Epigenetics and ncRNAs in Brain Function and Disease: Mechanisms and Prospects for Therapy

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Abstract The most fundamental roles of non-coding RNAs (ncRNAs) and epigenetic mechanisms are the guidance of cellular differentiation in development and the regulation of gene expression in adult tissues. In brain, both ncRNAs and the various epigenetic gene regulatory mechanisms play a fundamental role in neurogenesis and normal neuronal function. Thus, epigenetic chromatin remodelling can render coding sites transcriptionally inactive by DNA methylation, histone modifications or antisense RNA interactions. On the other hand, microRNAs (miRNAs) are ncRNA molecules that can regulate the expression of hundreds of genes post-transcriptionally, typically recognising binding sites in the 3' untranslated region (UTR) of mRNA transcripts. Furthermore, there are a myriad of interactions in the interface of miRNAs and epigenetics. For example, epigenetic mechanisms can silence miRNA coding sites, and miRNAs can be the effectors of transcriptional gene silencing, targeting complementary promoters or silencing the expression of epigenetic modifier genes like *MECP2* and *EZH2* leading to global changes in the epigenome. Alterations in this regulatory machinery play a key role in the pathology of complex disorders including cancer and neurological diseases. For example, miRNA genes are frequently inactivated by epimutations in gliomas. Here we describe the interactions between epigenetic and ncRNA regulatory systems and discuss therapeutic potential, with an emphasis on tumors, cognitive disorders and neurodegenerative diseases.

Keywords Epigenetics · Cancer · miRNA · ncRNA · Neurodegeneration

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In recent years the detailed analysis of genomic sequences has challenged our understanding of the relationship between genetic information and phenotype. For example, the genome of the nematode worm *Caenorhabditis elegans* encodes approximately the same number of genes as that of humans (~22,000), whereas the *Oryza sativa* (rice) genome contains approximately twice as many genes. Complexity is likely to scale up not only with the number of genes but mainly with the sophistication of the regulatory networks that govern gene expression. Observations like these highlight the importance of re-evaluating our understanding of cellular organization and disease pathology from a mere sum of gene products to a complex system of multiple, interacting regulatory networks. The study of these regulatory networks is the key to fully understanding cellular organisation and function, and the pathology of complex disorders like cancer and neurological disease, and ultimately for identifying new targets for therapeutic intervention. Epigenetic mechanisms and the regulation of multiple genes by single non-coding RNAs (ncRNAs) are at the core of this network.

Epigenetics is the study of mitotically and meiotically heritable changes in gene expression that are not encoded in the DNA itself [1]. These changes do not affect the nucleotide sequence but rather they alter other features such as the patterns of DNA methylation and histone modification that are inherited through cell divisions and across generations along with the genetic information [2, 3]. One of the most fundamental roles of epigenetics is the guidance of cellular differentiation in development [4]. Cascades of gene expression changes that are directed by epigenetic processes explain how cells and tissues with dramatically different phenotypic features can arise given that they all carry common genetic material [5].

Although the significance of epigenetics was first recognized in the context of cell differentiation we are now increasingly aware of the involvement of epigenetic mechanisms in the fast adaptive response to the environment or in the case of

injury and disease. It is now believed that epigenetic mechanisms can be responsible for the preadaptation of gene expression of newborns to the environment. A classic example of epigenetic inheritance in humans was described in an isolated community in northern Sweden. Famine experienced in the womb or as preadolescents in the 19th century was correlated with diabetes and cardiovascular diseases in their offspring born when food was plentiful [6–8]. Similarly, individuals conceived during the Dutch Hunger Winter during the Second World War showed hypomethylation at the insulin-like growth factor 2 gene (*IGF2*) and were more prone to obesity when tested decades later [9]. Another recently reported example is the transgenerational effects of posttraumatic stress disorder in babies of mothers exposed to the 9/11 terrorist attacks during pregnancy [10, 11].

