Epigenetic Regulation of Axon Outgrowth and Regeneration in CNS Injury: The First Steps Forward

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Abstract Inadequate axonal sprouting and lack of regeneration limit functional recovery following neurologic injury, such as stroke, brain, and traumatic spinal cord injury. Recently, the enhancement of the neuronal regenerative program has led to promising improvements in axonal sprouting and regeneration in animal models of axonal injury. However, precise knowledge of the essential molecular determinants of this regenerative program remains elusive, thus limiting the choice of fully effective therapeutic strategies. Given that molecular regulation of axonal outgrowth and regeneration requires carefully orchestrated waves of gene expression, both temporally and spatially, epigenetic changes may be an ideal regulatory mechanism to address this unique need. While recent evidence suggests that epigenetic modifications could contribute to the regulation of axonal outgrowth and regeneration following axonal injury in models of stroke, and spinal cord and optic nerve injury, a number of unanswered questions remain. Such questions require systematic investigation of the epigenetic landscape between regenerative and non-regenerative conditions for the potential translation of this knowledge into regenerative strategies in human spinal and brain injury, as well as stroke.

Keywords Epigenetics · Axonal regeneration · Histone modifications · DNA methylation · Spinal cord injury · Optic nerve crush

Introduction

A Limited Axonal Regenerative Program

Axonal injury in the central nervous system (CNS) leads to long-term functional impairment and disability in several neurologic disorders, including stroke, and traumatic brain or spinal cord injury. This is primarily owing to a lack of axonal sprouting and regeneration [1]. Importantly, in experimental models of spinal cord injury or stroke, successful enhancement of axonal sprouting and/or regeneration has been shown to improve neurologic function [2]. The reasons for such a limited endogenous regenerative response following injury are only partially understood, but seem to rely on 2 main components: (1) *the intrinsic properties of neurons*, which are deficient in triggering a regenerative response [2]; (2) *the glial environment*, which inhibits axonal re-growth [1].

While axonal regeneration is highly restricted in the CNS, spontaneous, though partial, axonal regeneration is possible in the peripheral nervous system (PNS). This is likely owing to the differences in both (1) the intrinsic properties of neurons and (2) the glial environment between CNS and PNS, which make the PNS more permissive to axonal regeneration. However, the intrinsic neuronal properties and the glial environment are not two completely independent entities. For example, the glial environment can affect the intrinsic properties of neurons, as is evident by the fact that the regenerative gene expression program is activated only when axons lie within the PNS, but not within the CNS. This phenomenon is exhibited by the dorsal root ganglia (DRG) neurons, which extend one axonal branch into the PNS and another into the CNS, and where only lesions to the peripheral branch trigger a pro-regenerative gene expression program [3, 4]. Moreover, within the CNS itself, specific neuronal subpopulations are more resistant than others to sprout

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or regenerate their axons following injury, with the corticospinal tract (CST) being the most refractory in comparison to the rubrospinal and raphespinal axons to regenerate [5–9]. Therefore, the canonical thinking that lack of axonal regeneration depends entirely upon the glial environment has been challenged by demonstrations of differences in the post injury regenerative capacity of descending supraspinal fiber tracts, i.e., CST, rubrospinal, and 5-hydroxytryptamine (serotonergic) raphespinal neurons, which all project into the CNS spinal cord environment [2].

At the molecular level, it is known that a robust regenerative transcriptional program [10] is initiated after peripheral axonal injury, and partially after injury in immature CNS neurons, but not after axotomy in the mature CNS [9, 11]. Within the CNS, the rubrospinal and spinal axons show a lesser degree of retraction, a better sprouting potential compared with the CST, and display more robust axon regeneration following a variety of neurotrophin-based and/or anti-scarring treatments [5, 7, 8, 10, 12–15]. As a partial explanation for such observed differences, several studies have demonstrated that following spinal lesions a subset of pro-regeneration associated genes (RAGs), including *Gap43*, *Jun*, *Lgals1*, *class II beta-tubulin*, and *alpha 1 tubulin*, is induced in the raphe and red nuclei, yet hardly in layer V neurons, from which the CSTs originate [16–22]. This suggests that retrograde signaling that stimulates gene expression is somewhat “weak” in CNS fiber tracts compared with peripheral nerves, and even more so in the corticospinal system.

