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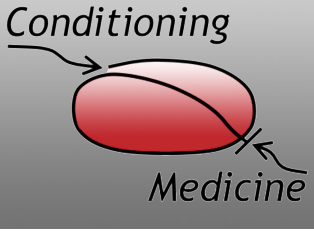

REVIEW ARTICLE | OPEN ACCESS

Nuances in the molecular controls of gene expression underlying preconditioning against cerebral ischemia

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Abstract: The molecular mechanisms underpinning neuroprotection afforded by preconditioning against cerebral ischemia have been a topic of interest since the first reports detailing that an endogenous induction of protective responses can occur. This review serves to update several modulators of gene expression that contribute to the induction of tolerance by various preconditioning stimuli. We focus here on updated information regarding key transcription factors, including HIF1-alpha, NF-kappaB, and NRF2, as well as the more recently explored topic of microRNAs.

Introduction

Mechanistic studies of brain conditioning that confers tolerance to noxious stimuli are very important for the development of various therapies. Therapies considered "conditioning" confer protection by invoking a "tolerant" state when, if a subsequent stronger challenge occurs, the organism incurs less deleterious outcomes than if the stronger challenge had occurred alone. Harnessing the endogenous programs that biological systems employ could not only uncover new therapeutic targets and timing strategies, but also may elicit fewer side effects to the organism.

Induction of a tolerant state is often viewed as biphasic, wherein distinct mechanisms are implicated in rapid and delayed tolerance windows. Delayed tolerance hinges on new protein synthesis and engenders neuronal protection days after the preconditioning stimulus. In contrast, rapid ischemic tolerance produces neuroprotection within minutes to hours, seemingly independent of new gene expression. The mechanisms of both types of brain tolerance involve a large number of molecular and signaling pathways. As the topic of this review centers around alterations in gene expression, we will focus primarily on the induction of delayed preconditioning, but will also draw into literature exploring related mechanisms in the rapid preconditioning window. In addition, given the number of substantial reviews, this article will focus on more recent findings, with references to more seminal papers and articles where relevant.

The definition of "conditioning" itself has yielded a generous amount of discussion, some of which certainly goes beyond the scope of this review. For the purposes of this review, we will focus primarily on stimuli that have been widely reported in the literature as conditioning (e.g., mild ischemia, sevoflurane, resveratrol, or mild inflammatory agents), and discuss newer aspects of the regulation of mechanisms underlying these paradigms that appear to be components of exerting tolerance in relation to the regulation of gene expression.

Alteration of gene expression as a key to understanding the tolerant state

One of the major themes in the molecular adaptive state that confers robust tolerance is the alteration of protein expression. Under classical molecular biology dogma, this process can be specifically controlled at the genome level, with activation or suppression of gene transcription via transcriptional factors or chromatin rearrangement, often controlled by posttranslational modifications on regulatory proteins. However, the recent discovery of the role of non-coding RNAs including microRNAs (miRNA) has revealed that genomic control of protein expression is only a part of the equation. We will discuss updates to the major mechanisms of genome-level transcriptional contribution to tolerance as well as more recent discussion on the potential involvement of miRNAs.

Genetic reprogramming

Early hypotheses regarding the molecular mechanisms of ischemic preconditioning suggested that alterations in gene/protein expression may be critical, particularly in the so-called "delayed" window of ischemic tolerance (Chen et al., 1997; Truettner et al., 2002; Gidday, 2006). Multiple studies

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proceeded to support this concept in various cerebral ischemic tolerance models, by using either an inhibitor of *de novo* gene expression (Barone et al., 1998), or by using expression arrays to mine differences between gene/gene family expression associated with the tolerant state (Bernaudin et al., 2002a; Marsh et al., 2009; Prasad et al., 2012). The use of expression profiling made clear that not only is induction of new protein expression apparent after preconditioning, but also suppression of proteins is associated with preconditioning. Over the course of the next decade of research, several key transcription factors were identified to be critically associated with preconditioning-induced neuroprotection, including recent updates on HIF-1, nuclear factor-κB (NK-κB) and CREB (Fig. 1), as well as NRF-2. We will focus on those more recent developments.

