neuroprotection and enhancing recovery have been the focus of intense preclinical studies, but translation of these treatments into clinical use has been disappointing thus far. The important role of epigenetic mechanisms in disease states is becoming increasingly apparent, including in ischemic stroke. These regulators of gene expression are poised to be critical mediators of recovery following stroke. In this review we discuss evidence for the role of epigenetics in neuroplasticity and the implications for stroke recovery.

Keywords

DNA methylation; Histone; MiRNA; Ischemia; Neurogenesis; Axon growth; Synaptogenesis

Introduction

Stroke is one of the leading causes of neurologic morbidity and mortality worldwide. United States data estimate the annual incidence of stroke in adults at nearly 800,000, with a corresponding economic burden surpassing $35 billion (Go et al., 2014). While more common in the elderly, stroke afflicts people across the entire age span including infants and children and thus represents an important cause of neurologic disability in the pediatric

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population as well. The past two decades have witnessed the advent of dedicated stroke centers, along with the widespread use of thrombolytic treatment. These advances have substantially improved our management of acute stroke, but little success has been realized in developing therapies that provide true neuroprotection and enhanced recovery. Recognizing the burden of stroke-related disability in our population and limitations of our ability to provide hyperacute therapies such as t-PA due to narrow therapeutic time windows, the development of neurorestorative therapies without such restrictive uses is imperative.

Epigenetics refers to changes in gene expression that are not based on mutation of the underlying DNA sequence (Ma et al., 2010). Epigenetic changes are generally considered to be long lasting and heritable through successive cell generations, but recent evidence also suggests the potential for previously unappreciated dynamic changes under certain conditions (Felling et al., 2012). Although the field of epigenetics is now well established, interest in the epigenetic mechanisms involved in stroke pathophysiology has only recently gained traction. Our understanding of the epigenetics of neural plasticity has been substantially informed by the study of learning and memory (Levenson and Sweatt, 2005). Using this knowledge as a basis to better understand the structural and functional changes that occur following stroke could provide innovative approaches to stroke recovery and rehabilitation because motor learning is a critical component of this process (Krakauer, 2006). The primary epigenetic mechanisms often considered involve DNA methylation, histone modifications including methylation and acetylation, and posttranscriptional mechanisms of regulation through small, noncoding RNAs. Recent reviews support the emerging interest in the relevance of this field to stroke pathophysiology, but these largely focus on the injury process (Qureshi and Mehler, 2010a,b, 2011). In this review we discuss the available evidence supporting epigenetic mechanisms of neuroplasticity, with emphasis on implications for stroke recovery. This is an emerging domain with the potential to offer important insight into the biology of regeneration and recovery after stroke.

A critical period of injury-induced plasticity

Stroke recovery is an incredibly complex process and therefore any discussion of underlying mechanisms requires a good framework. Most clinical measures of recovery focus on the ability to accomplish various tasks essential to everyday life. In this sense, recovery can be achieved a number of ways, the most efficient of which is arguably compensatory adaptation, or learning to accomplish the task in a different way. For instance, if I have suffered a left middle cerebral artery stroke and can no longer reach for an object with my right hand, the easiest way to obtain the desired object is to reach instead with my left hand. Much of today's clinical focus concentrates on such means of compensatory adaptation. This does not reflect any degree of real neurologic recovery, and the holy grail of brain recovery research is the true restoration of function to the injured brain. Stroke patients do exhibit a spectrum of true recovery, but this is frequently far too limited. Understanding the mechanisms underlying this spontaneous recovery is an essential prerequisite to augmenting it.

There is tremendous evidence that the majority of spontaneous recovery occurs within a defined period of time after stroke. A large study of the natural history of stroke demonstrated that patients reached their maximal improvement by 3 months regardless of the initial severity of their symptoms (Jorgensen et al., 1999). Additionally, animal models indicate that early initiation of rehabilitative therapies within the first days after stroke leads to better functional outcomes (Krakauer et al., 2012). Despite significant challenges in studying similar effects in human stroke patients, clinical studies have also demonstrated trends toward beneficial effects of early rehabilitation (Cifu and Stewart, 1999). Some have drawn comparisons between this early recovery phase after stroke and the critical periods of plasticity that occur during development (Nahmani and Turrigiano, 2014). This leads to 2 important concepts: 1. Interventions designed to truly target reduced neurologic impairment after stroke need to be implemented within this critical period; and 2. Understanding the molecular and cellular characteristics that define critical period may allow a similar window of opportunity to be recreated long after a stroke occurs.