From these findings, it is possible to deduce that this very restricted gene expression response following axonal injury is owing to the limited capacity of transcription factors to occupy target promoters. In fact, successful axon regeneration requires proper activation of the transcription program in the injured cell, which promotes cytoskeleton remodeling and axonal outgrowth [2]. A number of transcription factors have been implicated in successful axon regeneration in the CNS—in the facial nuclei of the brainstem, the dorsal column of the DRG system, or the optic nerve, but very rarely in the corticospinal system [2]. The most notable exception is the stereotaxic viral overexpression of retinoid acid receptor beta (RARβ) in layer V neurons in rats promoted some degree of axonal regeneration of the CST following spinal lesions [23]. This finding suggests that promoting axonal regeneration of the CST by driving a specific transcriptional cascade is possible. However, the benefits are limited, and the modification of a single factor is not sufficient to promote a robust regenerative response. Strategies that boost more globally the intrinsic potential of neurons to regenerate may be more effective. An indication of this was the modulation of phosphatase and tensin homolog-mammalian target of rapamycin signaling, thereby modifying protein synthesis, which recently led to very promising axonal regeneration of the CST that was, however, not correlated with functional

recovery [24]. Therefore, it is plausible to hypothesize that the axonal regeneration program is restricted by a “code” dictated by specific neuronal “epigenetic changes”, which influences the conformation of chromatin, and the rate of gene transcription and the accessibility to regenerative gene expression following axonal injury.

Epigenetics

Currently the field of “epigenetics” is one of the fastest growing research areas in biomedicine, which involves protein changes that affect gene regulation without altering the core DNA sequence. Originally, epigenetic changes were only associated with specific events during early embryogenesis, such as inheritable genomic imprinting. Two classical epigenetic mechanisms are cytosine-specific DNA methylation and histone protein tail modifications. More recently, non-coding RNAs, which also contribute to transcriptional regulation, have been suggested as another epigenetic mechanism [25–29].

The combination of specific patterns of epigenetic modifications leads to both global and promoter/gene specific changes. Globally, they contribute to dynamically affect chromatin structure by facilitating an overall condensed or relaxed chromatin conformation, which is tightly connected to gene expression regulation in a cell-type and developmental stage-dependent manner [30–33]. However, specific epigenetic marks may also occur more selectively on subsets of gene promoters affecting gene regulation of individual genes without producing detectable global change of chromatin conformation. Typically, epigenetic information is heritably encoded in addition to genomic sequence information, and is conserved through mitosis or meiosis. More so than just the DNA sequence, epigenetic modifications have a crucial influence on gene expression patterns, especially during embryonic development, where tight temporal and spatial regulation of gene expression is necessary. Moreover, it has become evident that epigenetic mechanisms are essential for genome structure and gene expression regulation, not only during embryonic development but also in cancer and in the pathogenesis of immunologic, cardiovascular, and developmental disorders, modulating gene expression response to external environmental signals to affect the cellular phenotype. Recently, chromatin modifications have been shown to play a fundamental role in the context of CNS development, adult neurogenesis, cognition, vision, learning and memory, and several developmental and neurodegenerative disorders [28, 34–37]. However, more recently, experimental evidence suggested a potential key function for epigenetic regulation in axonal outgrowth and regeneration in post-mitotic neurons of the adult CNS [38–42]. Consequently, epigenetic marks in adult CNS post-mitotic neurons might be more dynamic than previously believed, responding to neurophysiological changes or nerve

injury, thus representing a potential signaling mechanism for the regulation of axonal regrowth post-injury.