HIF-1

HIF-1 is a heterodimeric transcription factor that regulates the expression of hundreds of genes in response to reduced oxygen availability. The fully active HIF-1 is composed of the 120-kDa O_2-regulated HIF-1α subunit and a 91 to 94-kDa constitutively expressed HIF-1β subunit. Under normoxic conditions, HIF-1α is subjected to hydroxylation by prolyl-4 hydroxylase domain (PHD) family proteins. This hydroxylation generates a binding site for a component of the ubiquitin ligase complex, leading to polyubiquitination of HIF-1α and targeting it for proteasomal degradation. Under hypoxic conditions, the rate of hydroxylation and degradation declines. The non-hydroxylated proteins accumulate, dimerize with the HIF-1β subunit, and regulate a number of genes important in glycolysis, erythropoiesis, angiogenesis and catecholamine metabolism through several direct and indirect mechanisms (Semenza, 2011) (Fig. 1).

The HIF-1-mediated alterations in gene expression contribute to the brain's adaptive response to preconditioning. Under many preconditioning stimuli, evidence of transactivation by HIF-1 is noted, including nuclear translocation (Bernaudin et al., 2002b), DNA binding activity (Ruscher et al., 2002; Prass et al., 2003; Shao et al., 2005), and the increased expression of well-known target genes, including EPO, VEGF, and iNOS (Bernaudin et al., 2002b; Ruscher et al., 2002; Mu et al., 2003; Prass et al., 2003; Shao et al., 2005; Gu et al., 2008; Li et al., 2008; Chu et al., 2010; Lee et al., 2016), all of which play important roles in the establishment of tolerance under different stimuli. One of two cytokines with HIF-1α binding sequences in the promoter region, CXCL12β mRNA and total CXCL12 protein are upregulated in hypoxic conditioned cortex, and inhibition of the receptor for CXCL12 (CXCR4) effectively blocked intermittent hypoxic preconditioning-induced protection against subsequent transient focal ischemia (Selvaraj et al., 2017). Thus, CXCL12 may be an interesting downstream gene transactivated by HIF-1α that contributes to neuroprotective effects of preconditioning.

HIF-1 activation is typically characterized as neuroprotective and is implicated in preconditioning-afforded ischemic protection. Pharmacological inhibition of HIF-1α disrupts the neuroprotective effects of hypoxic and remote limb preconditioning (Lee et al., 2016; Selvaraj et al., 2017). However, the precise mechanism – including the cells or environment wherein HIF-1α activity occurs relevant to tolerance – is debatable in the context of cerebral ischemia. Although HIF-1α inactivation in calcium/calmodulin-dependent protein kinase (CaMK) II α-positive neurons significantly increased moderate ischemia-induced brain injury in mice, inactivation of HIF-1 in neurons does not affect the development of ischemic tolerance in response to hypoxic preconditioning (Baranova et al., 2007). Several in vitro studies indicate that HIF-1 activation in CaMKIIα-negative cells (e.g., glia) contributes to hypoxic preconditioning-induced neuroprotection. In primary cultured astrocytes, hypoxic preconditioning markedly increases the activity of HIF-1 (Ruscher et al., 2002), which not only mediates astrocyte tolerance against OGD (Liu and Alkayed, 2005) or oxidative injury (Chu et al., 2010) but also provides a paracrine neuroprotective effect on neurons (Ruscher et al., 2002). In addition, increased expression of CXCL12 following intermittent hypoxic preconditioning occurs in brain microvessels *in vivo*, and is associated with decreased leukocyte infiltration into the brain following a subsequent ischemic challenge (Selvaraj et al., 2017).

Many preconditioning stimuli, including hypoxia, remote limb ischemia, hyperbaric oxygen, and many pharmacological agents, induce an increase in HIF-1α protein expression (Hua et al., 2003; Liu and Alkayed, 2005; Mu et al., 2005; Jeyaseelan et al., 2008; Li et al., 2008; Limatola et al., 2010; Lee et al., 2016; Xia et al., 2017). Hypoxic preconditioning increases the mRNA expression of HIF-1α (Shao et al., 2005) and the expression of HIF-1β (Bergeron et al., 2000), suggesting that hypoxia may regulate HIF-1 activity at the transcriptional level. Inhibition of critical signaling pathways such as Erk1/2 block HIF-1α upregulation subsequent to thrombin (Hua et al., 2003) or isoflurane (Li et al., 2008) preconditioning. In addition, an inhibitor of PI3K significantly reduces the enhanced expression of HIF-1α induced by Ginkgolide B and ischemic preconditioning (Wu et al., 2009). However, the upregulation of HIF-1 is often transient, as expression levels of HIF-1α protein are highly impacted by the inhibition of its degradation. In this sense, although preconditioning often induces an upregulation of HIF-1, hypoxic preconditioning induces an upregulation of PHD-2, the predominant PHD isoform responsible for regulating (via degradation) the burst in HIF-1α protein expression after hypoxic stimuli in neonatal rat brain (Jones et al., 2006). Such hypoxia-induced modulation of PHD expression is absent in an HIF-1-deficient cell line (Hofbauer et al., 2003), indicating that HIF-1 may be regulating its own degradation mechanisms.

