**microRNA dysregulation in neurodegenerative diseases: a systematic review**

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## Introduction

Neurodegenerative diseases are defined by the progressive deterioration of neuronal structure and function eventually leading to neuronal loss that is thought to underlie most of the neurological impairments [1]. In addition to neuronal cell dysfunction and death, neurodegenerative diseases often share common cellular mechanisms and histopathological features regardless of their etiology [2]. For instance, aberrant protein processing, trafficking and aggregation in neural cells are observed in Alzheimer's disease (AD), amyotrophic lateral sclerosis (ALS), Huntington's disease (HD), Parkinson's disease (PD), prion diseases as well as in other neurodegenerative diseases [3-5]. This disruption in cellular protein homeostasis is often associated with extracellular plaque build-up that induce local inflammation through astrocyte and microglia activation [2, 3, 6-8]. Aberrant RNA metabolism is another common feature for several neurodegenerative diseases: ALS, ataxia, HD and myotonic dystrophy (DM) [9-11]. Furthermore, dysfunctional glutamate neurotransmission leading to neurotoxicity and oxidative injury are also a recurring theme in neurodegenerative diseases, including epilepsy [2, 12-15]. Shared pathophysiological processes in multiple neurodegenerative diseases raises the possibility that identifying common molecular regulation across various diseases may lead to approaches for therapies, which would be effective for multiple indications.

MicroRNAs (miRNAs) regulate hubs of gene expression and are dysregulated in many neurodegenerative diseases and their animal models [16-20]. miRNA biogenesis is a two-step cleavage process where a hairpin structure is made progressively shorter; the initial cleavage by ribonuclease Drosha and Dgcr8, and the second cleavage event by Dicer alone to form a miRNA duplex [21]. The duplex is loaded onto an Argonaute protein to form the core of the miRNA-induced silencing complex (miRISC), where a passenger strand of the duplex is ejected. The miRISC binds to the 3'-untranslated region (3'UTR) of target mRNA strands, leading to the degradation or translational repression of mRNAs. Disruption of the miRNA biogenesis machinery has been used to demonstrate the critical role of miRNAs in the central nervous system (CNS) [22-34]. Specifically, deletion of Dicer in different brain regions leads to brain atrophy, neurodegeneration, gliosis, locomotor deficits, and shortened lifespans [23, 25, 27, 28, 30-32]. Similarly, retinal deletion of Dicer or Dgcr8 lead to retinal degeneration and loss of visual function [22, 24, 26, 29, 33, 34]. Decreased Dicer expression has also been observed in patients with advanced aged-macular degeneration (AMD), epilepsy, and multiple sclerosis (MS) [29, 35-37]. Animal models of epilepsy and MS also exhibit decreased Dicer expression [37, 38]. Disruption of the miRNA-processing machinery provides proof-of-principle evidence that miRNAs are essential to nervous system function and integrity, and that disruption of miRNA expression could contribute to deficits identified in some neurodegenerative diseases.

Reviews of the literature describe the disruption of miRNA expression within specific neurodegenerative diseases but miRNA regulation across neurodegenerative diseases has not been systematically reviewed [39-41]. Identifying shared miRNA dysregulation across neurodegenerative diseases may lend insight into the conserved molecular pathways affected

during disease and potentially point towards novel targets for creating therapies aimed at general degenerative mechanisms involved in many neurodegenerative diseases. Here we perform a non-biased systematic review of studies identifying miRNA dysregulation in neurodegenerative diseases to facilitate identification of shared patterns of miRNA dysregulation across multiple neurodegenerative diseases. We recorded miRNA expression from articles that report miRNA expression spanning 12 neurodegenerative diseases and their animal models: AD, amyotrophic lateral sclerosis (ALS), AMD, ataxia, non-AD related dementia, DM, epilepsy, glaucoma, HD, MS, PD, and prion disorders. We describe seven individual miRNAs and one miRNA family occurring frequently within and across neurodegenerative diseases: miR-9-5p, miR-21-5p, miR-124-3p, miR-132-3p, miR-146a-5p, miR-155-5p, miR-223-3p, and the miR-29 family. Interestingly, three of these miRNAs were frequently upregulated in neurodegenerative diseases, while the other seven miRNAs did not exhibit a conserved direction of dysregulation. We also report that these miRNAs have roles in both the immune system and CNS, suggesting inflammation as a major component of neurodegenerative disease.

## Methods

*Search strategies*- A systematic and comprehensive search of the literature was conducted by a librarian (AA-Z) to retrieve articles discussing neurodegenerative conditions and miRNA. A search strategy was developed for Medline via Ovid and adapted to other databases: Embase, PSYCinfo and Biosis Previews, all also via Ovid. The Medline search strategy searched in controlled vocabulary where possible, along with text in the title, abstract, or author-supplied keyword fields. Searches conducted subsequent to Medline excluded the articles already found. The searches were initially run on 2017-07-13 and were updated with additional terms on 2017-11-07 and 2019-03-22. The updated Medline strategy is included in Supplementary File 1. Reference lists of included studies were also evaluated. Search terms were selected to be inclusive of all neurodegenerative diseases.

*Data management and screening*- Results of the literature searches (6421 publications) were imported into an Endnote library. Duplicate publications were removed prior to review. Two independent reviewers assessed the eligibility of articles using a policy of liberal acceleration: only one reviewer's approval was necessary to advance a publication to the next stage of screening, but both reviewers had to agree on the exclusion of a publication, as previously described for Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) [42, 43]. This strategy was chosen to ensure that every rejected article was validated as unrelated to the topic of this review by a second reviewer.

Articles went through four rounds of evaluation (Fig. 1). The first two rounds assessed the relevance of the article to the review topic (miRNA and neurodegenerative disease) and the type of article (review, conference abstract, primary research article, etc.) based on the information in their title and abstract. At the title and abstract stages, articles were excluded if they were not primary research articles, or if they did not refer to either miRNA or neurodegenerative disease.