What characteristics of the early post-stroke time period are so critical to the recovery process? The immediate post-stroke epoch can be conceptualized as a period of enhanced plasticity, in many ways resembling the time of neurodevelopment (Cramer and Chopp, 2000). This enhanced plasticity includes the generation of new cells and blood vessels, sprouting and growth of new axons, and modulation of new and existing synapses (Carmichael, 2006). How the mature brain can suddenly launch into a period of renewed growth and development remains largely mysterious. The possibility that key components in epigenetic regulation stand poised to mediate this process in response to injury is a promising concept. In this review we highlight epigenetic mechanisms that altered in the aftermath of stroke and are known to have important functions in neuroplasticity (Table 1).

Global epigenetic changes following stroke

Before discussing the roles of epigenetics specific to recovery, summarized in Table 1, we would like to introduce the global epigenetic changes induced by stroke and briefly mention the evidence that these may generally be involved in stroke physiology. These include some roles in neuroprotection and preconditioning, two processes that are certainly important to outcomes after stroke although not directly related to repair processes which by definition require injury to have occurred first. While the general mechanisms of epigenetic regulation are beyond the scope of this review, we do provide a brief introduction to each and refer the reader to excellent reviews of these topics for further detailed discussions.

DNA methylation

The methylation of cytosine residues was first observed by Johnson and Coghill (1925) but not implicated in the regulation of gene expression until posited by Holliday and Pugh (1975). The methylation of cytosine-guanine (CpG) dinucleotides by a family of methyltransferase enzymes (DNMTs 1-4) has since been well characterized (Goll and Bestor, 2005). The role of this DNA modification within CpG-rich islands near 5′ promoter sites has long been appreciated as an effective means of gene silencing (Bird, 1986), but more recently scientists have expanded the classical view of DNA methylation. The role of intragenic methylation, which in fact comprises most of the methylated residues under

homeostatic conditions, has garnered significant attention (Maunakea et al., 2010). Furthermore CpG dinucleotides may not be the exclusive site of methylation in the mammalian genome as previously thought (Ramsahoye et al., 2000), at least in neurons (Xie et al., 2012; Lister et al., 2013; Guo et al., 2014). Evidence of active demethylation of DNA has also called into question the stability of this epigenetic mark (Ma et al., 2009a; Guo et al., 2011a). These recent advances demonstrate the evolving nature of our understanding of DNA methylation.

Following stroke the level of global DNA methylation increases significantly in the infarcted tissue compared to the contralateral hemisphere (Endres et al., 2000). Interestingly, this occurred without measurable changes in DNMT protein or enzymatic activity. While the authors suggest that this may have been due to the technical limitations of the assays used, it may also reflect the complexity and regional specificity of events occurring throughout the brain following stroke. This is highlighted by the fact that DNMT1 reduction protects against stroke, but complete absence of the enzyme does not (Endres et al., 2001). The roles of DNA methylation following stroke are likely varied, and may play a part in both the injury process as well as recovery.

Chromatin modifications

DNA associates with histone proteins in subunits called nucleosomes that form chromatin. The chromatin organization dictates, in part, the access of the genetic code to a cell's transcriptional machinery. Histones can undergo a number of modifications that can allow or prevent transcription (Kouzarides, 2007). These modifications are catalyzed by a variety of enzymes often with reciprocal functions making the modifications largely reversible and allowing dynamic changes to gene expression in response to the cellular environment. Modulation of these enzymes and thus the underlying chromatin structure has produced interesting results in animal models of stroke, and the role of histone modifications in recovery after stroke has also been reviewed recently (J. Elder et al., 2013).

The polycomb group (PcG) and trithorax group (thxG) are families of proteins with reciprocal capabilities of repressing and activating gene transcription, respectively, by coordinating the posttranslational modification of histones (Schuettengruber et al., 2007). Bmi-1 is a PcG protein important in protecting neurons from oxidative stress (Chatoo et al., 2009). This prompted investigation of its role in ischemic preconditioning, a model of potent neuroprotection from ischemic stroke. In this study, levels of Bmi1 increased in animals subjected to both preconditioning and subsequent injurious ischemia, but not in animals subjected to either stimulus alone (Stapels et al., 2010). *In vivo* knockdown of *Bmi1* or another PcG protein, *Scmh1*, using siRNA essentially abolished the protection afforded by preconditioning, and overexpression of either protected cells in an *in vitro* model of oxygen glucose deprivation (Stapels et al., 2010).