DNA Methylation and Histone Modifications Regulate Gene Expression

The classical components of the epigenome include (1) *DNA methylation* and (2) *histone post-translational modifications* [43].

DNA methylation is mediated by the catalytic activities of DNA methyltransferase enzymes (DNMTs), including DNMT1, DNMT3A, and DNMT3B. Typically, the binding of methyl-CpG-binding domain (MBD) proteins (MBPs) to methylated DNA sites in gene promoters initiates a series of events, including the recruitment of histone-modifying enzymes, deacetylation of histone protein tails, and chromatin condensation around the gene promoter leading to transcriptional repression [37].

Histone post-translational modifications are dynamic events mostly regulated by histone acetyltransferase (HATs), histone deacetylases (HDACs), histone methyltransferases, and histone lysine demethylases, which form large multiprotein complexes in the proximity of gene regulatory regions [44, 45]. The degree of acetylation and methylation of chromatin is the result of a balance between these various chromatin-modifying enzymes leading to gene regulation—either activation or repression. Transcriptionally-active chromatin has a specific pattern of histone post-translational modifications (a “histone code”) and DNA methylation, which is the preferential chromatin state in early embryogenesis and the developing nervous system [46] where an active axon growth program is observed. Hypermethylated DNA in the proximal promoter region, as well as gene coding and noncoding sequences, is typically associated with low gene expression [37, 47]. However, full activation or repression of gene transcription is coupled with the acetylation status of nucleosomal histone proteins, especially within the vicinity of gene promoters. The attachment of MBPs to methylated DNA sites in gene promoters initiates a series of events, including recruitment of HDACs, deacetylation of histone protein tails, and chromatin condensation around the gene promoter, thus leading to transcriptional repression [48]. Covalent modifications of N-terminal histone protein tails are not limited to acetylation, but also include methylation, phosphorylation, and ubiquitination. Specific combinations of acetylated and methylated sites are associated with “open” or “closed” chromatin formations termed the “histone code”. This “code” mediates protein–protein interactions contributing to short-term, as well as long-term, regulation of transcription, representing a specific form of cellular memory.

DNA Methylation in Neuronal Plasticity and Axonal Injury

Most abundant in mammals, methylation of cytosine at the C5 position occurs predominantly in CpG dinucleotides, although there may also be substantial non-CpG methylation at cytosines [49]. Long stretches of DNA densely populated by CpGs are defined as CpG islands (CGIs). Two thirds of the ~25,000 CGIs in human (~23,000 in mouse) are found within the proximal promoter region of genes, including a majority of constitutively active house-keeping genes and about 40 % of tissue-specific genes [50–52]. About half of the CGIs associated with gene promoters are located around the transcription start site and often extend into the first exon or intron. However, CGIs are also found more distal to transcription start sites in intergenic or even intragenic regions, so-called “orphan CGIs” [53, 54].

While promoter CGIs influence gene expression, absolute gene expression levels do not generally correlate with the overall density of CGIs at gene promoters. Altogether, about 70 % of human gene promoters (50–60 % in mouse) exhibit a high concentration of CpG dinucleotides, mostly forming CGIs [55–57]. These genes are often associated with house-keeping functions, like gene expression mechanisms and DNA replication in the nucleus, metabolism, or cell cycle. Conversely, the other fraction of about 30 % of human gene promoters has a distinctly low CpG content, rarely exhibiting CGIs. Many of these genes fulfill more tissue-specific functions, often associated with physiological processes and stimulus response signaling [56, 57].

Differentially represented in proximity of CGIs, MBPs known to be involved in neuronal biology include MECP2 (Rett Syndrome) and MBD1. They interact with DNA methyltransferases, and supply a connecting platform for different chromatin-modifying enzymes and transcription factors within a transcription repressor complex bound to methylated DNA segments, and are strongly associated with gene repression [58–60]. MBD2 and MECP2 are known to interact with histone deacetylases HDAC1 and HDAC2, while MBD1 interacts with histone methyltransferase SUV39H1. Additionally, MBD proteins can bind to other auxiliary proteins such as the co-repressor SIN3A [61–63].