On the other hand, ncRNAs are also involved in the regulation of gene expression [12]. The term ncRNA encompasses a family of RNA molecules that are not translated into protein, including long ncRNAs (lncRNA), piwi-interacting RNA (piRNA), microRNAs (miRNAs), ribosomal RNA (rRNA), small interfering RNA (siRNA), small nucleolar RNAs (snoRNA) and transfer RNA (tRNA) [12]. MicroRNAs in particular are small RNA molecules (18–25 nucleotides) encoded by eukaryotic genomes [13–15]. Each one of these miRNAs can regulate the expression of hundreds of genes post-transcriptionally. After processing of the pre-miRNA into mature miRNA by the enzyme Dicer, one of the strands of the miRNA (the leading strand) is incorporated into the RNA-induced silencing complex (RISC) [16]. The leading strand is selected by its thermodynamic instability relative to the passenger strand and directs the complex to complementary mRNA [16]. MicroRNAs typically recognise binding sites in the 3' untranslated region (UTR) of mRNA transcripts. Generally, complete complementarity is required in the miRNA 'seed' region (i.e. positions 2–7 from the 5' terminus of the miRNA). Perfect base pairing between miRNA and target leads to mRNA cleavage by the slicer activity of Argonaute-2 (AGO2), whereas imperfect binding leads to translational repression and slicer-independent mRNA decay [17].

Since miRNAs can function by maintaining terminal differentiation and inducing developmental transitions, there has been a growing interest in miRNAs for stimulating stem cells to adopt specific lineages, reprogramming of differentiated cells to induce pluripotency [18–20] and, in general, for improving our understanding of stem cell biology [21–23]. As drivers or mediators of pathology, miRNAs are promising therapeutic targets. Deficiency in expression of a miRNA may be compensated by delivery of a miRNA mimic [24]. Conversely, miRNA inhibition strategies may be effective in cases where the expression of a miRNA is a causative factor in a disease [25]. Together with epigenetic mechanisms, non-coding RNAs like miRNAs form a regulatory network that modulate multiple phenotypic traits, or the pathology of

complex diseases such as neurological disorders and cancer. Here we discuss the interactions between these different regulatory elements in the central nervous system and how they can be targeted as potential therapies.

Epigenetic Regulation in the CNS

In eukaryotes, genomic DNA is packaged with histone proteins to form a dynamic polymer called chromatin. Histone proteins form a core with 1.67 superhelical turns of DNA wrapped around its surface to form a structure called a nucleosome. Chromatin acts to regulate gene expression in two ways. Firstly, the physical properties of chromatin directly regulate the accessibility of genomic DNA to the transcriptional machinery. Secondly, covalent modification of histone N-terminal tails regulates the pattern of DNA binding proteins at a specific locus, thereby enabling complex regulation of gene expression (the so-called histone code). The main mechanisms of epigenetic gene regulation include DNA methylation and histone modifications [1, 2].

Methylation

Methylation of cytosine to 5-methylcytosine, principally at CpG dinucleotides, is generally associated with inactive transcription. Aberrant methylation (i.e. epimutation) of CpG islands in gene promoters is a common feature of many cancers. In particular, in gliomas, *de novo* methylation inactivates DNA-repair and tumour suppressor genes leading to the accumulation of new mutations, uncontrolled cell proliferation and increased infiltration into the surrounding normal brain parenchyma [26]. Aside from gliomas, other diseases affecting the CNS have been linked to aberrant methylation. For example, alpha-thalassemia X-linked (ATR-X) syndrome causes intellectual disabilities due to methylation of ribosomal DNA and genomic repeats [27]. Similarly, Fragile X syndrome is caused by the methylation of CGG repeats in the 5' UTR of the *FMR1* gene which causes gene silencing and local chromatin reorganization into a chromosomal fragile site visible in the karyotype [28].

Alternatively, DNA hypomethylation has also been reported in gliomas. This leads to loss of imprinting, aberrant gene activation, chromosomal instability, and tumor expansion. DNA methylation is therefore a valuable molecular marker for early diagnosis and prognosis in cancer [26]. Additionally, a number of diseases are caused by aberrant activation of genes which are normally silenced by DNA methylation in the maternal or paternal copy (i.e. imprinted *loci*). For example, the pathogenesis of Prader-Willi syndrome is linked to aberrant methylation of a paternally imprinted region of chromosome 15 [29], which leads to diverse developmental abnormalities including cognitive disability [29, 30].

Interestingly, recent observations suggest the existence of significant crosstalk between methylation and RNA processing and, potentially, this process could also be involved in the regulation of gene expression in the CNS. Thus, it has been shown that differential enrichment for specific histone H3 modifications regulates the tissue-specificity of alternative splicing in the fibroblast growth factor receptor 2 gene (*FGFR2*) [31]. Also, allele-specific DNA methylation established during gametogenesis at imprinting sequences could lead to allele-specific polyadenylation as it occurs at the imprinted *Herc3/Nap115* [32] and *Mcts2/H13* [33] loci.