Nrf2

Another transcription factor that is highly sensitive to and activated by increased levels of oxidative stress, nuclear factor (erythroid-derived 2)-like 2 (NFE2L2, or Nrf2) is a member of a subset of the basic leucine zipper family of transcription factors. Similar to HIF-1α, Nrf2 is quickly degraded under normal conditions. In the context of oxidative stress, Nrf2 protein is stabilized and translocates to the nucleus, and promotes the expression of many antioxidant genes by binding to the antioxidant response element (ARE) promoter.

Several studies over the past few years implicate Nrf2 in upregulating the antioxidant response that helps to confer an ischemia-tolerant state under several non-ischemic but often-used preconditioning paradigms, including hyperbaric oxygen, sevoflurane, resveratrol and sulforaphane, as well as other pharmacological agents (Alfieri et al., 2013; Narayanan et al., 2015; Xue et al., 2016; Cai et al., 2017; Wu et al., 2017). Furthermore, tert-butylhydroquinone, a small molecule activator of Nrf2, mimics preconditioning when administered 1 to 3 days prior to a cerebral ischemic challenge by reducing subsequent ischemic injury; knockout of Nrf2 blocks the ability of tert-butylhydroquinone to exert ischemic protection (Shih et al., 2005).

Interestingly, as with HIF1, the point of action from where Nrf2 may exert its effects in ischemic tolerance may be at a non-neuronal location. Indeed, further studies with ischemic, resveratrol, and sulforaphane preconditioning point toward Nrf2 functioning within cerebral cells other than neurons. After sulforaphane preconditioning stimulus, expression of Nrf2 is particularly evident in cerebral microvessels; after the subsequent ischemic challenge (i.e., when exhibiting ischemic tolerance), Nrf2 is expressed robustly in the perivascular