Acetylation and deacetylation are histone modifications that are made by histone acetyltransferases (HATs) and histone deacetylases (HDACs), respectively. Ischemia alters the expression of multiple HDAC proteins (Baltan et al., 2011), and these have become popular targets for preclinical neuroprotection studies in stroke. Several studies now have demonstrated protective effects of HDAC inhibition in animal models of stroke with various

compounds including valproic acid, trichostatin A, and sodium butyrate (Kim et al., 2007; Wang et al., 2012; George et al., 2013). The action of HDAC enzymes is complex, with some subtypes exhibiting protective effects while others may promote cell death (Langley et al., 2008; Chuang et al., 2009). Furthermore, certain classes of HDAC enzymes are expressed outside of the nucleus where they influence the function of diverse proteins in a nonepigenetic manner (Cho and Cavalli, 2014). While many compounds used are often considered to be general inhibitors of HDACs, important work has demonstrated substantial class-specificity of many compounds (Bradner et al., 2010). This is a critical point of future investigation because some studies suggest that selective inhibition of specific HDAC isoforms, such as HDAC6, can offer neuroprotection without associated cell toxicity (Rivieccio et al., 2009). Further study of individual HDACs will help to refine our understanding of the complex role of these proteins in stroke pathogenesis and subsequent recovery.

MicroRNAs

Small noncoding RNAs are a relatively novel class of epigenetic regulators that exert their influence on the genome in complex and as yet incompletely understood ways. They function largely, although not exclusively, through posttranscriptional repression of gene expression (Hobert, 2008). The miRNAs interact with the Argonaute family of proteins to form the RNA induced silencing complexes (RISCs) which then bind to and silence specific mRNA transcripts (Carthew and Sontheimer, 2009). MiRNAs have emerged as important regulators during CNS development, but they are also modulated following a variety of CNS injuries which has gotten them significant attention as potential therapeutic targets (Bhalala et al., 2013).

Several groups have investigated the expression profiles of miRNAs following stroke in animal models (Dharap et al., 2009; X.S. Liu et al., 2011; Gubern et al., 2013) and humans (Tan et al., 2009). These exhibit variable changes in expression during the acute and recovery phases of stroke. Unfortunately, there is little overlap between these studies in terms of consistency of findings, which muddles the picture of which miRNAs are truly important in the evolution of stroke and recovery. The time periods examined in reference to injury and recovery are different. In fact there is likely significant overlap between these phases, and dissecting which miRNAs are involved in the regulation of each will be important work moving forward.

Determining regional changes in miRNA expression may be important in this effort. MiRNA 181a for instance increases in the ischemic core, but decreases in the penumbra (Ouyang et al., 2012). Inhibition of miRNA-181a through antagomir silencing (complementary miRNAs that bind to target miRNAs) reduces cell death in models of both focal and global ischemia with Grp78 and Bcl-2 being potential target transcripts of importance (Ouyang et al., 2012; Moon et al., 2013). Inhibition of let7f or miRNA-1 appears to provide neuroprotection via IGF1 pathways, substantially reducing infarct volume even when done 4 h after stroke, whereas inhibition of miR-124 does not (Selvamani et al., 2012). Conversely, viral mediated overexpression of miR-124 protects against both stroke *in vivo* and oxygen-glucose deprivation *in vitro* (Doeppner et al., 2013; Sun et al., 2013), but some

have demonstrated conflicting results showing that miR-124 actually promotes cell death by suppressing the expression of apoptosis inhibitors (X. Liu et al., 2013b; Zhu et al., 2014). Many miRNAs can target multiple different genes which may explain some conflicting findings, and trying to dissect the multiple functions that these regulators can serve in the pathophysiology of stroke will require sophisticated investigation.

Neurogenesis, gliogenesis, and angiogenesis

In multiple species including humans, neurogenesis continues to occur postnatally and throughout adulthood in distinct brain regions, specifically the subgranular zone (SGZ) of the hippocampus and the subventricular zone (SVZ) (Palmer et al., 1995; Eriksson et al., 1998; Gould et al., 1999). These neurogenic regions harbor populations of neural stem and progenitor cells (NSPs), and changes within this niche can modulate the process of neurogenesis. Following ischemic stroke, more cells are produced from these regions and exhibit altered paths of migration toward the region of injury (Felling et al., 2006; Arvidsson et al., 2002; Thored et al., 2006). While some of these cells do appear to incorporate into local circuitry (Hou et al., 2008), the vast majority of the cells die, and the extent to which this process contributes meaningfully to regeneration remains unclear. The concept of a “neurovascular unit” illustrates the coupling of neural cells with blood vessels, and many growth factors that influence neurogenesis also contribute to angiogenesis. Angiogenesis also occurs following stroke and should be considered an important component of the NSP niche (Ohab et al., 2006). Epigenetic changes provide an enticing mechanism by which stroke can influence changes within the NSP niche and thus influence the behavior of these cells.

