Ischemic Preconditioning Alters the Epigenetic Profile of the Brain from Ischemic Intolerance to Ischemic Tolerance

John W. Thompson · Kunjan R. Dave · Juan I. Young · Miguel A. Perez-Pinzon

Published online: 19 July 2013
© The American Society for Experimental NeuroTherapeutics, Inc. 2013

Abstract Ischemic preconditioning is an innate neuroprotective mechanism in which a sub-injurious ischemic exposure increases the brain's ability to withstand a subsequent, normally injurious ischemic insult. Part of ischemic preconditioning neuroprotection stems from an epigenetic reprogramming of the brain to a phenotype of ischemic tolerance, which results in a gene expression profile different from that observed in the non-injured and ischemia-injured brains. Such neuroprotective reprogramming, activated by ischemic preconditioning, requires specific changes in DNA accessibility coordinated with activation of transcriptional activator and repressor proteins, which allows for expression of specific neuroprotective proteins despite a general repression of gene expression. In this review we examine the effects of injurious ischemia and ischemic preconditioning on the regulation of DNA methylation, histone post-translational modifications, and non-coding RNA expression. There is increasing interest in the role of epigenetics in disease pathobiology, and whether and how pharmacological manipulation of epigenetic processes may allow for ischemic neuroprotection. Therefore, a better understanding of the epigenomic determinants underlying the modulation of gene expression that lead to ischemic tolerance or cell death offers the promise of novel neuroprotective therapies that target global reprogramming of genomic activity versus individual cellular signaling pathways.

Keywords Ischemic preconditioning · Stroke · Histone acetylation · DNA methylation · microRNA

Introduction

Cerebral ischemia results from the prolonged interruption of blood flow to the brain as a result of a stroke or cardiac arrest. Cerebral ischemia initiates a complex cascade of events leading to cell death both in the ischemic core and in the surrounding less severe ischemic tissue, where cell death continues for days and weeks following reperfusion. Most research aimed at preventing ischemia-mediated injury has focused primarily upon identifying the signaling pathways activated by ischemia/reperfusion and the pharmacological targeting of these pathways. However, this targeted approach has met with little success in the clinic [1].

The lack of therapeutic success in the clinic against stroke and cardiac arrest has led many researchers to study intrinsic mechanisms of neuroprotection. One such approach is known as ischemic preconditioning (IPC). IPC can be elicited by a brief non-injurious interruption of blood flow, which initiates a neuroprotective response that protects the brain against a subsequent injurious ischemic exposure. Several IPC models have been used in preclinical studies to induce a non-injurious ischemic stress—including transient intraluminal suture induced occlusion of the middle cerebral artery (MCAO), a model of focal ischemia; cardiac arrest, and bilateral carotid artery cross-clamping with hypertension, models of global ischemia and oxygen and glucose deprivation (OGD), an *in vitro* model of ischemia.

Numerous research groups have identified signaling pathways activated by IPC that lead to neuroprotection (reviewed in [2] and [3]) mediated partly through altered gene expression [4, 5]. Recent microarray analyses have revealed that the gene expression profiles of IPC, injurious ischemia, and IPC+injurious ischemia are very different [6–9]. For example,

J. W. Thompson (✉) · K. R. Dave · M. A. Perez-Pinzon
Cerebral Vascular Disease Research Laboratories,
Department of Neurology, Miller School of Medicine,
University of Miami, Miami, FL 33136, USA
e-mail: jthompson@med.miami.edu

J. I. Young
John P. Hussman Institute for Human Genomics and
Dr. John T. Macdonald Department of Human Genetics,
Miller School of Medicine, University of Miami,
Miami, FL 33136, USA

Stenzel-Poore et al. [9] compared gene expression of the ipsilateral cortex to the non-ischemic contralateral cortex 24 h following MCAO-induced preconditioning (15-min occlusion), injurious ischemia (60-min occlusion), or IPC followed 72 h later by injurious ischemia (ischemic tolerant). The genes regulated in each of these three conditions (IPC, injurious ischemia, and ischemic tolerant) were very different, with very little overlap among the three conditions. IPC induced expression of genes related to metabolic function and the cell cycle, whereas injurious ischemia upregulated genes involved in immune response and host defenses [9, 10]. In contrast to the major induction of gene expression observed in IPC and injurious ischemia, ischemia tolerance induced a distinct downregulation (77 %) of the differentially expressed genes [9, 10]. These suppressed genes encoded ion channels, transporters, and metabolic pathways, suggesting lowered cellular activity. These findings led to the notion that the brain's response to injurious ischemia is reprogrammed by preconditioning from one that leads to cell death to one that produces a neuroprotective phenotype [9, 10]. Mechanistically, the prevailing view is that epigenetic remodeling underlies the reprogramming of genome activity involved in acquisition and maintenance of the neuroprotective phenotype. These epigenetic changes have only recently begun to be investigated in the preconditioned brain.