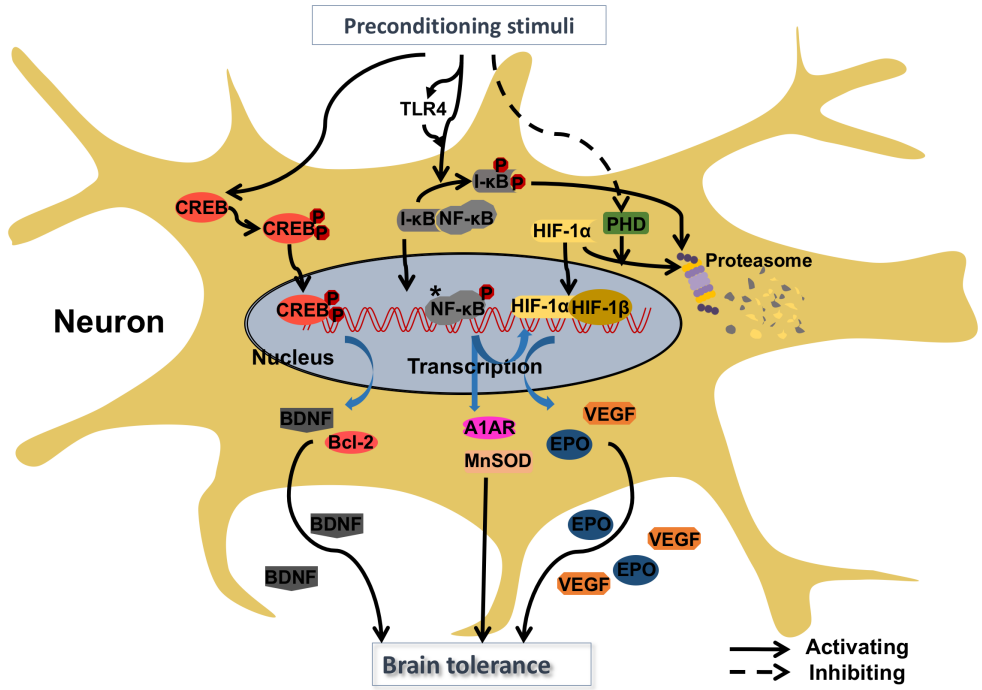

Figure 1. Neuroprotective pathways associated with transcriptional transactivation following preconditioning stimuli. Illustrated are some of the pathways leading to gene transactivation thought to underlie several preconditioning paradigms. While not reviewed in this article, cyclic AMP response element binding protein (CREB) has been implicated as a major transcriptional factor associated with many heavily reviewed neuroprotective pathways. In addition, nuclear factor-kappa B (NF-kB)-stimulated transactivation has been associated with a protective preconditioned state, despite the role of this protein in a deleterious aspect following severe ischemic insults (thus denoted with an asterisk). Hypoxia-inducible factor 1 (HIF-1) also appears to function in the induction of a preconditioned state, with activity associated with the transactivation of many classic protective molecules, including vascular endothelial growth factor (VEGF) and erythropoietin (EPO).

astrocytes in the peri-infarct area and the endothelium within the infarct (Alfieri et al., 2013). Furthermore, Nrf2 is necessary for oxygen-glucose deprivation-induced preconditioning in co-cultures containing neurons and astrocytes, as knockout of Nrf2 reduces the degree to which the preconditioning oxygen-glucose deprivation stimulus confers protection (Narayanan et al., 2017). Also supporting a role for Nrf2 in astrocytes, hyperbaric oxygen preconditioning co-cultures of spinal astrocytes/neurons leads to heightened Nrf2 activity and gene transactivation preferentially in astrocytes (Xu et al., 2014a).

NF-κB

NF-κB is a ubiquitously expressed, inducible transcription factor that consists of preformed DNA-binding dimers. Five different NF-κB subunits are found in mammals. The most common subunits expressed in neurons are p50 and RelA (p65), forming homo- and heterodimers in various combinations. In unstimulated cells, NF-κB remains in the cytoplasm tethered by the interaction with inhibitory I-κB proteins. Upon stimulation, I-κB is phosphorylated, polyubiquitinated, and then degraded by the proteasome, allowing NF-κB to be released and translocate into the nucleus to stimulate gene transcription (Fig. 1). However, the particular subsets of genes transactivated by NF-κB depends heavily on cofactors and post-translational modifications. NF-κB activation upregulates genes encoding proteins that involve both cell survival and cell death, suggesting a seemingly contradictory role for NF-κB. Also, NF-κB acts as a major mediator of pro-inflammatory responses in glial cells through regulating iNOS, proinflammatory cytokines and cell adhesion molecules.

NF-κB is activated in neurons and glial cells in brain disorders, including stroke. Mice deficient in the p50 subunit of NF-κB had a significant reduction in infarct size in both transient and permanent stroke models, indicating a detrimental role of NF-κB following cerebral ischemia (Schneider et al., 1999; Nurmi et al., 2004), possibly through potentiating the proinflammatory response (Wang et al., 2007). Preconditioning inhibits NF-κB activity after severe ischemia or epilepsy in models of brain tolerance (Blondeau et al., 2001). Likewise, anesthetic preconditioning with sevoflurane confers neuroprotection via suppressing an NF-κB-mediated inflammatory response after focal ischemic brain injury (Wang et al., 2011). However, NF-κB activity initiated following severe stroke appears to be different than NF-κB activity initiated after sublethal stressors such as preconditioning stimuli. Ischemic preconditioning or chemical preconditioning with different agents induces a rapid and transient activation of NF-κB by increasing its DNA binding activity and nuclear translocation in vivo and in vitro (Blondeau et al., 2001; Ravati et al., 2001; Jiang et al., 2003). Hypoxic preconditioning not only increases the phosphorylation of NF-κB (Bigdeli and Khoshbaten, 2008) but also persistently upregulates its expression (Rybnikova et al., 2008). Inhibition of NF-κB by its inhibitor diethyldithiocarbamate or a κB decoy DNA abolishes preconditioning-induced delayed neuroprotection, suggesting that NF-κB activation is required for the signal transduction that underlies the development of brain tolerance (Blondeau et al., 2001; Ravati et al., 2001). NF-κB can be post-translationally modified and regulated, which could impact the specific downstream targets for transactivation. Indeed, acetylation of RelA at a specific lysine residue (Lys310) appears to correlate with the deleterious role following lethal ischemia, whereas reduced acetylation at Lys310 is observed following a sublethal preconditioning ischemic stimulus (Lanzillotta et al., 2013). The toxicity of acetylation at Lys310 was confirmed in vitro using the RelA mutant K310R, which cannot be acetylated at Lys310. Overexpression of RelA increases toxicity following lethal oxygen-glucose deprivation, but overexpression of RelA-K310R does not exacerbate OGD toxicity, nor increase promoter activity at a target known to exert neurotoxicity (Lanzillotta et al., 2013). More detailed analyses of post-translational modifications under various preconditioning stimuli will likely contribute to our understanding about the timing and specificity of NF-κB actions.