DNA methylation

One way by which methylation silences gene expression is through recruitment of specific binding proteins to the promoter element. The methyl-CpG binding domain (MBD) family of proteins includes MBD1-4 and Mecp2 (Hendrich and Bird, 1998). Following transient ischemia, many of these proteins exhibit altered expression within subregions of the hippocampus, one of the important niches for postnatal neurogenesis. MBD1 and Mecp2 both remained unchanged initially but were elevated by 24 hours after ischemia, while MBD2 expression increased by 6 h after ischemia. In contrast, MBD3 was significantly reduced as early as 3 h after ischemia (Jung et al., 2002). These proteins can be important regulators in the process of neurogenesis. MBD1 is expressed in neural stem and progenitor cells. Mice deficient in this protein exhibit impaired neurogenesis as assessed by BrdU incorporation, partly explained by decreased survival of newly generated cells (Zhao et al., 2003). FGF-2 is an important growth factor for neurogenesis, and further study of Mbd1 knockdown demonstrated increased expression of FGF-2 along with specific hypomethylation of its promoter (Li et al., 2008).

The interrelationship between the expression of MBD1 and promoter methylation is intriguing and may represent a role for these proteins in either establishing or possibly protecting the methylation state. DNA methylation has classically been considered a very stable means of gene silencing, but recent evidence suggests that methylation states may be more dynamic than previously appreciated. The growth arrest and DNA-damage inducible

45 (Gadd45) family of proteins are important components to a process of active demethylation of cytosine residues (Barreto et al., 2007; Ma et al., 2009a,b). This process appears to be mediated through DNA repair pathways, and Gadd45 may function by coupling the necessary enzymatic machinery (Ma et al., 2009a,b; Schafer, 2013). Importantly, Gadd45-dependent demethylation appears to occur in specific genes, leaving global DNA methylation relatively unchanged (Jin et al., 2008; Engel et al., 2009). Gadd45b is essential to neurogenesis that occurs in the DG following electroconvulsive treatment, possibly via Tet1-mediated demethylation of important growth factors including FGF-1 and BDNF (Ma et al., 2009b; Guo et al., 2011b). Neurogenesis is similarly seen following various animal models of stroke, and ischemia substantially increases the expression of Gadd45 proteins in both the adult and perinatal rat brain after stroke (Chen et al., 1998; Schmidt-Kastner et al., 1998; Charriaut-Marlangue et al., 1999). Fig. 1 provides a mechanistic model by which Gadd45 mediated demethylation could lead to the increased expression specific genes important to neuroplasticity.

Histone modifications

As discussed previously, members of the polycomb group of proteins can influence the outcome of stroke. Their involvement in neurogenesis in the normal brain is well documented. Bmi-1 knockout reduces the self-renewal of neural stem cells in the developing brain without affecting the fate of lineage-restricted progenitors (Molofsky et al., 2003). Conversely overexpression of Bmi-1 enhances the self-renewal of NSCs in both the developing and adult brain through mechanisms that require the transcription factor Foxg1 (Fasano et al., 2009). These results, coupled with its role in histone modification, provide an appealing mechanism by which Bmi-1 can confer an “epigenetic memory” of differentiation state through subsequent cell divisions (Ringrose and Paro, 2007; Ng and Gurdon, 2008). Given that overexpression of Bmi-1 can substantially reduce infarction in stroke models (Stapels et al., 2010), the role of this protein in regulating neurogenesis following stroke is a worthwhile target of further investigation. EZH2 is a component of the polycomb repressive complex 2 (PRC2) that promotes epigenetic gene silencing by catalyzing histone H3 trimethylation (Cao et al., 2002). Downregulation of EZH2 can promote neuronal differentiation in human mesenchymal stem cells (hMSCs), and transplantation of hMSCs with knocked down EZH2 provides improved functional recovery after stroke compared to transplanted hMSCs with normal EZH2 function (Yu et al., 2013).

Oligodendrogenesis is also altered following stroke, and new evidence supports an important role for histone deacetylase activity in this process. In the early period following stroke, oligodendrocyte progenitors (OPCs) in the peri-infarct white matter demonstrate increased expression of HDAC1 and HDAC2, concurrent with increased proliferation. Mature oligodendrocytes, on the other hand, showed decreased HDAC1 and increased HDAC2 (Kassis et al., 2014). In addition to implying an important role for HDACs in oligodendrogenesis following stroke, this work demonstrates that even individual HDAC isoforms within the same class may have differential effects on cell maturation.