Histone Modification

Covalent modifications of the unstructured N-terminal tails of nucleosomal histone proteins exert regulatory effects on their bound genomic DNA. A wide variety of covalent modifications are observed including acetylation, methylation, phosphorylation, sumoylation and ubiquitylation. For example, reciprocal acetylation and methylation of histone H3 is associated with active and silent transcription, respectively. Transcriptionally active chromatin is acetylated at histone H3 lysine 9 and lysine 27, whereas the same lysine residues are di- and tri-methylated respectively at transcriptionally silent chromatin. These modifications alter the physical properties of chromatin. Positively charged histone lysine residues interact with the negatively charged DNA phosphodiester backbone allowing for close association and chromatin compaction. Acetylation reduces this positive charge and promotes the adoption of an open chromatin conformation more accessible to transcription factors and RNA polymerase II (RNAPII). Alternatively, differential histone covalent modification alters the association of chromatin with transcription factors and chromatin remodelling complexes which exert control of gene expression. Many disorders can be linked to aberrant histone modifications. For example, in the CNS, Rubinstein-Taybi syndrome, which is caused by mutations in the *CREBBP* gene that codes for a histone acetyltransferase and leads to problems associated with motor skills and long-term memory [34].

MicroRNA Regulation in the CNS

MicroRNAs play an important role in neurogenesis and a number of miRNAs are differentially expressed in disorders affecting the CNS [35–38]. Due to the widespread involvement of miRNAs in many disorders, several approaches are now being pursued in order to modulate the levels of aberrantly expressed miRNAs or block their pernicious effects as a gene therapy approach [25].

Numerous profiling studies have identified miRNAs that are involved in the pathophysiology of neurological disorders. For example, reduced expression of Dicer during the

pathogenesis of Huntington's disease leads to the inhibition of miRNA biogenesis [39]. Similarly, reduced levels of miR-133b and miR-34b/34c are observed in the brains of Parkinson's disease patients [40]. On the other hand, the cerebrospinal fluid (CSF) from individuals with Alzheimer's disease contains high levels of the miRNA let-7b which promotes neurodegeneration by activating the RNA-sensing Toll-like receptor 7 (*TLR7*). Furthermore, this neurodegeneration can be mirrored by delivering let-7b into the CSF of wild-type mice by intrathecal injection, thereby demonstrating how differential miRNA abundance can link the spurious activation of innate immune receptors with neurodegeneration [41]. Another example is the involvement of microvesicle-contained serum miRNAs which mediate the signalling of angiogenesis and thus promote uncontrolled cell proliferation in glioblastoma [42]. Additionally, serum miRNAs are promising non-invasive biomarkers for diverse diagnostic applications such as for multiple sclerosis [43] or for cancers [44–47]. It has been proposed that different subtypes of glioblastoma can be distinguished by their unique serum miRNA signatures [48].

Cross-Regulation of miRNAs and Epigenetic Mechanisms

There are a myriad of interactions between miRNAs and the various epigenetic gene regulatory mechanisms. The study of these interactions is of paramount importance for both understanding the pathology of complex diseases, and for identifying novel therapeutic targets. In Fig. 1 we describe the integration of miRNA and epigenetic regulation pathways. Additionally, as also shown in Table 1, there are miRNAs that have been implicated in regulating the expression of epigenetic effectors like polycomb group genes, DNA methyltransferases and histone deacetylases [82]. For example, miRNAs can silence the expression of epigenetic modifier genes like *MECP2* and *EZH2* by post-transcriptional regulation (Table 1, Fig. 1A). Given that these enzymes mediate genome-wide changes in gene expression, their dysregulation has been implicated in a number of pathological states (Table 1). For example, Rett syndrome, one of the most common forms of intellectual impairment in females, is caused by mutations in the *MECP2* (methyl CpG binding protein 2) that inhibits its normal function of binding to methylcytosines and silencing target genes which leads to numerous epigenetic abnormalities [30, 83]. Specifically, in neurogenesis, *MECP2* is a key player at the interface of epigenetics and miRNAs since this protein binds to methylcytosines and silences the expression of a number of miRNAs [82]. The reduction in the levels of one of these miRNAs, miR-137, induces the differentiation of neural stem cells (NSCs). The normal function of miR-137 is to repress the expression of the histone methyltransferase *EZH2* that would normally increase H3K27 trimethylation (Fig. 1A) [82].