Upstream regulators of NF-κB in preconditioning scenarios have been identified and reviewed, including TNF-α, EPO, NMDA receptors, toll-like receptor 4 (TLR4), and others (Ridder and Schwaninger, 2009). Ischemic preconditioning-induced NF-κB activation is impaired in TLR4-deficient mice, suggesting that the TLR4 signal is likely to participate in the regulation of NF-κB (Pradillo et al., 2009), which may then further the cytokine signaling by promoting the transcription of interleukin 1-beta.

Interestingly, there are likely several feedback loops involved in NF-κB signaling. For example, preconditioning-induced transient NF-κB activation may lead to direct transcriptional activation of the inhibitory protein I-κB, and thus inhibit its own activation after a subsequent injury (Blondeau et al., 2001). Crosstalk with other transactivators involved in preconditioning also seems likely to involve NF-κB. One of the targets of NF-κB transactivation is the gene that encodes the alpha subunit of HIF1, which is discussed above as a potential regulator of several forms of preconditioning. Together, these data suggest that NF-κB is at the intersection of cell death and survival pathways in cerebral preconditioning, but its role and precise mechanism and regulation need to be further evaluated.

Translational control of gene expression: miRNAs

One of the most rapidly growing areas in molecular research involves non-coding RNAs, including microRNAs. Although various species of non-coding RNAs have emerged, the majority of preconditioning research thus far has focused on the 22-nucleotide microRNAs. MicroRNAs (miRNAs) are a novel family of noncoding short RNA molecules that are able to rapidly regulate post-transcriptional gene expression, primarily by directly binding to complementary sequences in the 3' untranslated region (3'UTR) of target mRNAs and leading to their degradation of translational arrest. Although many genes that are involved in common cellular functions are not targeted by miRNAs, at least one-third of human genes can be targeted by miRNAs (Lewis et al., 2005). The preference of miRNA in targeting of non-"housekeeping" genes leads to the speculation that miRNAs serve as a rapid response to environmental changes that are distinct from (but perhaps still contributing to) long-term adaptive responses. Several brain-specific miRNAs have been discovered in mouse and human differentiating neurons, supporting the notion that these miRNAs serve to establish and maintain neuronal protein expression profiles, and function as effectors in synaptic plasticity.

miRNAs after injurious cerebral ischemia

miRNAs serve important roles in a variety of pathological processes including ischemic and traumatic brain injuries, as well as various neurodegenerative disorders (Saugstad, 2010). After focal cerebral ischemia, the cerebral miRNA expression pattern is significantly altered (Jeyaseelan et al., 2008; Dharap et al., 2009), prompting the investigation into several miRNAs for their roles in neuronal death and functional recovery after ischemia (Saugstad, 2010; Wang et al., 2017). Following transient MCAO, cerebral expression of miRNA130a increases (Sepramaniam et al., 2012; Wang et al., 2017), parallel to the increased leakage of the blood brain barrier (Wang et al., 2017). Both infarct and vascular leakage are attenuated by inhibition of miRNA130a using antagomir 130a (Wang et al., 2017). Many other studies have implicated other miRNAs in the context of cerebral ischemic injury and included the use of antagomirs (inhibitory molecules directed at specific miRNAs), including miRNA200a, miRNA106b-5p (Li et al., 2017), miRNA182 (Yi et al., 2017), miRNA124 (Liu et al., 2013), miRNA431 (Han et al., 2017), and miRNA130b (Zheng et al., 2017). Because the groups of targeted genes differ between miRNAs, some miRNAs promote ischemic injury (e.g., miRNA130a, miRNA182, miRNA106b-5p, miRNA182), while others can be used in protective efforts (e.g., miRNA431, miRNA130b).