Additional indirect evidence supporting a role for histone modification in post-stroke neurogenesis stems from the effects of HDAC inhibitors on stroke recovery. Valproic acid

(VPA) is a potent HDAC inhibitor and improves functional recovery after stroke (Wang et al., 2012). One possible mechanism is through the promotion of angiogenesis, as VPA treatment increased microvessel density and improved cerebral blood flow to the ischemic hemisphere 14 days after stroke (Wang et al., 2012). VPA is also known to promote neuronal differentiation in hippocampal progenitor cells (Hsieh et al., 2004) and thus may also mediate recovery through neurogenesis.

MicroRNAs

The recent explosion of miRNA profiling studies has yielded important results demonstrating the importance of these regulators in neurogenesis. MiR-124 is one molecule that has importance in the acute phase of stroke as discussed above, but also may influence repair after stroke by regulating the behavior of progenitor cells given its role as a neuronal fate determinant in the normal SVZ through targeting of the Notch ligand Jagged1 (JAG1) (Cheng et al., 2009). In the ischemic brain, miR-124a is reduced in the SVZ 7 days after stroke, corresponding to a time of significant neurogenesis (X. S. Liu et al., 2011). Introduction of exogenous miR-124a substantially reduces signaling through the Notch pathway, reduces progenitor proliferation, and induces neuronal differentiation, suggesting that the decreased expression of miR-124a observed after ischemia may be an important mediator of the progenitor cell response (X.S. Liu et al., 2011). MiR-9 is another microRNA transcript of potential importance to post-stroke repair. Loss of this transcript suppresses proliferation in cultured human neural progenitor cells and enhances the migration of these cells when transplanted into the ischemic brain (Delaloy et al., 2010). A potential target of miR-9 important in the ischemic brain is serum response factor, a transcription factor capable of promoting oligodendrocyte differentiation (Buller et al., 2012).

Axon sprouting and growth

The immediate border of an infarct is characterized by the formation of a glial scar in which upregulation of growth inhibitors such as myelin-associated proteins, extracellular matrix proteins, and other growth inhibitors prevent effective axon regeneration (Silver and Miller, 2004). Immediately adjacent to the glial scar however, is a region of periinfarct cortex characterized by the expression of multiple growth-promoting factors that is actually permissive to axon sprouting (Carmichael et al., 2005). Stroke induces extensive changes in the organization of affected networks (Napieralski et al., 1996; Carmichael et al., 2001; Dancause et al., 2005). Axon sprouting both in peri-infarct cortex as well as more remote areas of connected cortex is an important contributor to this remodeling process (Carmichael et al., 2005). Understanding the epigenetic mechanisms regulating the establishment of permissive and inhibitory environments for axon growth will help to further illuminate the means by which network remodeling occurs after stroke, and possible ways to facilitate it.

DNA methylation

Little is known about the importance of DNA methylation in regulating axon sprouting after stroke, but an important role can be inferred from studies in other models of injury. Small proline-rich repeat protein 1 (SPRR1) is expressed following axotomy and can promote axon outgrowth in this scenario (Bonilla et al., 2002). SPRR1 is also expressed at high levels

in the peri-infarct cortex early after stroke (Carmichael et al., 2005). Keratinocytes exhibit robust expression of SPRR1 when exposed to the hypomethylating agent 5-azacytidine, suggesting that its expression is influenced by methylation state (J. T. Elder and Zhao, 2002). Whether altered methylation of *SPRR1* following stroke is important in its expression still needs to be demonstrated.

Histone Modifications

SPRR1 can be induced by hypomethylating agents as discussed above, but its expression can also be modulated through histone modification. Similar to the effects of 5-azacytidine on keratinocytes, SPRR1 expression is increased in these cells following treatment with the HDAC inhibitor sodium butyrate (J.T. Elder and Zhao, 2002), but this has yet to be examined in the brain. GAP43 is a growth cone-associated protein that promotes neurite outgrowth by regulate cytoskeletal organization via protein kinase C signaling (Benowitz and Routtenberg, 1997). Gap43 expression decreases following neurodevelopment, but is highly induced in peri-infarct cortex after stroke (Skene et al., 1986; Carmichael et al., 2005). VPA is another commonly used drug that can inhibit HDACs, and VPA administration can induce the expression of GAP43 as well as other growth and survival proteins while promoting neurite outgrowth (Yuan et al., 2001). Supporting these findings in an optic nerve crush model of axonal injury, over expression of the HAT, p300, increases GAP43 and SPRR1 expression coincident with increased histone H3 acetylation and promotes axon regeneration (Gaub et al., 2011).