At the manuscript stage papers were evaluated using a more rigorous set of exclusion criteria: not a primary research article; no *in vivo* or *ex vivo* results; no reporting of differential expression data of mature miRNA or only reporting differential miRNA expression data taken from previous studies or from online repositories; not related to neurodegenerative disease; a duplicate; and/or inaccessible. These criteria were chosen to identify papers that reported new miRNA regulation data of biological material from neurodegenerative diseases and their models. Following the manuscript evaluation, papers were sorted into categories based on the neurodegenerative diseases studied. Any neurodegenerative disease category with fewer than 10 papers were excluded from further analysis as this was determined to be below the threshold for synthesis and analysis. This resulted in 641 accepted articles making up 12 disease categories: AD, ALS, AMD, ataxia, dementia, DM, epilepsy, glaucoma, HD, MS, PD, and prion disorders (Fig. 1).

Next, we wanted to determine what miRNAs were frequently dysregulated across these diseases. For each of the 641 accepted manuscripts, we recorded the miRNAs that were reported as significantly dysregulated, and the direction of dysregulation relative to control in a neurodegenerative disease and/or its animal model. We also list the identify of the profiled tissue

or bodily fluid (Supplementary File 2). When miRNAs were identified by microarray or next-generation sequencing, only miRNA regulation that was validated by qPCR was recorded. Studies incorporating miRNA overexpression or knockdown in an animal model of neurodegenerative disease were also recorded. miRNA annotation was confirmed using the miRbase Database [44]. We recorded a total of 2318 dysregulated miRNAs identified in the 641 manuscripts. We then assessed which miRNAs occurred in over half (7/12) of the disease categories and found 52 miRNAs that fit this criterion. Of these 52 miRNAs, some also occurred more frequently within the diseases. In order to identify the miRNAs occurring most frequently both across and within neurodegenerative diseases, we applied cut-offs per disease category based on the number of publications (Supplementary File 2, tab 1; Fig. 1). This identified 7 individual miRNAs that are miR-9-5p, miR-21-5p, miR-124-3p, miR-132-3p, miR-146a-5p, miR-155-5p, miR-223-3p. We also identified three miRNA families that occurred within our 52 miRNAs. From those three miRNA families, the miR-29 family occurred most frequently, and it was included for analysis with the 7 individual miRNAs.

*Heat map*- To determine if a miRNA was regulated in a distinct direction within our 10 assessed neurodegenerative diseases, either upregulated or downregulated during disease compared to control, we generated a heat map synthesizing their expression. If a study assessed the expression at multiple stages of diseases, or within multiple tissue, this was included as an additional data point. For each disease when a miRNA was upregulated in a distinct tissue, it was assigned 1, and if downregulated it was assigned -1. The sum was taken for each miRNA for a disease, and then divided by the amount of times it was identified as differentially dysregulated to determine a rank. If a miRNA has a rank closer to 1, this implies that it was mainly upregulated. If a miRNA has a rank closer to -1, this implies it was mainly downregulated. If a miRNA has a rank close to 0, this implies that it was reported as significantly upregulated and downregulated in an approximately equal number of tissues. Data that compared miRNA expression between patients treated with a disease-modifying therapies (DMTs) versus baseline were not included; nor were animal studies where the miRNA expression was manipulated. Data was compiled into an CSV file read by a python script with pandas (http://pandas/pydata.org/) to create a dataframe. We used Plotly's python API (http://plot.ly/) to create a heatmap from the dataframe [45].

## Results

Our initial search strategy of the systematic review resulted in a sum of 6421 publications, of which 989 were reviewed at the manuscript level (Fig. 1). Diseases that had fewer than 10 manuscripts were excluded as they were below our threshold for synthesis. This resulted with 641 manuscripts covering 12 disease categories: AD, ALS, AMD, ataxia, dementia, DM, epilepsy, glaucoma, HD, MS, PD, and prion disorders (Fig. 1).

To identify frequently dysregulated miRNAs spanning multiple neurodegenerative diseases, for each of the final 641 analyzed manuscripts, we identified the profiled tissue and if the miRNA was significantly upregulated or downregulated relative to control in neurodegenerative disease and/or its animal model. Animal models are an important consideration for some neurodegenerative diseases such as AMD, ataxia, dementia, glaucoma, epilepsy, HD, and prion disorders where there are few studies investigating dysregulation of miRNA in human material. All accepted manuscripts sorted by disease and denoting manuscripts that profiled miRNA expression in multiple neurodegenerative diseases are presented in Supplementary File 2. Studies incorporating miRNA overexpression or knockdown in an animal model of neurodegenerative disease were also recorded.

Using this set of 641 manuscripts we identified 10 miRNAs that were differentially regulated in at least 50% of the neurodegenerative diseases and/or their animal models that were also frequently dysregulated within the individual disease categories: miR-9-5p, miR-21-5p, miR-29a-3p, miR-29b-3p, miR-29c-3p, miR-124-3p, miR-132-3p, miR-146a-5p, miR-155-5p, and miR-223-3p. We reviewed the manuscripts describing regulation of these miRNAs during neurodegenerative diseases in greater detail and summarized the miRNA expression profiles in Table 1. We found that miRNA expression data from the animal models did not confound the human data, but rather supported it. To visualize the expression of these 10 miRNAs across neurodegenerative diseases and their animal models we generated a heat map to represent changes in expression (Fig. 2). A limitation of these studies is the small number of studies for some miRNA in individual diseases. For example, miR-29b-3p and miR-132-3p are reported in a single study for AMD as upregulated, resulting in a score of 1 on the heat map.