During the last few years, a substantial research effort specifically on MECP2 demonstrated an important role of MBPs, as well as DNA methylation, in brain-related functions and neural disorders. Either loss of function or increased MECP2 expression can cause various neuropsychiatric disorders, such as mental retardation, autism, and Angelman syndrome [64–66]. The most frequent and well characterized is the disruption or mutation of *Mecp2*, which affects DNA binding causing the X-linked Rett syndrome, a postnatal neurologic disorder, which is associated with cognitive and motor abnormalities, including deficient axon growth and autistic symptoms [59, 67, 68]. MECP2 is

expressed in neurons, as well as in glial cell types, such as astrocytes and oligodendrocytes; however, not in microglia, oligodendrocyte precursor cells, or Schwann cells [69–72]. Furthermore, differential DNA methylation was found for brain-derived neurotrophic factor (*Bdnf*), the expression of which is impaired in *Mecp2*-deficient mice and likely contributes to the deficient axon outgrowth observed therein [73]. Interestingly, neuronal activity-dependent membrane depolarization triggers calcium-dependent phosphorylation of MECP2 and its release from the *Bdnf* promoter, thus facilitating *Bdnf* transcription. In summary, BDNF synthesis in the neuron after depolarization correlates with decreased CpG methylation and the release of the MECP2–SIN3A repression complex from its promoter [74]. It would be intriguing to investigate such mechanisms directly after nerve injury where calcium-dependent depolarization occurs. Promoter binding of MECP2 to the *Bdnf* promoter can also be regulated by acetylation and deacetylation via the histone modifying enzymes EP300 and SIRT1, respectively [75], or by phosphorylation [76]. Surprisingly, binding of MECP2 was also reported earlier to repress *Bdnf* expression, depending on the specific promoter region likely regulating the expression of selected BDNF isoforms [77, 78]. Furthermore, MECP2 also forms a complex with the transcriptional activator cyclic adenosine monophosphate (cAMP) response element-binding 1 (CREB1) at promoters of activated target genes, like the growth-hormone release inhibitor somatostatin, but not at repressed target gene promoters [65]. As CREB1 seems to be an important transcriptional regulator of the axonal regeneration program in sensory neurons projecting into the spinal cord [4, 79, 80], it would be interesting to investigate the role of MECP2 signaling following nerve injury, as well as upon CREB1 co-regulation of target regeneration-associated genes. Therefore, we believe that although MBPs are essential during neurodevelopment and for cognitive functions [81–83], they may also be required for the regulation of axonal regeneration associated genes upon nerve injury.

DNMT3A and DNMT3B have similar and, in part, complementary roles suppressing tissue- and stage-specific genes during embryonic development, genomic imprinting, and for the silencing of repetitive elements. During early neurogenesis, DNMT3B is mainly expressed in embryonic and neural precursor cells. In contrast, DNMT3A is predominantly expressed in later developmental stages and in maturing neurons, oligodendrocytes, and astrocytes of the adult brain [84, 85]. Interestingly, DNMT1 is expressed in post-mitotic neurons of the adult CNS—at higher levels than either DNMT3A or DNMT3B. DNMT1 is important for the maintenance of DNA methylation after base-excision DNA repair and for maintaining, as well as re-establishing, high local levels of DNA methylation. DNMTs are abundantly expressed in the brain, spinal cord, and DRG [40,

86–88]. Coincidentally, global DNA methylation levels are higher in the adult brain than in other tissues [89–91]. Cytosine methylation seems to be dynamically regulated in the adult CNS, thus it might be directly involved in gene expression regulation upon physiological changes. Both DNA methylation and DNMT1 have been shown to influence neuronal plasticity and memory formation [92–95].