Fig. 1 Integration of microRNA and epigenetic regulation pathways. (A) MicroRNAs can regulate the expression of epigenetic modifier genes (such as *EZH2* or *MECP2*) by post-transcriptional gene silencing. MicroRNAs are transcribed as long primary (pri-miRNA) transcripts and then sequentially processed to generate the mature miRNA species. The miRNA RNA induced silencing complex (miRISC, which includes AGO2, Dicer and TARBP2) loaded with a mature miRNA searches the cytoplasm for its cognate target (e.g. the *EZH2* or *MECP2* mRNA). Silencing of *EZH2* or *MECP2* expression leads to global alterations in the epigenome. (B) MicroRNA genes are frequently inactivated by epimutations in cancers. Therefore, epigenetic alterations can affect

miRNA expression. Black lollipops indicate DNA methylation. (C) Endogenous miRNAs can induce transcriptional gene silencing of complementary target promoters. Pri-miRNAs are processed by the conventional miRNA processing pathway but the mature miRNA species is loaded into AGO1 instead of AGO2. AGO1 is the core component of the RNA Induced Transcriptional Silencing (RITS) complex which also includes HDAC1, SUV39H, EZH2 and DNMT3A. RITS is guided to a complementary promoter-associated RNA by its bound miRNA. Recruitment of RISC induces heterochromatin formation and promoter DNA methylation at the target promoter

On the other hand, epigenetic alterations can affect miRNA expression. Thus, miRNA genes are frequently inactivated by epimutations [83–85] (Fig. 1B). These epigenetic modifications have been also implicated in the aberrant expression of miRNAs and the molecular pathology of a number of diseases affecting the CNS. For example, it has been shown how the cross-regulation of epigenetic mechanisms and miRNAs in polyglutamine diseases like Huntington's disease contributes to disease pathology. Huntington's disease is caused by a trinucleotide expansion that leads to the formation of inclusions and subsequent neurodegeneration in the striatum and cortex. The product of the Huntington's disease gene, huntingtin, interacts with the transcriptional factor RE1-silencing transcription factor (REST) [85–87]. REST recruits histone deacetylases (HDAC) to promoters [87] and induces changes in the expression of neuronal miRNAs (miR-124, miR-134, miR-29a, miR-29b, miR-9/9*, miR-132 and miR-330-3p) [84, 85, 88]. The influence of these epigenetic mechanisms may also be inherited following cell division, or in certain cases, in the progeny. Interestingly, inherited changes in miRNA expression from previous generations could affect the phenotype and predisposition to disease of current generations. This has already been suggested to be the case in coding genes [6–8] and constitutes an interesting route for

the study of disease inheritance and the development of new therapies.

Finally, ncRNAs can be the effectors of transcriptional gene silencing targeting complementary promoters (Fig. 1C). In the post-genomics era it is now clear that the majority of transcription produces non-coding transcripts, many of which have been linked to the epigenetic control of gene expression (e.g long non-coding RNAs [89–91] or miRNAs targeting gene promoters [92–94], Fig. 1C).

It is important to point out that while miRNAs are particularly well understood examples of ncRNAs that are important in brain function and disease, there are a plethora of other ncRNAs expressed in the brain about which relatively little is known. It is likely that future studies will identify important roles for these transcripts in normal brain physiology and in disease. For instance, long non-coding RNAs (lncRNAs) are >200 nt RNA molecules which can regulate gene expression by acting as scaffolds for epigenetic modifying complexes and DNA methyltransferases [89]. In RNA-mediated epigenetic regulation, base-pairing between non-coding RNAs and their RNA or DNA targets confers gene-specific targeting and thereby allows for fine-grained control over gene expression and gene specific therapy.