As mentioned in the introduction of this section, the glial scar surrounding and infarct likely limits axonal regeneration due to the presence specific inhibitory factors such as chondroitin sulfate, myelin associated proteins, and proteoglycans. Interestingly, Gaub and colleagues used the HDAC inhibitor trichostatin A to examine effects on neurite outgrowth. They found that HDAC inhibition increased levels of the histone acetyltransferases CREB-binding protein/p300 (CBP/p300) and the p300-CBP-associated factor (P/CAF) as well as hyperacetylation of histone H3. This was accompanied by a reduction of growth cone collapse not only on permissive substrates, but also on inhibitory substrates containing myelin and chondroitin sulfate proteoglycans (Gaub et al., 2010). Recent data also demonstrate the importance of histone 4 acetylation levels in establishing a permissive environment for axon growth. H4 hypoacetylation is associated with diminished axon growth potential, but following a conditioning lesion paradigm of spinal cord injury H4 acetylation levels are restored, triggering the expression of multiple regeneration-associated genes through the activity of a transcriptional complex involving Smad1 (Finelli et al., 2013). It remains to be seen whether similar effects can be demonstrated in the brain after stroke.

MiRNA

Studies have demonstrated an important role for several miRNAs in the processes of axon outgrowth, guidance, and branching (Chiu et al., 2014). In the studies of miRNA expression profiles following ischemia, there are few reports of differential expression of any of these in either the acute or recovery phase of stroke. One exception is miR-9 which is downregulated in ischemic white matter (Buller et al., 2012). MiR-9 is expressed in the

axons of primary cortical neurons in the developing brain where it represses microtubule associated protein 1b (Map1b) translation. Inhibition of miR-9 by RNA interference resulted in significantly increased axon length but reduced branching patterns, effects that were dependent on the regulation of Map1b translation (Dajas-Bailador et al., 2012). Another observation of these experiments is that brain derived neurotrophic factor (BDNF), a signaling molecule important in axon development, influences miR-9 in a biphasic manor. Short pulses of BDNF decreased miR-9 expression and resulted in axon growth; whereas, longer pulses of BDNF actually increased the expression of miR-9 and promoted multiple branch points in axons (Dajas-Bailador et al., 2012). Given these modes of action, a role for miR-9 in regulating axon regeneration in the setting of stroke is very plausible but requires specific investigation.

Synaptic plasticity

Synaptic plasticity is central to the establishment and maintenance of neural networks. On one hand, Hebbian plasticity dictates that the strength of a synapse is dependent on its activity, manifested in the processes of long term potentiation and long term depression. Homeostatic mechanisms of plasticity function to maintain average neuronal excitability, thereby balancing activity-dependent synaptic changes that would have otherwise proceeded unchecked and providing stability to the system (Nelson and Turrigiano, 2008). Both of these mechanisms are important considerations in spontaneous recovery from stroke (Murphy and Corbett, 2009). Stroke results in marked functional cortical remapping both in animal models as well as human patients (Chollet et al., 1991; Clarkson et al., 2013). These changes are accompanied by increased synaptic protein expression in peri-infarct tissue as well as more remote contralateral brain several weeks after stroke (Stroemer et al., 1995). Epigenetic regulation is critical to synaptic plasticity and warrants specific investigation in the setting of stroke recovery (Guzman-Karlsson et al., 2014).

DNA methylation

We have previously discussed the role of DNMT activity in protecting against ischemia, but little is known regarding the importance of DNMT's in the recovery phase. There is evidence, however, that DNMTs are important for synaptic plasticity. Inhibition of DNMT activity blocks long term potentiation in the hippocampus and results in decreased methylation of the *reelin* and *BDNF* promoters, genes known to be involved in the induction of synaptic plasticity (Levenson and Sweatt, 2005). Furthermore mice deficient in *DNMT1* and *DNMT3a* exhibit impaired learning and memory along with abnormal hippocampal plasticity (Feng et al., 2010). Given this data, it would be interesting to examine the effects of DNMT deficiency on synaptic remodeling after stroke.

Following stroke, neurons in the peri-infarct cortex exhibit intrinsic hyperexcitability (Schiene et al., 1996; Brown et al., 2009). This increased excitability is intriguing because neuronal activity has recently been linked to the dynamic regulation of DNA methylation states (Guo et al., 2011a; Felling et al., 2012). Neuronal activity has been shown to promote the specific demethylation of *BDNF* through the activity of Gadd45b and TET1 (Ma et al., 2009a,b; Guo et al., 2011b). In *Gadd45b* knockout mice, changes in dendritic morphology typically induced by electroconvulsive treatment do not occur (Ma et al., 2009a,b). It is

possible that these mechanisms of demethylation are important in the recovery of stroke, but this has yet to be studied.