Epigenetics

Epigenetics is concerned with mitotically heritable changes in transcriptional potential. Mechanisms that underlie epigenetic regulation, through determination of the accessibility of DNA to the transcriptional machinery, include direct modification of DNA by methylation and a variety of chemical modifications of the proteins regulating chromatin structure, including histones and many nonhistone proteins. Noncoding RNAs (ncRNAs) mediate an additional mode of epigenetic regulation by influencing gene expression at multiple levels, including modulation of chromatin structure, RNA splicing, and mRNA stability and translatability.

These mechanisms ultimately regulate gene transcription, replication, and repair. Therefore, epigenetic changes allow cells to adapt and respond to changes in their internal and external environment [11].

The current hypothesis states that the regulation of chromatin modifications by epigenetic factors during both normal and ischemic conditions directly influences the accessibility of the DNA to proteins that regulate gene expression, and lead to a cell death or survival phenotype [12]. Research from different groups has shown that IPC and injurious ischemia leading to epigenetic changes participate in cerebral protection or damage, respectively [6–9]. Below we

provide a summary describing epigenetic changes in DNA methylation, histone post-translational modifications, and non-coding RNA expression following IPC and injurious ischemia, which may play important roles in the genetic reprogramming of the cell to a phenotype of ischemic tolerance or intolerance.

DNA Methylation

DNA methylation of cytosine residues in gene promoter regions is an important and well-characterized epigenetic mechanism for regulation of gene expression (Fig. 1). In general, DNA methylation at promoter regions represses gene expression whereas DNA hypomethylation is associated with gene transcription. The level of DNA methylation is regulated by the balance of the activities of DNA methyltransferases, which transfer a methyl group from S-adenosylmethionine to cytosine residues, and DNA demethylases, which remove methyl groups from the DNA. *De novo* methylation of DNA is mediated by DNA methyltransferase (DNMT)3a and 3b, whereas DNMT1 maintains the DNA methylation [13, 14].

Cerebral ischemia affects the extent of DNA methylation [15, 16]. In a mouse model of focal cerebral ischemia, Endres et al. [16] observed that cerebral ischemia/reperfusion led to increased DNA methylation in brain tissues. The functional effect of this increased methylation on cell survival was demonstrated in DNA methyltransferase knockout mice, where reduced DNA methylation and lower cerebral ischemic damage were observed following 30 min of MCAO. They also demonstrated that inhibiting DNA methyltransferase using 5-aza-2'-deoxycytidine was neuroprotective against cerebral ischemic damage and was associated with reduced levels of DNA methylation. This study concluded that cerebral ischemia-induced DNA hypermethylation plays a key role in pathways leading to cerebral ischemic injury [16]. However, complete inhibition of DNA methylation is not advantageous as, in a follow-up study, the same group demonstrated that lower levels of DNMT1, but not complete absence of DNMT1, protected neurons from ischemic brain injury [17], suggesting complex mechanisms of methylation-mediated damage/neuroprotection. In a different study, Hu et al. [18] demonstrated that OGD-induced suppression of thrombospondin-1 (TSP-1) (a potent angiostatic factor) was dependent upon DNA methylation of the TSP-1 promoter region. OGD was observed to increase methylation levels, which coincided with decreased TSP-1 mRNA and protein expression. Reperfusion following OGD resulted in decreased methylation, and increased TSP-1 mRNA and protein expression. They hypothesized that OGD leads to lower TSP-1 expression via increased methylation of the promoter region of TSP-1, which may, ultimately, increase angiogenesis [18, 19].

Fig. 1 Epigenetic control of gene expression. The genomic expression profile of a cell is primarily determined by histone post-translational modifications, DNA methylation, and microRNA (miRNA) expression. Exposure to injurious ischemia alters the epigenetic profile of the cell leading to cell death, which can be reduced by histone deacetylase

(HDAC) inhibitors, sirtuin 1 activation by ischemic preconditioning or resveratrol treatment, and by reducing the activity of DNA methyltransferase. Part of the epigenetic reprogramming also involves altered miRNA expression, which regulates target gene expression. *Ac* acetylation, *HAT* histone acyltransferase, *CH3* methylation

A recent study has also demonstrated that genetic alterations in the DNA methylation pathway increase an individual's risk of stroke [15, 20]. Casas et al. [20] performed a meta-analysis of epidemiological studies and found that methylene-tetrahydrofolate reductase (an enzyme involved in the formation of cellular reservoirs of methyl groups) deficiency increases an individual's risk of stroke [15]. However, the effect of methylene-tetrahydrofolate reductase deficiency on ischemic damage, following a stroke, is not known.

These studies suggest that DNA hypermethylation plays an important and complex role in cerebral ischemia-induced damage, which could be reduced by DNMT inhibition (Table 1). The effect of IPC on DNA methylation and the contribution of DNA methylation to IPC-mediated ischemic tolerance are currently undefined.