To generate hypotheses about how these 10 frequently dysregulated miRNAs may contribute to neurodegenerative disease we thoroughly reviewed the papers and their known mechanism of action. Figure 3 summarizes the validated targets and pathways identified by these studies, breaking down the pathways by immune and neural components.

*Upregulated miRNAs*

Within the top ten miRNAs, we found the miRNAs miR-146a-5p, miR-155-5p, and miR-223-3p to be generally upregulated across the 12 investigated diseases ("miRNA sum" ≥ 0.5; Fig. 2) (Table 1). Upregulation of these miRNAs across neurodegenerative disease raises the possibility that they may contribute to common mechanisms underlying disease pathogenesis and the possibility that they are upregulated as a response to neurodegeneration itself. Here we discuss the known functions of these three miRNAs in more detail.

*miR-155-5p*

miR-155-5p is described as a master regulator of the immune response, specifically driving myeloid cell polarization to a pro-inflammatory state [46, 47]. Deletion of miR-155 prolonged survival in $SOD1^{G93A}$ mice, prevented Experimental Autoimmune Encephalomyelitis (EAE) induction, and attenuated microgliosis and neuronal loss in an α-synuclein-driven PD model [48-56]. In the PD model, miR-155-5p deletion was associated with a reduction of MHCII expression in microglia and attenuated iNOS upregulation in response to α-synuclein[48]. Improvements in the $SOD1^{G93A}$ and EAE models upon miR-155 deletion were associated with decreased expression of proinflammatory cytokines interferon-γ (IFN-γ) and interleukin-17 (IL-17) (Fig. 3) [49-56]. miR-155 deletion also causes increased expression of targets that block myeloid cell proliferation and survival, Th17 development, oxidative stress, and blood brain barrier (BBB) permeability [53, 56-58]. Increased miR-155-5p expression across the profiled neurodegenerative diseases, and its well-known roles in promoting neuroinflammation raises the possibility that increased miR-155-5p expression may exacerbate neurodegenerative disease pathology via an inflammatory mechanism.

*miR-223-3p*

miR-223-3p is a well-characterized hematopoietic miRNA, regulating myeloid cell and granulocyte differentiation, and dendritic cell activation [59-62]. In MS and EAE, miR-223-3p upregulation is associated with pathogenic polarization of immune cells and increased inflammation. *miR-223 -/-* EAE animals possess diminished populations of pathogenic Th1 and Th17 cells, due to a larger population of myeloid-derived regulatory cells and reduced dendritic cell activation[62-64]. These lines of evidence suggest that upregulation of miR-223-3p within immune components may be deleterious in neurodegenerative disease. However, early work characterizing miR-223-3p suggest that the roles of miR-223-3p may be more nuanced since both overexpression or deficiency of miR-223 leads to granulocyte overproduction and widespread inflammation [59, 60]. miR-223-3p targets and inhibits components within the NF-κβ pathway, including TRAF6, Tab1, and Cul1a/b, which are part of the canonical pathway; and IKKα, which controls both canonical and non-canonical activation of NF-κB [65, 66]. Through suppression of the canonical NF-κB pathway, miR-223-3p expression is shown to dampen neutrophil activation, suggesting anti-inflammatory effects [66]. Conversely, miR-223-3p also targets IKKα, an anti-inflammatory factor that may prevent spontaneous activation of

macrophages, thus promoting inflammation [65]. The involvement of miR-223-3p in both the pro- and anti-inflammatory NF-κB cascades demonstrates the divergence in its immune regulatory roles.

In addition, miR-223-3p appears to promote neural repair and regeneration. In an animal model of ischemia, *miR-223 -/-* mice showed contextual memory deficits, enhanced excitotoxicity, and neuronal death [67]. miR-223-3p overexpression blocked these effects by targeting GluR2 and NR2B, AMPA receptor and NMDA receptor subunits, respectively (Fig. 3). Similarly, miR-223 overexpression in the retina and optic nerve blocked the formation of EAE-driven pathological axonal swellings, attributed to decreased excitotoxicity by reduced GluR2 and NR2B expression [68]. In models of regeneration, miR-223-3p is upregulated, specifically in mouse DRGs post sciatic nerve lesion, and in the regenerating optic nerve of zebrafish [69, 70]. These results suggest miR-223-3p may play a direct role in the neuronal response to injury.

*miR-146a-5p*

miR-146a-5p is also a major regulator of the NF-κB pathway [71]. The pre-miR-146a gene is positively regulated by NF-κB transcription factor and the mature miRNA-146a-5p product operates in a negative feedback loop by inhibiting IRAK1 and TRAF6, two upstream NF-κB signaling components (Fig. 3) [71]. This regulatory loop operates as a brake against rampant myeloid-driven inflammation. Indeed, *miR-146a -/-* animals had worsened motor pathology with a massive 10-fold increase in myeloid cell proliferation and T-cell activation caused by relief of inhibition on IRAK1 and TRAF6 [72, 73]. miR-146a-5p targeting of IRAK1 and TRAF6 also reduces T-cell adhesion *in vitro*, indicating that miR-146a-5p may impair the ability of immune cells to cross the BBB [74]. These functions of support a model whereby upregulation of miR-146a-5p may play a neuro-protective role in neurodegenerative disease. This is further supported by a report that miR-146a-5p overexpression promotes remyelination of axons in the cuprizone model of demyelination [75].

miR-146a-5p overexpression can also indirectly lead to the promotion of pro-inflammatory non-canonical NF-κB signaling [76]. Specifically, downregulation of TRAF6 and IRAK1 of the NF-κB pathway by miR-146a-5p overexpression is compensated by an upregulation in IRAK2, in turn activating myeloid cells and promoting cytokine release that perpetuates TLR-IL-1R-mediated NF-κB activation, as shown in AD models [76]. Thus, miR-146a-5p may be a protective response to disease by limiting NF-κB signaling and promoting remyelination. However, the complexity of the negative feedback loop between miR-146a-5p and NF-κB suggests that increased levels of miR-146a-5p can indirectly contribute to other pro-inflammatory responses. Increased levels of miR-146a-5p coincide with increased AD clinical scores[77]; and in AD mouse models, miR-146a-5p positively correlates with clusters of activated microglia[78]. In EAE, an animal model of MS, stress-exposure that exacerbated disease also further increased miR-146a-5p expression[73, 79]. Thus miR-146a-5p upregulation may have a dichotomous role with both pro-inflammatory and anti-inflammatory responses with divergent effects in neurodegenerative disease progression.