Histone modifications

We have already discussed the potential role for HDAC inhibitors in protecting against stroke, but abundant evidence demonstrates that histone acetylation is important in synaptic plasticity and may therefore be important in the recovery phase as well. Use of HDAC inhibitors enhances LTP and memory formation in the hippocampus (Vecsey et al., 2007). Conversely, another group was able to perform essentially the reverse experiment by genetically reducing CBP, thereby lowering histone acetylation, and demonstrated impaired LTP (Chatterjee et al., 2013). Anatomically, HDAC inhibition significantly increases dendritic spine formation in hippocampal neurons, an effect that was demonstrated to be specifically related to HDAC2 using genetic knockout or overexpression (Guan et al., 2009). Investigating the effects of these types of modulation on synaptic plasticity in relation to stroke recovery will provide important mechanistic insight.

MiRNA

The immediate early gene *Arc* is important regulator of synaptic plasticity (Shepherd and Bear, 2011). *Arc* expression is decreased in the ischemic core, but significantly increased in the peri-infarct cortex soon after stroke likely due to glutamate release and neuronal activation (Berger et al., 2003). Evidence now implicates multiple miRNAs in the regulation of Arc, at least one of which is differentially expressed after stroke. MiR-324 was among a group of 16 miRNAs studied that could inhibit the expression of a reporter containing the *Arc* 3′ untranslated region (Wibrand et al., 2012). A member of the miR-324 family also exhibits decreased expression in the brain during the recovery phase after ischemia (F.J. Liu et al., 2013). This presents a plausible mechanism by which Arc expression could be increased to regulate synaptic plasticity after stroke.

MiR-124 is highly specific to neurons and has been implicated in various aspects of stroke pathophysiology. It is also a key component of a pathway involved in long term potentiation. This pathway involves the exchange protein directly activated by cAMP (EPAC) and Zi268, and disruption of the pathway through knockout of EPAC results in impaired LTP and overt learning deficits that can be reversed with knockdown of miR-124 (Yang et al., 2012). MiR-181 has been shown to influence the degree of infarction, and its expression decreases in the penumbra following ischemia (Ouyang et al., 2012). Other studies have shown it to be an inhibitor of dendritic spine formation in the nucleus accumbens (Saba et al., 2012), therefore, decreased expression may contribute to synaptic plasticity after stroke.

Concluding remarks

Epigenetic mechanisms have established roles in neuroplasticity within the normal brain. These have been demonstrated through studies of neurodevelopment, learning and memory. The role of such mechanisms in neuroplasticity following injury have not been clearly defined. Epigenetic changes have been shown following stroke, but mostly in the context of

injury evolution. In this review we have examined the intersection of these evolving fields of investigation, and proposed mechanisms by which epigenetic players may influence post-stroke remodeling and plasticity (Fig. 1). These roles need to be examined independently in the context of the injured brain to provide a more comprehensive understanding of how repair processes are initiated in the acute phase of stroke, and how these contribute to later recovery.

While we have focused on positive aspects of plasticity, an important point to note is that the emerging concept of “maladaptive plasticity” suggests that some aspects may actually inhibit spontaneous recovery or otherwise lead to negative consequences such as epileptogenesis (Takeuchi and Izumi, 2012; Jang, 2013). Additionally while certain types of plasticity are enhanced following stroke, other types such as visual plasticity may actually be impaired (Jablonka et al., 2007; Greifzu et al., 2011, 2014). The role of epigenetic regulation in these facets of stroke recovery must also be explored.

We have only begun to scratch the surface in understanding the complex epigenetic changes in the ischemic brain. In contrast to the injured brain, there is an abundance of literature describing the epigenetic mechanisms responsible for the normal functions of plasticity during neurodevelopment and learning. In this review we have broken down the cellular aspects of stroke recovery into parallel physiological processes and identified potential mechanisms with evidence in both stroke and plasticity. This framework helps to identify plausible candidates important to stroke-induced plasticity, but in many cases the roles have yet to be directly investigated. Furthermore, as discussed in the introduction, stroke is a disease with devastating impact across the lifespan. Aging has profound influence on the epigenome (Issa, 2014), and the mechanisms underlying stroke induced plasticity in the aged brain are unlikely to be the same as those in the young and developing brain, thus necessitating consideration of effects of developmental maturation on recovery from stroke.

Epigenetic mechanisms of regulation are potent mediators of changes in gene expression that can be influenced by both intrinsic and extrinsic factors. This places them in a prime position to facilitate increased plasticity following stroke. As discussed, clinical and scientific observations suggest a critical period of plasticity following stroke during which true neurologic repair is a possibility. Further investigation of histone modifications, DNA methylation and demethylation, and miRNA regulation following stroke will provide important insights into the basic mechanisms of such plasticity and further enhance our understanding of the brain's inherent regenerative capacity. Clinical epigenetics is a rapidly advancing field, spurred largely by advances in cancer medicine. Combining these tools with a sophisticated understanding of the underlying biology of spontaneous neurologic remodeling could dramatically improve our clinical approach to stroke patients.