Histone Modifications

The compaction of DNA within the nucleus is achieved by its association with an octamer of 4 positively-charged core histone proteins, H2A, H2B, H3, and H4, which make up the nucleosome [21, 22]. Post-translational modifications [e.g.,

acetylation, phosphorylation, methylation, ubiquitylation, and small ubiquitin-related modifier (SUMO)ylation] of the histone proteins alter the DNA-nucleosome and internucleosomal interactions, thereby affecting both global and local chromatin structure [23]. Therefore, the chromatin structure is in a continuous dynamic state of repression and accessibility for particular genes, and is regulated by the pattern of histone post-translational modifications, referred to as the histone code [24]. The activity of epigenetic factors that write/erase and read the histone code allows for the translation of cell signaling pathways into altered genomic expression profiles.

There is extensive evidence that regulation of histone post-translational modifications contribute to the etiology and progression of ischemic damage in the brain [25–28].

Acetylation

One of the most extensively studied histone modifications in general and in regard to cerebral ischemia is acetylation. Acetylation neutralizes the positive charge of lysine residues on the histone tail thereby opening local chromatin configurations, relaxing the DNA:nucleosomal association, and allowing nucleosomal repositioning thereby creating accessibility to transcription factors and associated proteins.

Table 1 Pharmacological targeting of epigenetic mediators for ischemic neuroprotection

Epigenetic target	Drug	Ischemic model	Outcome	Reference
DNA methyltransferase	5-aza-2'-deoxycytidine	Mouse, 30 min MCAO	Reduced DNA methylation and infarct volume	[16]
Class I, II, and IV HDACs	Suberoylanilide hydroxamic acid Valproic acid	Mouse, 6 h of MCAO	Increased histone acetylation and reduced infarct volume	[29]
		Rat, 1 h MCAO	Increased histone H3 acetylation and reduced infarct volume	[30]
		Rat, permanent MCAO	Increased histone acetylation and reduced infarct volume	[32]
		Mouse, permanent carotid artery occlusion followed by 30 min of hypoxia (6 % oxygen)	Reduced infarct volume	[31]
	Trichostatin A	Rat, permanent MCAO	Increased histone acetylation and reduced infarct volume	[32]
		Mouse, 1 h MCAO	Reduced infarct volume	[34]
	Sodium butyrate	Rat, permanent MCAO	Increased histone acetylation and reduced infarct volume	[32]
		Rat, 4 h MCAO	Reduced infarct volume	[33]
Class III HDAC	Resveratrol	Rat, organotypic hippocampal slices exposed to 40 min oxygen and glucose deprivation	Increased SIRT1 activity and reduced cell death	[39]
	Resveratrol	Rat, 8 min of asphyxia cardiac arrest	Increased SIRT1 activity and reduced cell death in the CA1 region of the hippocampus	[38]

Therefore, histone acetylation is usually associated with gene expression. The level of acetylation is determined by the balance of the activities of histone acetyltransferase and histone deacetylase (HDAC) enzymes. Numerous studies have demonstrated that inhibition of HDACs reduces infarct size. For example, in the mouse brain Faraco et al. [29] demonstrated that histone H3 acetylation levels were reduced following 6 h of MCAO. Treating the mice with the HDAC inhibitor suberoylanilide hydroxamic acid maintained histone H3 acetylation levels following MCAO and reduced the level of ischemic damage. Similar protection of histone acetylation levels and reduction in infarct volume was also observed in rats treated with the HDAC inhibitor valproic acid following 1 h MCAO [30]. In fact, numerous cerebral ischemia studies in both mice and rats have demonstrated that HDAC inhibition maintains histone acetylation levels, reduces infarct volume, and improves functional recovery following ischemia [29–34]. Other HDAC inhibitors that have been shown to protect against neuronal ischemic damage include sodium 4-phenylbutyrate, trichostatin A and sodium butyrate [29–34] (Table 1). The studies discussed above suggest HDAC inhibitors alter the epigenetic profile of the brain from ischemic damage to survival by maintaining histone acetylation levels and by altering transcriptional activity thereby decreasing neurodegenerative protein expression while increasing pro-survival protein expression [29–34].

In the abovementioned studies, HDAC inhibitors were administered during or shortly after the induction of ischemia. Administration of sodium butyrate 24 h prior to and then again

4 h prior to permanent MCAO reduced infarct size by approximately 50 % at 24 h when compared with vehicle-treated controls [33]. This finding suggests that HDAC inhibition may be able to precondition the brain against ischemic damage, as has been demonstrated in the heart using the HDAC inhibitor trichostatin A [35].