Table 1 MicroRNAs targeting genes involved in epigenetic regulation pathways: B lymphoma mo-MLV insertion region 1 homolog (*BMI1*), histone-lysine N-methyltransferase (*EZH2*), jumonji domain containing 1 (*JMJD1A*), histone deacetylase 1 (*HDAC1*), histone deacetylase 4 (*HDAC4*), DNA methyltransferase 3A (*DNMT3A*), DNA methyltransferase 3A (*DNMT3B*), methyl CpG binding protein 2 (*MECP2*) and Yin and Yang 1 (*YY1*)

Target genes	miRNAs	References	Reported effects
<i>Bmi1</i>	miR-183	[49]	Derepress expression of p53 and inhibit tumorigenicity
	miR-128	[49–51]	
	miR-194	[52]	
	mir-200c	[49, 53]	
	miR-218	[54]	
<i>DNMT3A</i>	miR-203	[49]	Induce global hypomethylation and expression of tumor suppression genes
	miR-29	[55–57]	
	miR-29c	[58]	
	miR-370	[59]	
	miR-450a	[60]	
<i>DNMT3B</i>	miR-29	[55–57]	Induce global hypomethylation and expression of tumor suppression genes
	miR-148	[61]	
	miRNA-29b	[62]	
<i>EZH2</i>	let-7a	[63]	Decrease histone methylation and increase expression of tumor suppressor genes
	miR-26a	[64–67]	
	miR-101	[68]	
	miR-138	[69]	
	miR-124	[70]	
<i>HDAC1</i>	miR-214	[71]	Induce growth arrest by decreasing histone deacetylation
	miR-449	[72]	
<i>HDAC4</i>	miR-874	[73]	Promote myogenesis and impairs transcriptional activity of B-cell lymphoma 6 (<i>BCL6</i>)
	miR-1	[74]	
<i>JMJD1A</i>	miR-155	[75]	Decrease histone demethylation and hypoxic gene expression
	miR-627	[76]	
<i>MECP2</i>	miR-155	[77]	Promote demethylation and cell differentiation
	miR-132	[78]	
<i>YY1</i>	miR-483-5p	[79]	Promote cell differentiation in muscle and tumours
	miR-29b	[80]	
	miR-7	[81]	

Epigenetic Therapy

Small Molecules

Conventional pharmaceuticals which modulate the activity of the readers and writers of the epigenetic code are a means of inducing large-scale changes in the epigenome. Small-molecule inhibitors of histone deacetylases (HDACs), histone acetyltransferases (HATs), histone lysine methyltransferases (KMTs) and protein arginine methyltransferases (PRMTs) are already in clinical use or in the drug development pipeline [1, 95]. Given the importance of epigenetics in oncogenic transformation, many of these molecules are aimed at treating tumours [26, 96]. One of the most studied is Vorinostat (suberoylanilide hydroxamic acid), a histone deacetylase inhibitor that is currently FDA-approved for the treatment of leukemia but is also being tested in brain tumor clinical trials [97, 98]. Vorinostat activates the expression of genes involved in cell differentiation by inhibiting HDACs, which have been found to be overexpressed in tumours [99]. Vorinostat is being tested for its single-agent activity and also in combination with

compounds like the proteasome inhibitor Bortezomib where it has been found to induce sensitization of malignant human glioma cells to apoptosis [98].

HATs are also involved in the normal function of the CNS and they have been shown to be vital for memory formation and neuronal plasticity [100]. The loss of HAT activity has been implicated in the pathogenesis of a number of neurodegenerative diseases. Histone deacetylase (HDAC) inhibitors that decrease HDAC activity and, therefore, increase histone acetylation represent a promising therapeutic strategy [100]. For example, valproic acid is a HDAC inhibitor and one of the most widely prescribed anti-epileptic drugs for its anticonvulsant properties. Although its precise mechanism of action remains unclear it is thought to involve an increase of the neurotransmitter gamma-aminobutyric acid (*GABA*) [101]. HDAC inhibitors are also being studied as possible treatments for polyglutamine diseases like Huntington's disease [102].

Other well-known epigenetic therapeutics are inhibitors of DNA methylation like the nucleoside analogues 5-aza-CR and 5-aza-CdR [95]. These compounds have been approved by the FDA as treatments for leukemia and myelodysplastic

syndromes [103]. DNA methylation also plays an important role in glioblastoma [104] and these compounds are now also under investigation as possible therapies for these cancers. Thus, treatment of glioma cells with 5-aza-dC was able to reactivate the expression of the brain angiogenesis inhibitor (*BAIL*), which is normally silenced in gliomas, by recruitment of the methyl-CpG-binding domain protein 2 (*MBD2*) to the hypermethylated *BAIL* promoter [105].