miRNAs as part of the preconditioning machinery

Can manipulation of miRNAs underlie preconditioning-afforded neuroprotection? In addition to research directed at alteration of miRNAs following injurious ischemia, miRNAs also appear to be capable of impacting endogenous neuroprotection in the brain's response to ischemic preconditioning (Dharap and Vemuganti, 2010; Lee et al., 2010; Lusardi et al., 2010). miRNA profiles are responsive to a variety of preconditioning stimuli, and likely involve differences between cell type and proximity to the ischemic insult (Bell et al., 2017). In addition, the timing of the effects of miRNAs could encompass a wide timeframe, as some changes in miRNA expression persist even at 3 days after preconditioning ischemia.

Currently, over 500 different miRNAs have been found to be differentially expressed under various preconditioning stimuli (Shi et al., 2013; Bell et al., 2017), as well as alterations in miRNA profiles occurring as a result of ischemic post-conditioning (Miao et al., 2016). Despite the identification of a small subset of altered miRNAs occurring in more than one study, variability remains in the experimental observations of direction of gene expression change and specific miRNA involved. This variability may be due to several factors, including the heavy use of culture models rather than consistent animal models, as well as differences between nuances of the preconditioning stimuli.

This rapidly evolving field has identified several potential miRNAs that may modulate preconditioning. Using a mouse model of ischemic preconditioning, Lee et al. found that, from a total of 360 miRNAs, two miRNA families (miR-200 and miR-182) are particularly upregulated within three hours after ischemic preconditioning (Lee et al., 2010). Transfections of these two miRNA family members protect mouse neuroblast cells against OGD damage by upregulating HIF-1α (Lee et al., 2010), a surprising observation given that members of miR-200 are among miRNAs that are upregulated after lethal ischemia (Stary et al., 2015); (Di et al., 2014). In addressing the associated targets of miR-200 that might contribute to tolerance, it is currently thought that PHD2, a known target of the miR-200 family and a factor in the degradation of HIF-1α, may be selectively repressed at the translational level and therefore may allow sustained expression of HIF-1α contributing to ischemic preconditioning (Lee et al., 2010; Di et al., 2014). Other miRNAs, including miR-182 and miR-429, are upregulated in the early phase after ischemic preconditioning (Lee et al., 2010), and may contribute to the tolerant state, despite having deleterious effects following stronger ischemic challenges (Yi et al., 2017). In addition to these particular miRNAs, miRNA-21 may have particular implication in promoting ischemic tolerance, as it is consistently and robustly upregulated over the initial three days following ischemic preconditioning (Dharap and Vemuganti, 2010); a detailed review of this particular miRNA was recently published (http://www.conditionmed.org/Data/View/180?type=200).

The global transcriptional regulator methyl CpG binding protein 2 (MeCP2) is both a target of miRNA translational repression and a regulator of miRNAs themselves. In a study in 2010, Lusardi et al. identified putative regions in MeCP2 that could be targeted by miRNAs, including mi-132, which provided a hypothesis to the correlation observed after ischemic preconditioning between increased MeCP2 proteins that were independent of changes in MeCP2 mRNA levels and decreased expression of specific miRNAs (Lusardi et al., 2010). Supporting this postulation, others found that repression of a reporter protein engineered downstream of the MeCP2 3' UTR depends on miR-132 (Su et al., 2015). Similarly, hypertension

preconditioning decreases 10 miRNAs that target MeCP2, implying that MeCP2 protein expression would be predicted to increase following hypertension preconditioning (Dharap and Vemuganti, 2010). Although miR-132 is not among the miRNAs that decrease following hypertension preconditioning, the perceived effects should, in theory, be similar.