Recently, it has been demonstrated that the class III nicotinamide adenine dinucleotide (NAD⁺)-dependent HDAC, sirtuin 1 (SIRT1), is required for preconditioning-mediated ischemic tolerance. SIRT1 is a member of the sirtuin family of deacetylase and ADP-ribosylase enzymes, which are involved in numerous cellular activities, including the cellular stress response, genome stability, and energy metabolism [36, 37]. In contrast to ischemic neuroprotection by class I, II, and IV HDAC inhibitors (which do not inhibit sirtuins), our laboratory, and others, have demonstrated that SIRT1 activation is required for ischemic tolerance [38, 39]. Our laboratory has demonstrated both *in vivo* and in organotypic hippocampal slices that nuclear SIRT1 activity is increased at 48 h following IPC and that blocking SIRT1 activation with sirtinol abrogated IPC-mediated ischemic neuroprotection [38, 39]. We have also demonstrated that preconditioning could be induced, both *in vivo* and in hippocampal organotypic slices with the SIRT1 activator, resveratrol. Resveratrol-mediated neuroprotection was lost in the presence of sirtinol, confirming a role for SIRT1 in resveratrol-mediated ischemic neuroprotection [38, 39]. Using a different model of preconditioning, Yan et al. [40] demonstrated that hyperbaric oxygen preconditioning increased SIRT1 protein

expression and that inhibition of SIRT1 deacetylase activity with EX527 blocked hyperbaric oxygen preconditioning neuroprotection against MCAO-mediated damage. It is interesting to note that transgenic mice overexpressing neuronal-specific SIRT1 are not protected against ischemic damage, suggesting that other signaling pathways may work in conjunction with SIRT1 for the induction of ischemic tolerance [41].

The conflicting requirements of SIRT1 activation for ischemic tolerance and HDAC (class I, II, and IV) inhibition for ischemic neuroprotection may stem, in part, from the NAD⁺ requirement for SIRT1 enzymatic activity. The coupling of SIRT1 activity to cellular NAD⁺ levels places SIRT1 in a position of nutrient sensing and energy metabolism by the cell. Therefore, SIRT1 alters the cellular physiology for ischemia-induced energy deprivation by increasing mitochondrial biogenesis and efficiency, increasing antioxidant protein expression, and by activation of hypoxia-inducible transcription factor pathways, to name a few (reviewed in [36] and [37]). In the ischemia/reperfused heart, SIRT1 is activated and provides a degree of protection [42], but not at the level observed when SIRT1 is activated prior to the injurious ischemic insult.

SIRT1 neuroprotection may stem, in part, from alteration of the epigenetic profile of the cell through the deacetylation of both histone and non-histone proteins. For example, SIRT1 is known to deacetylate histone H3 at lys9 and 14, and histone H4 at lys16 [43]. In the heart, SIRT1 decreases p300 protein levels (a histone acetyltransferase) [44], which, combined with histone deacetylation, would result in a more repressive and transcriptionally silent chromatin structure. SIRT1 has also been demonstrated to bind to and deacetylate DNMT, thereby increasing or decreasing its methyltransferase activity depending upon the lysine residue deacetylated [45]. As discussed in the previous section, DNA methylation is a major epigenetic factor leading to gene silencing. SIRT1 has also been demonstrated to deacetylate methyl-CpG-binding protein 2 (MeCP2). Deacetylated MeCP2 is released from methylated CpG sites within the brain-derived neurotrophic factor promoter, thus leading to increased expression of brain-derived neurotrophic factor, a known neuroprotective protein [46]. SIRT1 may also regulate a neuroprotective phenotype through the deacetylation and thus activation or inhibition of transcription factors, transcriptional co-activators, and nuclear receptors [37]. Based upon these studies it appears that SIRT1 may regulate the epigenetic status of the cell at various levels, including global changes in chromatin configuration, more specific modulation of histone modifications and DNA methylation at specific gene promoters, and modulating the activity of transcription factors (Fig. 2).

These studies suggest that the epigenetic profiles of ischemic protection by HDAC inhibitors and IPC are quite different, with HDAC inhibitors maintaining acetylation levels and IPC decreasing acetylation levels via SIRT1 activation [42].

The similarities and differences in the epigenetic profile of IPC and HDAC inhibition during ischemia are unclear, but suggest that multiple gene expression profiles exist with each leading to ischemic neuroprotection.

Methylation

Cortical spreading depression (CSD) is one of the stimuli by which ischemia tolerance can be induced in brains [47, 48]. In the rat brain, 24 h after CSD, Rana et al. [47] evaluated the effects of CSD preconditioning on histone H3 lysine 4 (H3K4) and histone H3 lysine 9 (H3K9) di-methylation level in loci affected by CSD-induced preconditioning. CSD resulted in decreased H3K4, but increased H3K9 dimethylation of 2 long interspersed sequences, suggesting alteration of epigenetic regulation of retrotransposable sequences. Selective analysis of 2 neuroprotective genes (*inducible nitric oxide synthase* and *hypoxia-inducible factor-1 α*) also confirmed decreased H3K4 and increased H3K9 dimethylation. The study concluded that CSD-induced preconditioning can alter expression of neuroprotective genes and retrotransposable sequences by histone methylation-mediated epigenetic regulation.