*Mixed Regulation*

The remaining miRNAs, miR-9-5p, miR-21-5p, the miR-29 family, miR-124-3p, and miR-132-3p demonstrate mixed level of expressions across tissue and animal models of the 12 investigated neurodegenerative diseases (-0.5 < "miRNA sum" < 0.5; Figure 2). Their regulation and functions are further described below.

*miR-9-5p*

Many miR-9-5p functions are related to CNS development, including the proliferation and differentiation of neural stem cells, and levels remain enriched throughout adulthood (Fig. 3) [80, 81, 82]. In $SOD1^{G93A}$ mice, an ALS model, degeneration of motor neurons promotes the proliferation and differentiation of neuronal progenitors. This effect has been proposed as a compensatory neurogenesis response against motor neuron loss [83, 84]. Elevated levels of miR-9-5p within CNS tissue may reflect the accelerated proliferation and differentiation of progenitors [85, 86]. Accordingly, miR-9-5p upregulation within CNS tissue is also negatively correlated with STAT3 activation, a factor known to inhibit neuronal cell fate [85, 87]. The role of miR-9-5p in modulating neuronal fate in neurodegenerative disease is further supported by miR-9-5p targeting transcription factor REST [88]. REST suppresses neural fate and promotes miR-9-5p expression, which targets and inhibits REST expression in a negative feedback loop. This homeostasis is disrupted in HD, contributing to disease pathogenesis [88]. Neurodegenerative diseases AD, HD, MS and PD also show signs of altered neurogenesis [89-92]. This raises the interesting possibility that dysregulation of miR-9-5p and subsequent changes in neuronal progenitor populations may be a common altered mechanism between neurodegenerative diseases.

*miR-21-5p*

In animal studies modelling aspects of MS (EAE) and PD (MPTP), miR-21-5p downregulation appeared to be neuroprotective[93-95]. miR-21-5p expression is downregulated in an EAE-resistant rat strain, and *miR-21 -/-* mice were resistant to EAE induction and exhibited defects in Th17 differentiation (Fig. 3) [94, 95]. In MPTP-treated mice, miR-21-5p knockdown resulted in increased neuronal survival [93]. This neuroprotective effect was mediated by increasing the expression of the miR-21-5p target LAMP2A. LAMP2A promoted autophagy of α-synuclein, thereby limiting pathogenic aggregation [93]. miR-21-5p downregulation also relieved its target PPARα, a transcription factor that promotes the expression of neuroprotective factors such as brain-derived and glial derived neurotrophic factors (BDNF and GDNF, respectively), and limited the expression of neuroinflammatory factors including NF-κB [96]. These studies suggest that miR-21-5p inhibition dampens the inflammatory response and promotes neuroprotection.

Conversely, miR-21-5p was often downregulated in AD patients and animal models [97-100]. In an animal model of AD, overexpression of miR-21-5p promoted functional recovery [101]. Likewise, overexpression of miR-21-5p in a mouse model of glaucoma, induced by increased intraocular

pressure (IOP), prevented loss of retinal neurons [102]. Increased IOP in the glaucoma model is associated with increased Aβ-deposition in apoptotic retinal neurons, which can be targeted to reduce disease pathogenesis [103]. Thus, in AD and glaucoma models, miR-21-5p dysregulation is likely related to Aβ pathology. Ultimately, the dichotomous dysregulation of miR-21-5p points to a complex role in neurodegenerative disease and additional research in its functional contributions to disease would offer clarity.

*miR-29 family*

The miR-29 family consists of two miRNA clusters, miR-29ab1 and [104]. The clusters produce miR-29a, miR-29b and miR-29c; these miRNAs share the same seed sequences and thus the ability to target the same genes. Animal studies suggest miR-29 is necessary for proper motor function. miR-29ab loss-of-function shows severe motor impairment [105, 106]. Disease modifying treatments of both PD and MS patients resulted in miR-29a-3p upregulation relative to untreated controls[107-109]. This suggests that these disease-modifying treatments (DMTs) may aid in restoring normal expression of miR-29a-3p. However, challenging this are experiments showing that miR-29ab1 deletion in EAE mice led to an improvement in the EAE disease course [104]. This could be a result of a negative feedback loop formed by miR-29 and IFN-γ, where IFN-γ induces pathogenic Th1 biology in EAE mice (Fig. 3) [104, 110]. This falls in line with decreased miR-29-a-3p and miR-29c-3p in the peripheral blood mononuclear cells (PBMCs) of MS patients following treatment with the DMT IFN-β [111].

*miR-124-3p*

miR-124-3p expression is predominantly expressed in the CNS and most articles profiled its dysregulation in CNS tissue (Table 1) [112]. Overexpression of miR-124-3p led to symptomatic recovery in animal models of AD, AMD, glaucoma, HD, MS, and PD [112-120]. Functionally, miR-124-3p has been implicated in apoptotic signaling, autophagy, neurogenesis, glutamate signaling, and immune modulation (Fig. 3). miR-124-3p overexpression in these animal models appears to regulate these functions to limit or prevent disease pathogenesis.