Therefore, it is likely that rapid and dynamic changes of promoter DNA methylation patterns might also contribute to transcriptional regulation of axon regeneration-associated genes upon peripheral nerve injury. DNA methylation was increased after mild focal brain ischemia following middle cerebral artery occlusion, which was partly dependent on DNMT1 activity. Blocking of DNMT1 activity protected injured neurons improving their survival [86]; however, axon sprouting was not examined. More recently, it has been proposed that DNA methylation is involved in enhancement of CNS repair via folate metabolism and a functional methylation cycle. Folic acid is a methyl donor substrate needed to produce cellular S-adenosyl-methionine that, in turn, is the substrate for DNMTs [40, 96]. Systemic delivery of folate-induced axonal regeneration in rats with combined spinal cord and sciatic nerve injury via the high-affinity folate receptor 1. The effect of folate was dependent on a functional methylation cycle, and affected global and gene-specific DNA methylation, as well as with the *de novo* DNA methyltransferases. However, the specific cellular subtype—glial or neuronal—responsible for these expression changes was not identified, and direct evidence that folate exerts its regenerative effects via DNA methylation of regeneration-associated promoters in neurons was not provided. Altogether, these findings make it worthwhile to investigate the specific role of DNA methylation in axonal regeneration and regulation of RAG expression.

An emerging epigenetic mechanism, still largely unclear, is the occurrence of active DNA demethylation. Recently, GADD45, a DNA damage response gene, has emerged to actively mediate DNA demethylation, coupling it with DNA repair [97]. Given that expression of GADD45 is strongly induced in damaged sensory dorsal root ganglia, as well as in lumbar motor neurons after sciatic nerve injury [98] its potential role in DNA demethylation of injury responsive genes, including RAGs, deserves further investigation.

Histone Modifications in Neuronal Plasticity, Axonal Injury, and Regeneration

Genome-wide studies have confirmed that histone acetylation usually correlates with gene activation. Specifically, histone H3 acetylation at lysine residues 9, 14, 18, and 27 (H3-K9/14/18/27 ac) and H4 acetylation is often found in active promoter regions and around the transcription start

site. As well as acetylation of histones, transcriptional activity also correlates with methylation of histone H3-K4 and K36, and relaxed chromatin at gene promoters [99, 100]. Acetylation neutralizes the positive charge of histone lysine or arginine residues, and, as a result, the negative backbone of DNA is bound less efficiently by acetylated histones [31, 101, 102]. Type A HATs are localized in the nucleus and can be classified according to their conformational structure and sequence homology: GCN5-related N-acetyltransferases, MYST, and CREB binding protein (CBP)/P300 families [103]. Most of these enzymes are able to modify multiple histone tail residues and are found together with other chromatin modifying enzymes in large multiprotein complexes, where they also function as transcriptional co-activators [44, 104]. Well characterized are KAT2B [lysine (K) acetyltransferase 2b; CBP/P300-associated factor], more commonly known as PCAF and belonging to the GCN5-related N-acetyltransferase family, and the complex CBP/P300 (CREBBP/EP300; KAT3A/KAT3B). PCAF is known to promote transcriptional activation and acetylate transcription factors as a co-activator, and by acetylating the core histones H3 and H4. Known target sites of PCAF are histone H3 lysine residues 9, 14, and 18 (H3-K9/K14/K18) whereby PCAF is the primary acetyltransferase for H3-K9, while the other H3 target sites are shared with GCN5A (KAT2A) and CBP/P300 [105–108]. *Pcaf*-knockout mice do not show an obvious phenotype, but they do show deficits in memory formation and stress response [109].