SUMOylation

SUMO conjugation is another epigenetic factor affecting gene expression through alteration of the histone code, chromatin-modifying enzymes, promoter-specific transcription factors, and chromatin-associated proteins [49]. SUMOylation is shown to regulate HDAC recruitment to promoters, and regulation of mono- or di-methylation of H3K4 via affecting histone demethylase LSD1 [49]. It is interesting to note that SIRT1 is post-translationally SUMOylated, which increases its deacetylase activity and its anti-apoptotic activities [50]. SUMOylation plays an important role in neuroprotection in models of hibernation and ischemia tolerance. Hibernators naturally tolerate severe reductions in cerebral blood flow during torpor [51, 52]. For example, our laboratory previously observed that arctic ground squirrels (*Urocitellus parryi*) are remarkably tolerant to global cerebral ischemia during euthermia [53, 54]. Massive protein SUMOylation (including increased SUMO signal in cell nuclei) is reported to occur during torpor in 13-lined ground squirrels (*Spermophilus tridecemlineatus*) [52]. In the same study they also observed that preconditioning increased SUMO conjugation levels in SHSY5Y neuroblastoma cells. SHSY5Y neuroblastoma cells overexpressing Ubc9 (the SUMO-specific E2-conjugating enzyme) were highly resistant to OGD compared with wild-type cells. This study suggests that SUMOylation is necessary and sufficient for the induction of tolerance against OGD-induced damage [52]. In another study Lee et al. [55] observed that,

Fig. 2 Sirtuin 1 (SIRT1) regulates both global and local mechanisms of gene repression and expression. SIRT1 leads to both global and local gene repression through deacetylating histone H3 and H4, and through deacetylating DNA methyltransferase 1 (DNMT1) on lysine residues located within the DNMT1 catalytic domain thereby increasing DNMT1 activity, leading to DNA hyper-methylation and gene repression.

Conversely, SIRT1 can increase gene expression through deacetylating lysine residues within a lysine–glycine repeat region in DNMT1 thereby reducing DNMT1 activity leading to DNA hypomethylation, and through the deacetylation-dependent activation of transcription factors (TF) and inactivation of transcriptional repressors such as methyl-CpG binding protein 2 (MeCP2)

following OGD preconditioning, cultured cortical neurons had elevated SUMO-1 conjugation levels compared with non-preconditioned cells. They also observed that cortical neurons and SHSY5Y cells overexpressing SUMO-1 or SUMO-2 tolerated severe OGD. Overall, these studies demonstrate that preconditioning-induced alterations in protein SUMOylation may play an important role in induction of tolerance against severe cerebral ischemia. However, downstream targets of protein SUMOylation remain to be identified in models of cerebral ischemia tolerance.

ncRNA

A third mechanism of epigenetic regulation is that of ncRNA. There is increasing evidence that ncRNA, by mechanisms not yet fully defined, recruit histone-modifying enzymes and DNA methyltransferases to specific genomic loci thereby affecting epigenetic memory, chromatin architecture, and transcriptional regulation of the cell [56, 57]. Several types of ncRNA have been identified, with microRNA (miRNA) being the best described.

Numerous studies have demonstrated that miRNA expression is altered in the brain following ischemia and contributes to the etiology of ischemic injury. In a MCAO model of cerebral ischemia it was shown that miRNA expression is highly selective and temporally regulated [58]. Changes in the miRNA transcriptome were observed as early as 3 h following reperfusion and lasted for at least 72 h. Ischemia/reperfusion-induced changes in miRNA expression profiles correlated with altered expression of the predicted miRNA target proteins, many of which are involved in transcriptional regulation, inflammation, ionic flux, neuroprotection, and receptor function [58]. These cellular functions are associated with the ischemia/reperfusion pathological response, suggesting altered miRNA expression may contribute significantly to the ischemia/reperfusion damage.

Although ischemia has been demonstrated to alter the expression profile of numerous miRNAs, only a few of these have been experimentally demonstrated to functionally contribute to ischemic cell damage. For example, miR-15a expression is increased in vascular endothelial cells following acute OGD, and was demonstrated to inhibit expression of

the anti-apoptotic gene B-cell lymphoma 2 (*bcl-2*) leading to cell death. Inhibition of miRNA-15a expression *in vivo* increased *bcl-2* protein levels, and reduced cerebral infarct volume in mice following 60 min of MCAO [59]. Similarly, miR-497 expression was increased following transient MCAO and was demonstrated to inhibit *bcl-2* and *bcl-w* expression. Knockdown of miR-497 in mice prevented the downregulation of *bcl-2* and *bcl-w* protein levels in the infarcted region, and attenuated infarct volume and improved neurologic outcome following ischemia [60].

miRNAs have also been demonstrated to be involved in the epigenetic reprogramming of the brain in ischemic tolerance. Several studies have demonstrated that IPC alters the miRNA transcriptome. For example, Dharap and Vemuganti [61] found that 26 miRNAs were upregulated and 25 miRNAs downregulated in the rat cerebral cortex following IPC, with 20 of these miRNAs maintaining altered expression for 3 days. In the mouse cortex, Lusardi et al. [62] compared miRNA expression from preconditioned and injurious ischemic groups, and found that miRNA expression is preferentially upregulated by IPC, but predominately downregulated by injurious ischemia. Bioinformatic analysis of miRNA expression revealed that IPC differentially regulates miRNAs that primarily target transcriptional regulators. For example, one of the most notable downregulated miRNAs was miR-132, which regulates MeCP2 expression, a major regulator of gene expression. Consistent with the miRNA findings, MeCP2 protein expression was increased in the IPC brain and was required for ischemic tolerance, as knocking out MeCP2 expression in mice abrogated IPC-mediated neuroprotection [62].