In an AD model, miR-124 overexpression reduced expression of BACE1; decreased pathological tau; restored autophagy function by reducing Bax; and restored or improved behavioural outputs such as memory [112, 113, 119]. In a PD animal model, miR-124-3p directly targeted and inhibited Bim, a protein that mediates translocation of Bax to both mitochondrial and lysosomal membranes, mediating both apoptotic and autophagic pathways, respectively [121]. Thus, upregulation of miR-124 may inhibit apoptotic processes through inhibition of Bim and indirectly through Bax.

In a PD model, miR-124-3p delivery to the subventricular zone by nanoparticles enhanced neurogenesis and neural cell differentiation by targeting cell-fate proteins Sox9 and Jagged1 (Fig. 3) [114]. *In vitro* these nanoparticles also promoted axonogenesis through modulation of the c-Jun N-terminal kinase (JNK) pathway. Likewise, in ALS and HD mouse models, Sox9

downregulation was associated with increased miR-124-3p [85, 116]. Similar to miR-9-5p, this suggests miR-124-3p may enhance neurogenesis and differentiation of NPCs, perhaps to compensate for neuronal loss within ALS and HD mouse models.

miR-124-3p is also suggested to limit glutamate excitotoxicity by targeting AMPA receptors (AMPAR) (Fig. 3) [122-124]. In an AD mouse model, miR-124-3p upregulation in the brain was associated with a decrease in target PTPN1 [122]. PTPN1 inhibition lead to decreased AMPAR membrane-insertion, resulting in AMPAR downregulation. miR-124-3p can also directly target GluR2, an AMPAR subunit[123]. This was observed in an animal model of dementia and in demyelinated MS lesions[123, 124]. In dementia, miR-124-3p overexpression or GluR2 knockdown rescued behavioural deficits; and in the lesions of human MS and in demyelinated mouse hippocampi, increased miR-124-3p was associated with AMPAR downregulation[123, 124]. Remyelination of mouse hippocampus reversed these changes. These findings suggest a neuroprotective mechanism in which miR-124-3p downregulates AMPAR to reduce glutamate excitotoxicity in regions of demyelination.

Finally, miR-124-3p was identified to have an immunomodulatory effect both in MS and PD, where its overexpression reduced macrophage/microglia activation to limit disease progression (Fig. 3) [115, 120]. In sum, overexpression of miR-124-3p appears to predominantly promote functional repair in various animal models of neurodegenerative disease.

*miR-132-3p*

miR-132 and miR-212 form a cluster, where the two miRNAs have similar sequences and thus seed regions; however, miR-132 is the major functional species in the brain [125]. miR-132-3p was predominantly downregulated in CNS tissue (Table 1), suggesting it may be required for proper CNS function. miR-132-3p expression is promoted by CREB – a transcription factor typically associated with promotion of neurotrophic factors – and is downregulated by REST (Fig. 3) [126]. Through CREB, miR-132-3p promotes neurite outgrowth, dendritic growth, and maintenance of the circadian clock [127-129]. The importance of miR-132-3p has been demonstrated through loss-of-function experiments. miR-132-3p deletion promotes apoptosis in neurons [130]. AD mouse models crossed with *miR-132/212 -/-* mice display worsened long-term memory, enhanced Aβ burden, and increased tau pathology; where multiple genes of the tau subnetwork are miR-132-3p targets [125, 131, 132].

EAE mice treated with anti-inflammatory agent tetrachlordodibenzo-p-dioxin (TCDD) exhibited decreased clinical deficits and these treatment effects were lost with miR-132 loss-of-function, thus demonstrating that miR-132-3p is involved in anti-inflammatory attenuation of EAE by Th2-promoting TCDD [133]. TCDD upregulation of miR-132-3p decreases target acetylcholinesterase, relieving hydrolysis of acetylcholine, a key suppressor of pro-inflammatory cytokines [126, 133]. However, like TCDD, proinflammatory FICZ also promoted miR-132-3p upregulation[133, 134]. miR-132-3p's protective roles in inflammatory modulation is further

complicated by its involvement in Th1 and Th17 immune cells. *miR-132/212 -/-* mice showed a resistance to EAE induction, affiliated with lower frequencies of Th1 and Th17 cells (Fig. 3) [134]. Th1/Th17 dominant paradigms, expressing higher IL-17, IFN-γ, and TNF-α levels, have elevated miR-132-3p [135-137]. These results demonstrate that miR-132-3p has context-dependent roles in both anti-inflammatory and pro-inflammatory pathways.

*Disease trends*

Interestingly, global trends for the predominant miRNAs taken collectively were uncovered within some disease categories. In the AMD, MS and prion disease categories, the predominant miRNAs surveyed were more frequently reported to be upregulated ("Disease sum" $\geq$ 0.5). Inversely, in DM and HD disease categories the different miRNAs surveyed were predominantly reported to be downregulated ("Disease sum" $\leq$ - 0.5; Figure 2). For the remaining (7 out of 12) disease categories, no clear global trends emerge out of the reports for the miRNAs surveyed (- 0.5 < "Disease sum" < 0.5; Figure 2). Whether this suggests anything about the pathogenesis of the disease is unclear. It is possible that there are disease mechanisms that generally interfere with miRNA homeostasis.

## Discussion

MiRNAs are pervasive post-transcriptional regulators and their involvement in neurodegenerative disorders is supported by a vast literature. With this systematic review of the literature, we identified miRNAs that are commonly dysregulated across neurodegenerative diseases. We identified miR-9-5p, miR-21-5p, the miR-29 family, miR-124-3p, miR-132-3p, miR-146a-5p, miR-155-5p, and miR-223-3p as the miRNAs predominantly reported to be dysregulated in 12 categories of neurodegenerative disease and related animal models (Fig.1).