NGF receptor signaling, stimulated by neurotrophins, induces phosphorylation and nuclear translocation of PCAF with H3-K9 acetylation in neuronal-like PC-12 cells during neurite outgrowth [110]. Interestingly, the PCAF–CBP/P300 complex interacts with transcriptional activators, such as FOS, JUN, MYB, HIF1A, TRP53, or KLF13, some of which are known to be involved in axonal regeneration [101, 111–114]. EP300 has functions in neuronal differentiation acting as a co-activator of NEUROD1 [115], and generally acetylates histones as a tag for transcriptional activity. In addition to H3 acetylation, CBP specifically binds to phosphorylated CREB1, an important factor in axonal regeneration, and enhances its transcriptional activity towards cAMP-responsive genes. *CREB1* induction by serotonin in sensory neurons leads to promoter histone acetylation of *CREB1* target genes via CBP recruitment. This causes increased long-term potentiation, a form of synaptic plasticity, which is counteracted by the CREB2/HDAC5 complex with subsequent promoter histone deacetylation leading to long-term depression [116]. However, CREB1 also becomes activated upon peripheral injury of rat DRG together with JUN, RELA, ATF2, or ATF3. Thus, epigenetic regulation of CREB1-mediated transcription might be relevant for sensory nerve injury and axonal regeneration [2, 117, 118].

Opposing the effects of HATs, HDACs deacetylate lysine residues of histones H2A, H2B, H3, and H4, although with

modest substrate specificity, yet having a general repressive effect on transcription. HDACs are usually recruited to chromatin as part of larger multiprotein complexes, for example as components of the NuRD, SIN3A, or RCOR1 complexes [119–121]. HDAC1 and HDAC2, the best characterized members, interact with histone acetyltransferases (PCAF or CBP/P300), other histone deacetylases (HDAC7, SIRT1), histone methyltransferases (SUV39H1, SETDB1), histone demethylases (KDM4A/5A), and with transcription factors. Additionally, they also interact with DNA methylation associated proteins (DNMT1/3A, MECP2, MBD2/3), thus linking different transcription repression mechanisms. Because of their interactions, enzymes of complementary mechanisms for establishing or erasing histone marks work together dynamically, allowing for a balance between transcriptional activation and repression [37, 100]. Histone deacetylation has a recognized role in the differentiation of adult neurogenesis. Inhibiting HDAC activity by valproic acid leads to decreased proliferation of adult progenitors in the hippocampus, together with increased neuronal differentiation, without increased gliogenesis [122]. In cortical neurons, HDAC3 interacts with nuclear receptor co-repressor 2 which controls neuronal responsiveness of several transcription factors, thus regulating neurogenic and neuroprotective pathways [123]. Nuclear receptor co-repressor 2 further interacts with HDAC1/2 and with retinoic acid receptors. For example, retinoid acid receptor beta (RARβ) is involved in the prevention of regeneration-inhibiting signals from CNS myelin upon nerve injury [124]. A balance between HDAC1/2 and EP300 interactions with RELA is required during Schwann cell differentiation and for proper myelination in the PNS, which would also be a major requirement during axonal regeneration. In support of this, mice lacking HDAC1/2 exhibited a severe myelin deficiency, with Schwann cells remaining at an immature stage [125]; however, their role in myelination after nerve injury and regeneration is still unknown. Thus, gene promoter acetylation or deacetylation are essential to drive the regeneration response following peripheral nerve injury and may be prevented upon central nerve lesion.

Interestingly, HDACs may also have non-nuclear functions mainly by acetylating tubulin in the cytoplasm or in the growth cones of extending axons. HDAC6 inhibition was recently reported to promote survival from oxidative stress and neurite outgrowth in DRG sensory neurons when plated on inhibitory substrates such as myelin and chondroitin sulfate proteoglycan [126]. Importantly, modulation of HDAC6 seemed to mediate its effects via transcription-independent mechanisms, possibly via acetylation of tubulin. However, whether HDAC6 inhibition was sufficient to enhance axonal regeneration across an injured CNS was not investigated.

Histone modifications play an essential role in neurophysiological processes such as memory and neuronal plasticity, as well as a role in neurodegenerative disorders [127, 128].