Conclusions

Ischemic tolerance represents the end product of numerous signaling pathways, which transiently act in concert to modify the activity of epigenetic proteins, which, in turn, collectively alter the genomic expression profile of the cell, leading to neuroprotection. Therefore, the activation or inhibition of gene sets versus individual signaling pathways may represent a viable approach for the therapeutic intervention for cerebral ischemia. However, we are clearly only in the infancy of our understanding of the multitude of epigenetics mechanisms mediating ischemic tolerance or injury. The participation of DNA, histone, and non-histone chromatin-associated proteins, as well as ncRNAs, underscores a level of complexity of the epigenetic response of the cell to ischemic stress that will take an important effort to understand. The continued failure of clinical trials for stroke beckons for alternative approaches for ischemic neuroprotection which may be based on this newly acquired knowledge of epigenetics. As discussed in this review, the data collected thus far

demonstrate that further investigations into the epigenetics of ischemic cell death and IPC-mediated ischemic neuroprotection are well justified and may well lead to novel targets for ischemic neuroprotection.

Acknowledgments This work was supported by National Institutes of Health grants NS45676, NS054147 and NS34773 (to M.A.P.P.), and NS073779 (to K.R.D.) and by a Postdoctoral award from the American Heart Association, Greater Southeast Affiliate (to J.W.T). The authors declare no competing financial interests. Full conflict of interest disclosures are available in the electronic supplementary materials for this article.

Required Author Forms Disclosure forms provided by the authors are available with the online version of this article.

References

- O'Collins VE, Macleod MR, Donnan GA, Horky LL, van der Worp BH, Howells DW. 1,026 experimental treatments in acute stroke. *Ann Neurol* 2006;59:467–477.
- Iadecola C, Anrather J. Stroke research at a crossroad: asking the brain for directions. *Nat Neurosci* 2011;14:1363–1368.
- Lin HW, Thompson JW, Morris KC, Perez-Pinzon MA. Signal transducers and activators of transcription: STATs-mediated mitochondrial neuroprotection. *Antiox Redox Signal* 2011;14:1853–61.
- Barone FC, White RF, Spera PA, et al. Ischemic preconditioning and brain tolerance: temporal histological and functional outcomes, protein synthesis requirement, and interleukin-1 receptor antagonist and early gene expression. *Stroke* 1998;29:1937–1950.
- Roth S, Li B, Rosenbaum PS, et al. Preconditioning provides complete protection against retinal ischemic injury in rats. *Invest Ophthalmol Vis Sci* 1998;39:777–785.
- Benardete EA, Bergold PJ. Genomic analysis of ischemic preconditioning in adult rat hippocampal slice cultures. *Brain Res* 2009;1292:107–122.
- Feng Z, Davis DP, Sasik R, Patel HH, Drummond JC, Patel PM. Pathway and gene ontology based analysis of gene expression in a rat model of cerebral ischemic tolerance. *Brain Res* 2007;1177:103–123.
- Prasad SS, Russell M, Nowakowska M, Williams A, Yauk C. Gene expression analysis to identify molecular correlates of pre- and post-conditioning derived neuroprotection. *J Mol Neurosci* 2012;47:322–339.
- Stenzel-Poore MP, Stevens SL, Xiong Z, et al. Effect of ischaemic preconditioning on genomic response to cerebral ischaemia: similarity to neuroprotective strategies in hibernation and hypoxia-tolerant states. *Lancet* 2003;362:1028–1037.
- Simon RP, Meller R, Zhou A, Henshall D. Can genes modify stroke outcome and by what mechanisms? *Stroke* 2012;43:286–291.
- Feinberg AP. Phenotypic plasticity and the epigenetics of human disease. *Nature* 2007;447:433–440.
- Langley B, Gensert JM, Beal MF, Ratan RR. Remodeling chromatin and stress resistance in the central nervous system: histone deacetylase inhibitors as novel and broadly effective neuroprotective agents. *Curr Drug Targets CNS Neurol Disord* 2005;4:41–50.
- Egger G, Jeong S, Escobar SG, et al. Identification of DNMT1 (DNA methyltransferase 1) hypomorphs in somatic knockouts suggests an essential role for DNMT1 in cell survival. *PNAS* 2006;103:14080–14085.
- Okano M, Bell DW, Haber DA, Li E. DNA methyltransferases Dnmt3a and Dnmt3b are essential for de novo methylation and mammalian development. *Cell* 1999;99:247–257.