One of the main findings of this review is that three miRNAs, miR-146a-5p, miR-155-5p, and miR-223-3p, are predominantly upregulated across the neurodegenerative disease categories. However, the other miRNAs identified as predominant in the reviewed literature are either subject to conflicting reports within disease categories or exhibit opposing trends between categories. We also identified directional regulation of the predominant miRNAs within diseases. MS, prion, and AMD disease categories demonstrated a general upregulation of the predominant miRNA species. Conversely, HD and DM demonstrated an overall downregulation of these species. This may indicate specific disease mechanisms have overarching effects on miRNA homeostasis. Indeed, one can speculate that HD and DM, both being trinucleotide repeat disorders resulting in aberrant RNA and protein production and degradation, may result in similar interference in miRNA homeostasis. However, since miRNAs are frequently studied in isolation, there is very little known about the mechanisms behind the dysregulation of multiple miRNAs in neurodegenerative diseases. Since the scope of this article was limited to the predominant miRNAs across neurodegenerative diseases as well, we cannot say whether this dysregulation is conserved when looking at the whole list of miRNAs dysregulated within a specific disease.

We also summarized pathways known to be targeted by the miRNAs commonly dysregulated in neurodegenerative diseases (Fig. 4). There lies important functional overlap between these miRNAs in regulating differing cellular pathways with multiple miRNAs often converging on the same pathways. Pathways targeted by multiple miRNAs include Aβ genesis, regulation of AMPAR subunits, autophagy homeostasis, apoptosis, microglial activation, NF-κB signaling, BBB maintenance, and neurogenesis. The largest shared convergence occurs with Aβ genesis, where miR-146a-5p, the miR-29 family, miR-124-3p, and miR-9-5p all limit Aβ genesis. miR-9-5p, miR-124-3p, miR-29a-3p, miR-29b-3p, and miR-29c-3p target BACE1. Target site cooperation is a fundamental characteristic of miRNA-mediated silencing suggesting these miRNAs are likely to be functioning synergistically to facilitate repression of their shared targets[138, 139]. We also see miRNAs converging on the same pathways but with alternate outputs. For example, miR-29 family members, miR-223-3p, miR-155-5p, and miR-132-3p all regulate T cell activation and proliferation. However, while miR-223-3p and miR-155-5p promote a Th1/Th17 profile, the miR-29 family inhibits Th1 promotion by blocking IFN-γ signaling. miR-132-3p promotes or blocks development of a Th1/Th17 inflammatory milieu depending on the stimuli. We suggest that these miRNAs likely function cooperatively to directly repress

individual targets, as well as indirectly by targeting different mRNAs from within the same pathways.

In summarizing the known functions of these predominant miRNAs, we identified roles for each miRNA in regulating distinct neuronal and immune aspects of disease. A major pathway intersecting both immune system regulation and the CNS was the NF-κB pathway (Fig. 4). miR-146a-5p and miR-223-3p targeted multiple components of this pathway regulating both pro-inflammatory and anti-inflammatory responses[140, 141]. However, the other predominant miRNAs could contribute indirectly by regulating promoters of NF-κB signaling such as Aβ, excess glutamate, and inflammatory cytokines[141]. Non-canonical NF-κB signaling in microglia mediates the production of more inflammatory cytokines and neurotoxic molecules such as glutamate and ROS, which only feeds back into pathological microglial activation[141, 142]. Microglia represent the innate immune cells of the CNS contributing to both defence and maintenance of the CNS [143]. We identified miR-9-5p, miR-124-3p, miR-132-3p, miR-155-5p, and miR-223-3p as regulators of microglial activation. Our systematic review thus complements previous reports of miR-9-5p, miR-124-3p, miR-132-3p, miR-146a-5p, and miR-155-5p as "NeurimmiRs", which are defined as gatekeepers of both the nervous and immune system [144]. We suggest that miR-21-5p, the miR-29 family, and miR-223-3p to be similarly considered NeurimmiRs as they also simultaneously modulate immune cell activation and neuronal function. Many of the predominant miRNAs also were involved in T-cell differentiation and activation further emphasizing their immune roles. Overall these results suggest there is a robust immune response during neurodegenerative disease that may be underestimated in the literature.

In summary, we identified trending miRNAs across neurodegenerative disease. We noted overlapping functions for these miRNAs, suggesting that they work in concert across the diseases. We also noted a strong role for each miRNA in both the neuronal and immune compartments during disease. There are many ways to parse miRNA dysregulation within and across neurodegenerative diseases. By conducting a systematic review of articles discussing miRNA dysregulation in neurodegenerative disease, we have made available a wealth of information to be further exploited in the interest of identifying miRNA dysregulation within or across neurodegenerative disease (Supplementary File 2). Our analysis supports the hypothesis that the identification and future characterization of miRNAs involved in pathological mechanisms common to multiple neurodegenerative diseases may help improve our understanding of commonalities in disease pathogenesis and may aid in novel hypotheses relating to cross cutting DMTs.