An example in neuronal plasticity is the response to social defeat stress, which includes consequent epigenetic remodeling such as increased methylation of histone H3 at the RAG promoter of *Bdnf*, thereby downregulating it [129]. This epigenetic regulation of *Bdnf* may provide insights into the molecular regulation of axonal regeneration upon nerve injury, where BDNF signaling promotes neuronal survival and axonal growth.

We have recently found that the HATs CBP/P300 and PCAF form a transcriptional complex with the transcription

factor TRP53 in primary neurons to enhance promoter accessibility on select pro-regeneration promoters [38, 113, 130]. This transcriptional complex activates the expression of several RAGs, including RAB13, CORO1B, and GAP43, triggering an intrinsic pro-axonal outgrowth program. This was observed *in vitro* as well as *in vivo*, where this transcriptional complex, including each single component, TRP53, CBP/P300, or PCAF, was required for neurite outgrowth, while TRP53 was needed for axonal regeneration following facial nerve axotomy [39, 113, 130], as well as for CST and

Fig. 1 Diagram summarizing putative epigenetic pathways downstream inhibitory [central nervous system (CNS) lesion] versus regenerative [peripheral nervous system (PNS) lesion] signaling. This was modeled after obtaining data from *in vivo* paradigms of axonal injury, as well as in cultured neurons during neurite outgrowth. The CNS lesion is likely to convey inhibitory epigenetic changes that do not allow regenerative gene expression (left), which is triggered after PNS lesion (right). *RTN4* reticulon 4, *MAG* myelin-associated glycoprotein, *OMGP* oligodendrocyte myelin glycoprotein, *CSPG* chondroitin sulfate proteoglycan, *BDNF* brain-derived neurotrophic factor, *NGF* nerve growth factor, *IL-6* interleukin-6, *TNFRSF19* tumor necrosis factor receptor superfamily, member 19 (TROY), *NGFR* nerve growth factor receptor, *RA* retinoic acid, *RARB* retinoic acid receptor beta, *PI3K* phosphatidylinositol 3-kinase, *PTEN* phosphatase and tensin homolog, *RHOA-GTP* ras homolog gene family, member A-Guanosine triphosphate, *cAMP* cyclic adenosine monophosphate, *P-MAPK8* phosphorylated mitogen-activated protein kinase 8 (JNK1), *JAK*

Tyrosine-protein kinase, *ROCK* rho-associated coiled-coil containing protein kinase, *LIMK* LIM-domain containing protein kinase, *AKT* v-akt murine thymoma viral oncogene homolog/Protein kinase B (PKB), *TRP53* transformation related protein 53, *ATF3* activating transcription factor 3, *STAT3* signal transducer and activator of transcription 3, *JUN* Jun oncogene, *CREB1* cAMP response element-binding 1, *PCAF* *P300/CBP-associated factor/**K(lysine) acetyltransferase 2B (KAT2B)*, *CBP* CREB binding protein, *GSK3B* glycogen synthase kinase 3 beta, *HDACs* histone deacetylases, *KLF4* kruppel-like factor 4, *PKA* protein kinase A, *p-MAPK1/3* phosphorylated mitogen-activated protein kinase 1/3 (ERK2/1), *HKMT* histone lysine methyltransferase, *RCOR* REST corepressor 1, *Pol II* RNA polymerase II, *H3K9* histone-3 lysine-9, *me* methyl-group, *ac* acetyl-group, *MBP* methyl-CpG binding domain protein, *CpG* cytosine-guanidine dinucleotide, with phosphate, *KDMs* histone lysine demethylase, *SWI/SNF* SWItch/Sucrose NonFermentable chromatin remodelling subunits, *MYC* myelocytomatosis oncogene