15. Qureshi IA, Mehler MF. Emerging role of epigenetics in stroke: part 1: DNA methylation and chromatin modifications. *Arch Neurol* 2010;67:1316–1322.
16. Endres M, Meisel A, Biniszkiwicz D, et al. DNA methyltransferase contributes to delayed ischemic brain injury. *J Neurosci* 2000;20:3175–3181.
17. Endres M, Fan G, Meisel A, Dirnagl U, Jaenisch R. Effects of cerebral ischemia in mice lacking DNA methyltransferase 1 in post-mitotic neurons. *Neuroreport* 2001;12:3763–3766.
18. Hu CJ, Chen SD, Yang DI, et al. Promoter region methylation and reduced expression of thrombospondin-1 after oxygen-glucose deprivation in murine cerebral endothelial cells. *J Cereb Blood Flow Metab* 2006;26:1519–1526.
19. Udali S, Guarini P, Moruzzi S, Choi SW, Friso S. Cardiovascular epigenetics: From DNA methylation to microRNAs. *Mol Aspects Medicine* 2013;34:883–901.
20. Casas JP, Hingorani AD, Bautista LE, Sharma P. Meta-analysis of genetic studies in ischemic stroke: thirty-two genes involving approximately 18,000 cases and 58,000 controls. *Arch Neurol* 2004;61:1652–1661.
21. Williamson WD, Pinto I. Histones and genome integrity. *Front Biosci* 2012;17:984–995.
22. Li G, Reinberg D. Chromatin higher-order structures and gene regulation. *Curr Opin Genet Develop* 2011;21:175–186.
23. Bannister AJ, Kouzarides T. Regulation of chromatin by histone modifications. *Cell Res* 2011;21:381–395.
24. Jenuwein T, Allis CD. Translating the histone code. *Science* 2001;293:1074–1080.
25. Fessler EB, Chibane FL, Wang Z, Chuang DM. Potential roles of HDAC inhibitors in mitigating ischemia-induced brain damage and facilitating endogenous regeneration and recovery. *Curr Pharm Design* 2013 Feb 19 [Epub ahead of print].
26. Shimamura M, Sato N, Nakagami H, Taniyama Y, Morishita R. Development of nucleic acid drugs for neurological disorders. *Curr Topics Med Chem* 2012;12:1621–1629.
27. Chen YT, Zang XF, Pan J, et al. Expression patterns of histone deacetylases in experimental stroke and potential targets for neuroprotection. *Clin Exp Pharmacol Physiol* 2012;39:751–758.
28. Noh KM, Hwang JY, Follenzi A, et al. Repressor element-1 silencing transcription factor (REST)-dependent epigenetic remodeling is critical to ischemia-induced neuronal death. *PNAS* 2012;109:E962–E971.
29. Faraco G, Pancani T, Formentini L, et al. Pharmacological inhibition of histone deacetylases by suberoylanilide hydroxamic acid specifically alters gene expression and reduces ischemic injury in the mouse brain. *Mol Pharmacol* 2006;70:1876–1884.
30. Ren M, Leng Y, Jeong M, Leeds PR, Chuang DM. Valproic acid reduces brain damage induced by transient focal cerebral ischemia in rats: potential roles of histone deacetylase inhibition and heat shock protein induction. *J Neurochem* 2004;89:1358–1367.
31. Qi X, Hosoi T, Okuma Y, Kaneko M, Nomura Y. Sodium 4-phenylbutyrate protects against cerebral ischemic injury. *Mol Pharmacol* 2004;66:899–908.
32. Kim HJ, Rowe M, Ren M, Hong JS, Chen PS, Chuang DM. Histone deacetylase inhibitors exhibit anti-inflammatory and neuroprotective effects in a rat permanent ischemic model of stroke: multiple mechanisms of action. *J Pharmacol Exp Ther* 2007;321:892–901.
33. Langley B, D'Annibale MA, Suh K, et al. Pulse inhibition of histone deacetylases induces complete resistance to oxidative death in cortical neurons without toxicity and reveals a role for cytoplasmic p21(waf1/cip1) in cell cycle-independent neuroprotection. *J Neurosci* 2008;28:163–176.
34. Yildirim F, Gertz K, Kronenberg G, et al. Inhibition of histone deacetylation protects wildtype but not gelsolin-deficient mice from ischemic brain injury. *Exp Neurol* 2008;210:531–542.
35. Zhao TC, Cheng G, Zhang LX, Tseng YT, Padbury JF. Inhibition of histone deacetylases triggers pharmacologic preconditioning effects against myocardial ischemic injury. *Cardiovasc Res* 2007;76:473–481.
36. Morris KC, Lin HW, Thompson JW, Perez-Pinzon MA. Pathways for ischemic cytoprotection: role of sirtuins in caloric restriction, resveratrol, and ischemic preconditioning. *J Cereb Blood Flow Metab* 2011;31:1003–1019.
37. Zhang F, Wang S, Gan L, et al. Protective effects and mechanisms of sirtuins in the nervous system. *Prog Neurobiol* 2011;95:373–395.