## Figures

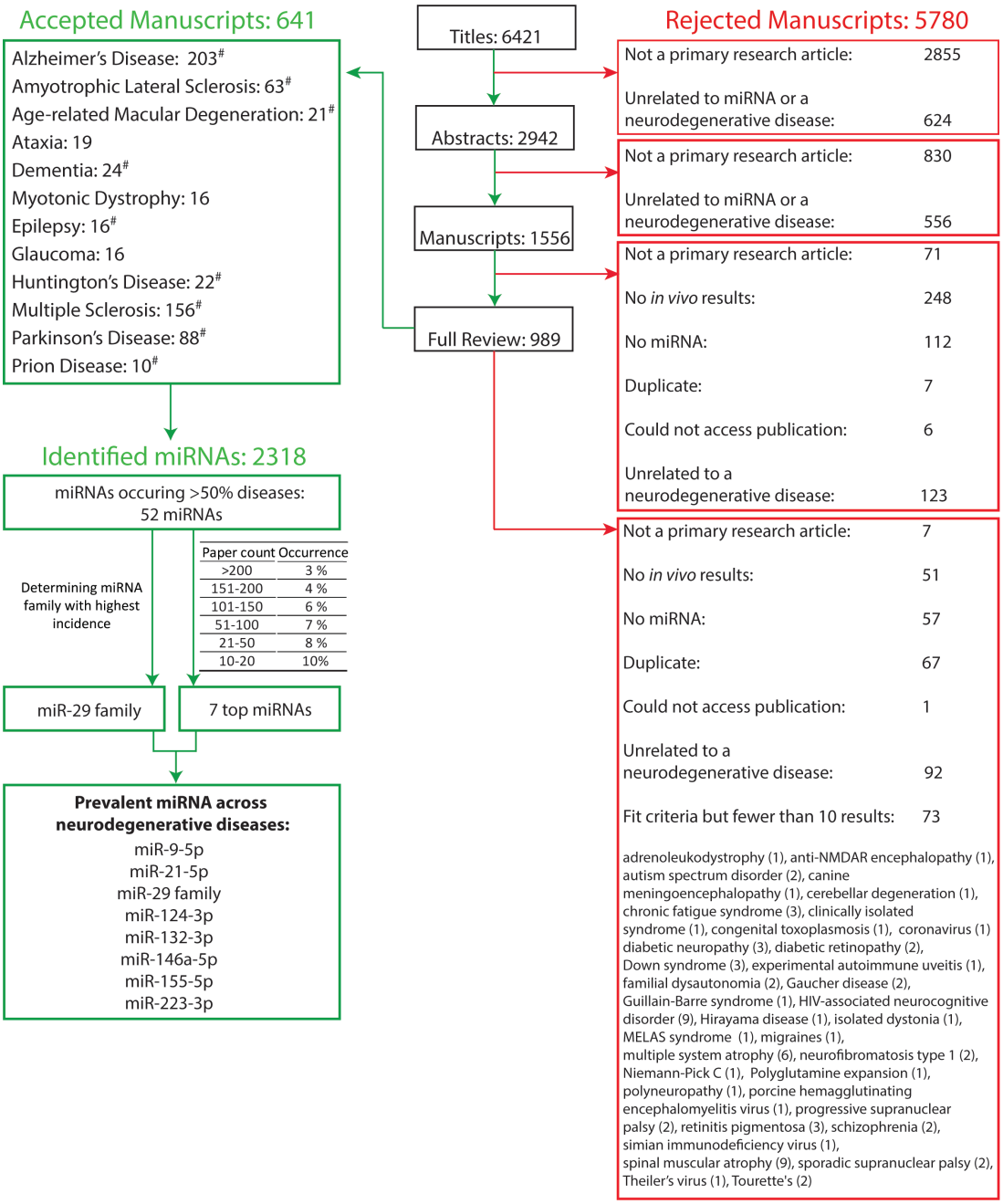


**Figure 1. PRISMA flowchart of the systematically reviewed manuscripts with differential miRNA expression in neurodegenerative disease and their animal models.** Initial library searches identified 6421 articles. Accepted papers were categorized based on the neurodegenerative disease, and disease categories with less than 10 results were excluded from the review. Diseases marked with # indicates shared papers between assessed diseases. Supplementary File 2 contains all accepted manuscripts.