serotonergic sprouting after spinal cord injury [131]. TRP53 may also transcriptionally activate cyclic guanosine monophosphate (cGMP)-dependent protein kinase I (cGKI; PRKG1) to inhibit semaphorin 3A-mediated growth cone collapse in DRG neurons and explants, suggesting a potential role in axonal regrowth by counteracting inhibitory signals [132]. However, the direct interaction of TRP53 with transcriptional co-factors such as HATs or HDACs upon cGMP-dependent protein kinase I expression has not been investigated. In addition, we have explored the possibility that histone modifications and specific HATs are associated with the capacity of CNS neurons to regenerate their axons after injury. Indeed, we found that after optic nerve crush, retinal ganglia cells that do not regenerate their axons displayed decreased expression of the HAT EP300 and of the corresponding acetylation on histone H3-K18 [39]. To demonstrate the functional relevance of these observations, we overexpressed an EP300 expressing viral vector to find that EP300 can promote axonal regeneration in the optic nerve after optic nerve crush. EP300 overexpression also triggers a pro-regeneration gene expression program via modifying the rate of histone acetylation on selected gene promoters, including *Gap43*, *Coro1b*, and *Sprr1a* [39]. Interestingly, when we delivered the HDAC I/II inhibitor trichostatin A *in vitro* after optic nerve crush, we observed enhanced survival of retinal ganglia cells, but not axonal regeneration. This suggests that the identification of key epigenetic molecules that respond to nerve injury signals is needed to tailor specific axonal regeneration enhancing strategies rather than more global approaches.

Here we propose a model summarizing the potential molecular regulation of epigenetic pathways in non-permissive (CNS lesion) versus permissive (PNS lesion) conditions to axonal regeneration (see Fig. 1).

Conclusions and Perspectives

Although epigenetic changes seem to contribute to the regenerative capacity of injured axons, studies that address modifications of the epigenome following axonal injury in limiting conditions to axonal regeneration as in CNS neuronal populations, such as the corticospinal tracts, via unbiased high-throughput approaches are necessary. These studies may allow the elucidation of the obscure reasons for axonal regenerative failure following CNS injury and, hopefully, open novel therapeutic possibilities in several neurologic disorders such as stroke, and traumatic spinal and brain injury.

In addition, we still are not aware of the nerve injury-related signals that may influence epigenetic changes, thus affecting the gene expression regenerative program. To this end, we have recently investigated whether specific changes

of the neuronal epigenome could be a hallmark of regeneration permissive (PNS injury) versus regeneration restrictive axonal injury (CNS injury). We employed a systematic approach to understanding the epigenetic environment, including at promoters of regeneration-associated genes, in pseudo-unipolar DRG neurons. We examined both DNA methylation and histone modifications in DRG neurons in regard to gene regulation following sciatic or spinal axonal injury, and found that specific epigenetic signals are, indeed, associated with axonal regrowth and may promote axonal regeneration in the CNS (personal communication).

Additionally, axonal injury triggered signals, including mitogen-activated protein kinase-dependent cascades, cAMP-related phosphorylation of transcription factors, such as CREB, or phosphorylation of STAT-3, are molecular pathways previously described to support axonal regeneration of the sciatic nerve [133] and may contribute to regenerative gene expression changes within the epigenetic machinery. In fact, the activity of these transcription factors is conditional to permissive epigenetic marks such as histone H3 K9 acetylation, which is, in turn, potentially influenced by phosphorylation of key HATs, including P300, CBP, and PCAF.

Moreover, it would be worth investigating the relationship between the reactive glial environment, including astrocyte and macrophage/microglia membranes, or secreted molecules and axonal retrograde transport, which may induce signal specific epigenetic modifications. In fact, the neuronal gene expression program is continuously shaped by the interaction with the glial environment, which, after CNS injury, is mainly inhibitory to axonal regrowth. Epigenetic regulation may lie at this crossroad between environmental stimuli and neuronal gene regulation following injury, allowing for the fine-tuning of the neuronal short and long-term response, both by influencing cell fate and axonal growth. Finally, manipulation of key selected signals involved in epigenetic long-term modifications may allow for regenerative gene reprogramming in injured neurons, which would benefit neurologic recovery.

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