The major limitation of small molecule-mediated epigenetic therapies is that they are non-specific in nature and therefore have the potential for multiple off-target gene expression effects. For example, epigenetic activation of oncogenes could promote oncogenic transformation. Similarly both DNA methyltransferase inhibitors and HDAC inhibitors can cause apoptosis through p53 activation. In the case of epigenetic therapies for neurodegenerative disorders, which require long-term treatment, this p53 activation could lead to increased loss of neurons and thereby exacerbate pathology [106]. Consequently, off-target effects induced by epigenetically active small molecule pharmaceuticals must be considered carefully.

Gene-Specific Epigenetic Therapy

Small interfering RNA (siRNA) molecules are known to silence gene expression at the post-transcriptional level in a process called RNA interference (RNAi) which utilises the host cell miRNA processing machinery. In 2004, Morris et al. [107] showed that siRNAs could also induce transcriptional gene silencing (TGS) by an epigenetic mechanism in human cells. Since this landmark study there have been numerous further reports of TGS in mammalian cells and much of the mechanistic detail has now been elucidated.

In this pathway, siRNAs or miRNAs are loaded into AGO1. AGO1 is the core component of the RNA iInduced transcriptional silencing (RITS) complex which is analogous to RISC that mediates conventional RNAi. RITS also includes the histone deacetylase, HDAC1, the histone methyltransferases SUV39H1 and EZH2 [108], and the DNA methyltransferase DNMT3A. AGO1 facilitates the interaction between the small RNA effector and the promoter-associated RNA target [106]. Subsequently, recruitment of RITS induces heterochromatin formation and promoter DNA methylation at the target promoter. Because RITS is guided to a complementary promoter-associated RNA by its bound small RNA, targeting a promoter with a small RNA TGS effector can lead to chromatin remodelling (promoters become enriched in the silent state chromatin marks H3K9me2 and H3K27me3) and, in some cases, promoter DNA methylation [108, 109].

One of the main advantages of inducing epigenetic changes is that the silencing can be long-term [108], and therefore repeated administration of the effector molecules is not needed. TGS can be induced using siRNAs, antisense oligonucleotides [109], expressed antisense RNAs [110] or short hairpin RNAs

[111, 112]. And there are also examples of the successful implementation of this approach *in vivo* by local delivery in muscle [113], retina [112] and xenograft models [114]. The specificity of TGS is due to sequence complementarity between the small RNA effector and the target locus. However, although TGS is assumed to be highly specific, there have been no studies to date that have tested this in a systematic way.

A similar approach consists of targeting natural antisense transcripts (NATs) [90, 91, 115] (that can also be promoter-associated) in order to activate gene expression. Many NATs act as epigenetic silencers of their corresponding sense-orientated protein-coding gene. By targeting the NAT with small interfering RNAs or antisense oligonucleotides, this epigenetic silencing is relieved leading to an increase in sense transcription and corresponding gene activation. The NAT is transcribed in the opposite direction to its corresponding protein-coding gene and recruits the histone methyltransferase enhancer of EZH2, which is part of the polycomb-repressive complex2 (PRC2). EZH2 alters the chromatin structure by adding the repressive mark H3K27me3 and inducing transcriptional silencing. In the case of neurological disorders, the effectiveness of this approach has been recently validated *in vivo* [90]. This study by Modarresi et al. [90] was the first demonstration of oligonucleotide-mediated transcriptional gene activation in brain. Delivery of locked nucleic acid (LNA) gapmer antisense oligonucleotides by intracerebroventricular delivery using an osmotic minipump was shown to up-regulate expression of brain-derived neurotrophic factor (*BDNF*), glial-derived neurotrophic factor (*GDNF*) and the Ephrin receptor B2 (*EPHB2*). The authors also observed concomitant stimulation in neuronal outgrowth and survival.

MicroRNA Therapy

In some cases, compensation for the loss of a particular miRNA or up-regulation of a protective miRNA could be used to treat disease [116]. For instance, miR-223 can be neuroprotective following stroke [117]. This miRNA represses expression of the glutamate receptor subunits GluR2 and NR2B in the CNS, decreasing neuronal calcium influx and thereby minimizing neuronal cell death following ischemia [117]. Mimicking the function of beneficial miRNAs, such as miR-223, can be achieved by overexpressing the miRNA precursor or as chemically synthesized oligonucleotides [118, 119]. A number of studies have demonstrated the feasibility of this approach. For example, miR-137 mimics negatively regulate neural stem cell proliferation [120]. Additionally, miR-132 mimics have been shown to block ocular dominance plasticity produced by monocular deprivation when delivered to the mouse visual cortex [121].