38. Della-Morte D, Dave KR, DeFazio RA, Bao YC, Raval AP, Perez-Pinzon MA. Resveratrol pretreatment protects rat brain from cerebral ischemic damage via a sirtuin 1-uncoupling protein 2 pathway. *Neuroscience* 2009;159:993–1002.
39. Raval AP, Dave KR, Perez-Pinzon MA. Resveratrol mimics ischemic preconditioning in the brain. *J Cereb Blood Flow Metab* 2006;26:1141–1147.
40. Yan W, Fang Z, Yang Q, et al. Sirt1 mediates hyperbaric oxygen preconditioning-induced ischemic tolerance in rat brain. *J Cereb Blood Flow Metab* 2013;33:396–406.
41. Kakefuda K, Fujita Y, Oyagi A, et al. Sirtuin 1 overexpression mice show a reference memory deficit, but not neuroprotection. *Biochem Biophys Res Commun* 2009;387:784–788.
42. Nadtochiy SM, Yao H, McBurney MW, et al. SIRT1-mediated acute cardioprotection. *American journal of physiology Heart Circ Physiol* 2011;301:H1506–512.
43. Imai S, Armstrong CM, Kaerberlein M, Guarente L. Transcriptional silencing and longevity protein Sir2 is an NAD-dependent histone deacetylase. *Nature* 2000;403:795–800.
44. Kuno A, Hori YS, Hosoda R, et al. Resveratrol improves cardiomyopathy in dystrophin-deficient mice through SIRT1 protein-mediated modulation of p300 protein. *J Biol Chem* 2013;288:5963–5972.
45. Peng L, Yuan Z, Ling H, et al. SIRT1 deacetylates the DNA methyltransferase 1 (DNMT1) protein and alters its activities. *Mol Cell Biol* 2011;31:4720–4734.
46. Zocchi L, Sassone-Corsi P. SIRT1-mediated deacetylation of MeCP2 contributes to BDNF expression. *Epigenetics* 2012;7:695–700.
47. Rana G, Donizetti A, Virelli G, et al. Cortical spreading depression differentially affects lysine methylation of H3 histone at neuroprotective genes and retrotransposon sequences. *Brain Res* 2012;1467:113–119.
48. Matsushima K, Schmidt-Kastner R, Hogan MJ, Hakim AM. Cortical spreading depression activates trophic factor expression in neurons and astrocytes and protects against subsequent focal brain ischemia. *Brain Res* 1998;807:47–60.
49. Ouyang J, Gill G. SUMO engages multiple corepressors to regulate chromatin structure and transcription. *Epigenetics* 2009;4:440–444.
50. Yang Y, Fu W, Chen J, et al. SIRT1 sumoylation regulates its deacetylase activity and cellular response to genotoxic stress. *Nat Cell Biol* 2007;9:1253–1262.
51. Dave KR, Christian SL, Perez-Pinzon MA, Drew KL. Neuroprotection: lessons from hibernators. *Comp Biochem Physiol B Biochem Mol Biol* 2012;162:1–9.
52. Lee YJ, Miyake S, Wakita H, et al. Protein SUMOylation is massively increased in hibernation torpor and is critical for the cytoprotection provided by ischemic preconditioning and hypothermia in SHSY5Y cells. *J Cereb Blood Flow Metab* 2007;27:950–962.
53. Dave KR, Anthony Defazio R, Raval AP, et al. Protein kinase C epsilon activation delays neuronal depolarization during cardiac arrest in the euthermic arctic ground squirrel. *J Neurochem* 2009;110:1170–1179.
54. Dave KR, Prado R, Raval AP, Drew KL, Perez-Pinzon MA. The arctic ground squirrel brain is resistant to injury from cardiac arrest during euthermia. *Stroke* 2006;37:1261–1265.
55. Lee YJ, Castri P, Bembry J, Maric D, Auh S, Hallenbeck JM. SUMOylation participates in induction of ischemic tolerance. *J Neurochem* 2009;109:257–267.
56. Mattick JS, Amaral PP, Dinger ME, Mercer TR, Mehler MF. RNA regulation of epigenetic processes. *BioEssays* 2009;31:51–59.

57. Bernstein E, Allis CD. RNA meets chromatin. *Genes Develop* 2005;19:1635–1655.
58. Dharap A, Bowen K, Place R, Li LC, Vemuganti R. Transient focal ischemia induces extensive temporal changes in rat cerebral microRNAome. *J Cereb Blood Flow Metab* 2009;29:675–687.
59. Yin KJ, Deng Z, Hamblin M, et al. Peroxisome proliferator-activated receptor delta regulation of miR-15a in ischemia-induced cerebral vascular endothelial injury. *J Neurosci* 2010;30:6398–6408.
60. Yin KJ, Deng Z, Huang H, et al. miR-497 regulates neuronal death in mouse brain after transient focal cerebral ischemia. *Neurobiol Dis* 2010;38:17–26.
61. Dharap A, Vemuganti R. Ischemic pre-conditioning alters cerebral microRNAs that are upstream to neuroprotective signaling pathways. *J Neurochem* 2010;113:1685–1691.
62. Lusardi TA, Farr CD, Faulkner CL, et al. Ischemic preconditioning regulates expression of microRNAs and a predicted target, MeCP2, in mouse cortex. *J Cereb Blood Flow Metab* 2010;30:744–756.