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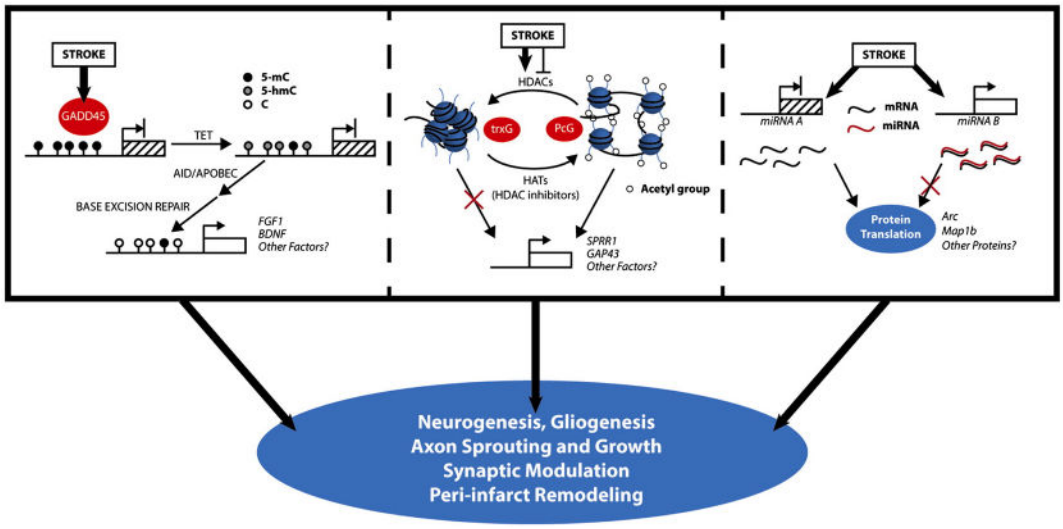

Figure 1.

Proposed mechanistic models by which epigenetic changes can influence neuroplasticity following stroke. Active demethylation of DNA leads to increases in gene expression which promotes plasticity. Shifts in histone acetylation may increase or decrease the transcription of specific growth factors. Altered expression of specific non-coding miRNAs can result in increased or decreased translation of proteins. The specific mechanisms reviewed here have strong evidence in neuroplasticity and have been observed to be altered by stroke. The importance in modulating plasticity after stroke requires further specific investigation.

Table 1

Selected epigenetic mechanisms influencing neuroplasticity. The mechanisms listed in this table have plausible roles in stroke recovery based on alterations of the key molecular players following stroke.

	Neurogenesis, gliogenesis, angiogenesis	Axon growth	Synaptic plasticity
DNA methylation	• Increased MBD1 enhances survival of NSPs (Zhao et al., 2003) • Gadd45 promotes active demethylation of growth factor promoters (Ma et al., 2010)	• Hypomethylation of SPRR1 increases expression and promotes axon outgrowth (Bonilla et al., 2002)	• Altered DNMT activity influences synaptic remodeling (Li et al., 2008) • Gadd45 influences synaptic remodeling (Ma et al., 2010)
Histone Modification	• Bmi-1 regulates NSP division following stroke (Fasano et al., 2009; Moon et al., 2013) • Decreased expression of EZH2 promotes neuronal differentiation of NSPs (Yu et al., 2013) • HDAC inhibitors promote neurogenesis (Jablonka et al., 2007)	• Histone acetylation increases expression of SPRR1 to promote axon outgrowth (George et al., 2013) • Histone acetylation increases expression of GAP43 and other proteins (George et al., 2013) • HDAC inhibition improves growth cone stability (Go et al., 2014)	• Histone acetylation (or HDAC inhibition) increases synaptogenesis (Chatterjee et al., 2013; Guo et al., 2011a; Vecsey et al., 2007)
MicroRNA	• Reduced expression of miR-124 increases NSP proliferation (Cheng et al., 2009; Ma et al., 2009a) • MiR-9 alters expression of serum response factor, promoting oligodendrogenesis (Buller et al., 2012; Delaloy et al., 2010)	• Modulated by BDNF, miR-9 regulates translation of Map 1b and regulates axon regeneration (Dajas-Bailador et al., 2012)	• Decreased miR-324 increases Arc translation (Wibrand et al., 2012) • Decreased of miR-124 increases LTP (Yang et al., 2012) • Decreased miR-181 in penumbra enhances synaptogenesis (Saba et al., 2012)