In another twist, a subsequent number of studies outside of ischemia note that MeCP2 functions not only as a global transcriptional regulator at the level of the genome, but also specifically inhibits the machinery necessary for the maturation of many miRNAs (Cheng et al., 2014). Thus, the indirect induction of MeCP2 *via* downregulation of suppressive miRNAs that target MeCP2 may further decrease the maturation and activity of a number of other miRNAs. The suppression of those mature miRNAs by MeCP2 may lead to rapid expression of survival-related proteins, or prevention of upregulation of deleterious miRNAs by subsequent stronger ischemic insults. Interestingly, in a completely different system using T cells, overexpression of MeCP2 increases expression of Eif2c2, a component of the machinery used by miRNAs to silence targeted mRNAs (Koelsch et al., 2013). In a different system, miR-21 is identified as a main component of Eif2c2 complexes (Mase et al., 2012). As discussed briefly above and in recent reviews (Xu et al., 2014b) (Yi et al., 2017), miR-21 is associated with neuroprotective effects, including the indirect perpetuation of HIF1a expression. Thus, increased expression of MeCP2 in the preconditioned state may induce both downregulation and upregulation of a wide variety of effector miRNAs that may underlie neuroprotection during tolerance, supported by the observation that MeCP2 knockout mice were more sensitive to mild ischemic insults (e.g., preconditioning) (Lusardi et al., 2010).

An important note must be underscored: miRNA profiles following ischemic episodes can be sexually dimorphic (Lusardi et al., 2014). Given the differences between male and female brain response to ischemic injury, further studies into miRNA regulation and targeting may contribute to the understanding of sex-related differences in ischemic sensitivity and response. Additionally, it is important to note that not all studies have found significant changes in miRNA profiles correlating to non-ischemic preconditioning, suggesting that alteration of miRNAs during the preconditioning window is not an absolute requirement to confer a tolerant state. A study using resveratrol as the preconditioning stimulus found no significant changes in miRNA profiles in brain in the preconditioned state using arrays encompassing all known mouse miRNAs compared to vehicle groups (Lopez et al., 2016). This was surprising, as resveratrol-treated rats displayed differential miRNA expression in heart tissue (Mukhopadhyay et al., 2010). Additionally, the toll-like receptor 9 agonist, cytosine-phosphate-guanine (CpG) can be administered systemically as a preconditioning stimulus against cerebral ischemia, but it does not significantly alter miRNA expression patterns in the preconditioning window (Vartanian et al., 2015). However, following a subsequent stronger ischemic challenge, differential expression of a subset of miRNAs occurs between saline- and CpG-preconditioned groups (Vartanian et al., 2015); this particular subset of miRNAs is associated with neuroprotective effects via indirect upregulation of protective genes. Thus, although ischemic preconditioning itself does appear to be associated with alterations in miRNA profiles, other preconditioning stimuli do not appear to necessitate miRNA differential expression patterns prior to the subsequent ischemic insult in order to confer tolerance, but may still impact miRNA expression and activity during the post-severe ischemic conditions.

Given that the role of miRNAs following stroke itself is still fairly nascent, the upcoming years should provide a more complete view of the contributions of miRNAs to protection afforded by preconditioning stimuli. In addition, exciting and newly emergent studies have identified several other functional non-coding RNA molecules that likely contribute to ischemic injury or recovery, including long non-coding RNA (lncRNA) and PIWI-interacting RNA (piRNA) (Chandran et al., 2017) (Dharap et al., 2011). It will be interesting to see the development of the role of these molecules in preconditioning scenarios.

Conclusion

A number of transcription factors and regulators, including CREB, SIRT1, and other canonical pro-survival effectors of gene expression, as well as alteration to the genome (e.g., DNA methylation and histone modification) have been investigated in terms of preconditioning and induction of the tolerant state, and have been heavily reviewed (Thompson et al., 2013). Despite the identification of these molecules' general impact on the induction of ischemic tolerance, the recent work clearly identifying cell-specific contributions to overall brain tolerance and the emergence of tools that can target specific cell populations demands that much more work is needed to clearly understand the different molecular regulation of gene expression in cell populations – based on cell type, proximity to ischemia, temporal setting, etc. In addition, we are constantly learning of new mechanisms for cellular adaption, such as the modulation of the expression of a wide array of proteins via microRNAs or the suppression of other gene products via the actions of other non-coding RNA species. With these new discoveries, we then further expand our concept of how cells adapt to environmental stressors to better survive subsequent challenges. Thus, the study of the molecular adaptation induced by preconditioning not only may indicate levels at which therapeutic interventions for severe ischemia can be targeted, but also provides interesting insights into the endogenous adaptive responses by cells and tissues to environmental stressors.

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