Some small molecules seem to also be able to enhance the biogenesis of a particular subset of miRNAs. As such, small

molecule libraries have been screened in order to identify compounds that modulate the biogenesis of particular miRNAs [122–124]. For example, the antibiotic enoxacin has been found to enhance miRNA biogenesis by binding to the RISC component TARRBP2 (TAR RNA-binding protein 2) which leads to increased levels of tumor suppressor miRNAs [125] and miRNAs involved in neural development [126].

Conversely, the aim of some therapies is to decrease the levels of miRNAs or block their effects at the mRNA level. Currently, the most advanced approach is silencing of miRNAs using antisense oligonucleotides, termed antimiRs. AntimiRs have been successfully implemented in a number of animal models including non-human primates [127, 128]. A remarkable variant of this strategy is the use of tiny (8mer) LNA oligonucleotides that are able to block the action of whole families of miRNAs by binding to their common seed sequences [25]. AntimiRs have been also been studied in the context of neurodegenerative diseases like Friedreich's ataxia (FRDA), the most common inherited ataxia. FRDA is caused by an intronic repeat expansion in the frataxin (*FXN*) gene that induces a loss of function pathology. The protein levels of the *FXN* gene are reduced and levels of miR-886-3p are increased in patient samples suggesting that this small RNA is involved in the pathology of FRDA. Treatment with an antimiR targeting miR-886-3p led to the increase of *FXN* expression demonstrating that this miRNA is a promising therapeutic target for FRDA [129]. The most advanced miRNA based therapy is Miravirsen which is currently in late-stage clinical trials for the treatment of chronic hepatitis C virus (HCV) infection. Miravirsen is a LNA modified phosphorothioate oligonucleotide that targets and blocks miR-122 (a miRNA required for HCV viral replication) [130].

A number of different oligonucleotide chemistries have been used to inhibit miRNA activity in cell lines or *in vivo* following intracerebroventricular injections. For instance, 2'-O-methyl oligonucleotides have been shown to inhibit let-7b and miR-124, thereby inducing proliferation of neural stem cells [131]. Treatment with LNA antisense oligonucleotides decreased the levels of miR-21 in glioblastoma cell lines [132] and miR-219 in cortex [128, 133]. Similarly, peptide nucleic acids (PNAs) delivered into neurons decreased miR-326 activity and caused an increase in expression of synaptic plasticity genes [134].

An alternative approach to inhibiting miRNA regulation is the use of miRNA protectors. These protectors are oligonucleotides complementary to the miRNA binding site on the 3' UTR of the target mRNA. These oligonucleotides compete with the miRNAs for the binding site on the mRNA, preventing the post-transcriptional inhibition of the target mRNA. The main advantage of this approach is that it limits the non-specific (off target) effects of other miRNA therapies like miRNA mimics that can target hundreds of genes. As an alternative to oligonucleotide-based therapies, miRNA sponges with multiple miRNA binding sites [135, 136] have

been also used this time to sequester miRNAs. For example, delivery of a miR-9 sponge resulted in modified motor neuron subtypes in the developing spinal cord [137]. Finally, an important point when designing therapies aimed to correct both ncRNA or epigenetic changes is to establish whether each of these changes are in fact a pathological cause or form part of the physiological protective response to the disease. Therefore, therapies should be based on the deep knowledge of the neurophatology provided by basic science rather than on down- or upregulated levels in affected tissues.

Conclusion

NcRNA / epigenetic regulation forms a network of interactions which shape the landscape of gene expression and development. Both epigenetic mechanisms and miRNAs play a fundamental role in neurogenesis and normal brain function. Additionally, there are a myriad of interactions at the interface of miRNAs and epigenetics. Thus, chromatin remodelling can occur in miRNA coding sites, miRNAs can silence the expression of epigenetic modifier genes and endogenous miRNAs can be the effectors of transcriptional gene silencing targeting complementary promoters to result in epigenetic modification. Furthermore, subtle alterations in the enzymes required for the epigenetic DNA methylation and chromatin remodelling or for miRNA expression or processing can lead to complex diseases such as neurological disorders and cancer. Work done in multiple laboratories worldwide is improving our understanding of this complex regulatory network that will likely lead to the development of new disease biomarkers and therapies.

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