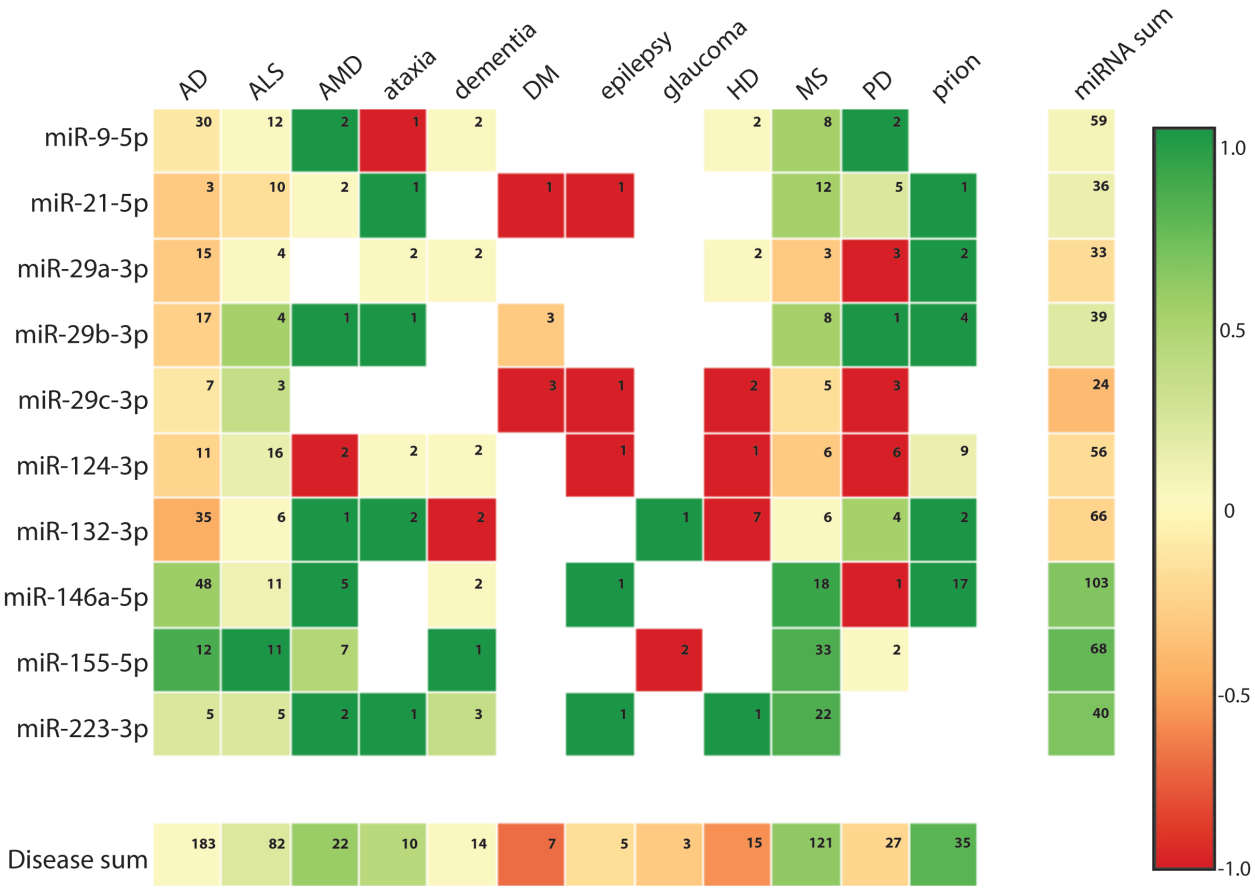


**Figure 2. Heat map summary of the direction in which trending miRNAs are expressed across neurodegenerative disease.** miRNAs with a rank of 1 were exclusively upregulated in a disease, -1 downregulated, and 0 implies the miRNA was equally upregulated and downregulated. Multiple values were taken from individual manuscripts when multiple time points and tissues were assessed. The number of values used to generate the heat map are listed within each rectangle.

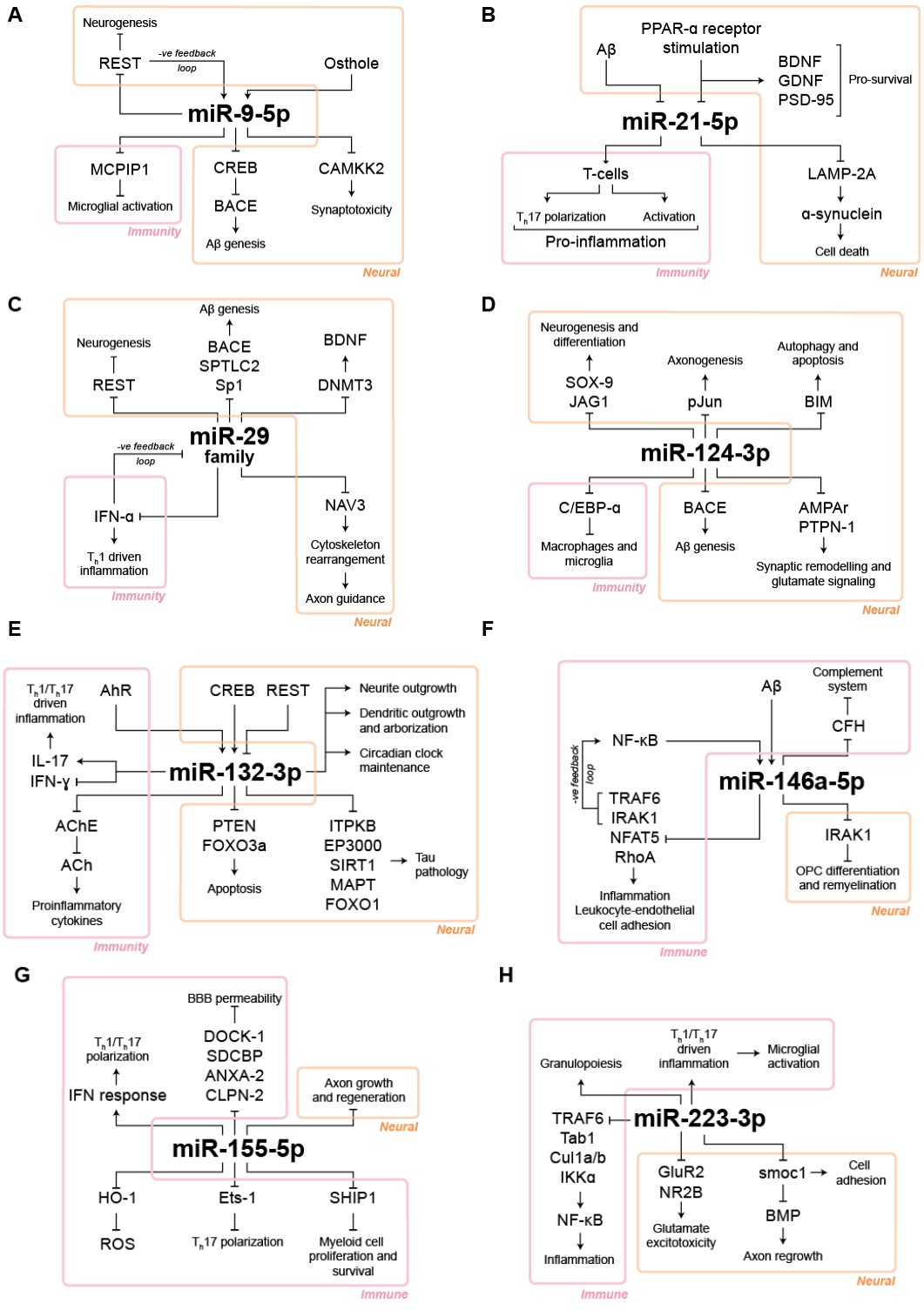


**Figure 3. Summary flow chart of the neural and immune components regulated by key miRNAs identified as regulated across multiple neurodegenerative diseases**. These miRNAs may act as underlying connections between the neural and immune components of neurodegenerative disease. The references for mRNA targets and/or specific signaling cascades are listed in Supplementary File 4.

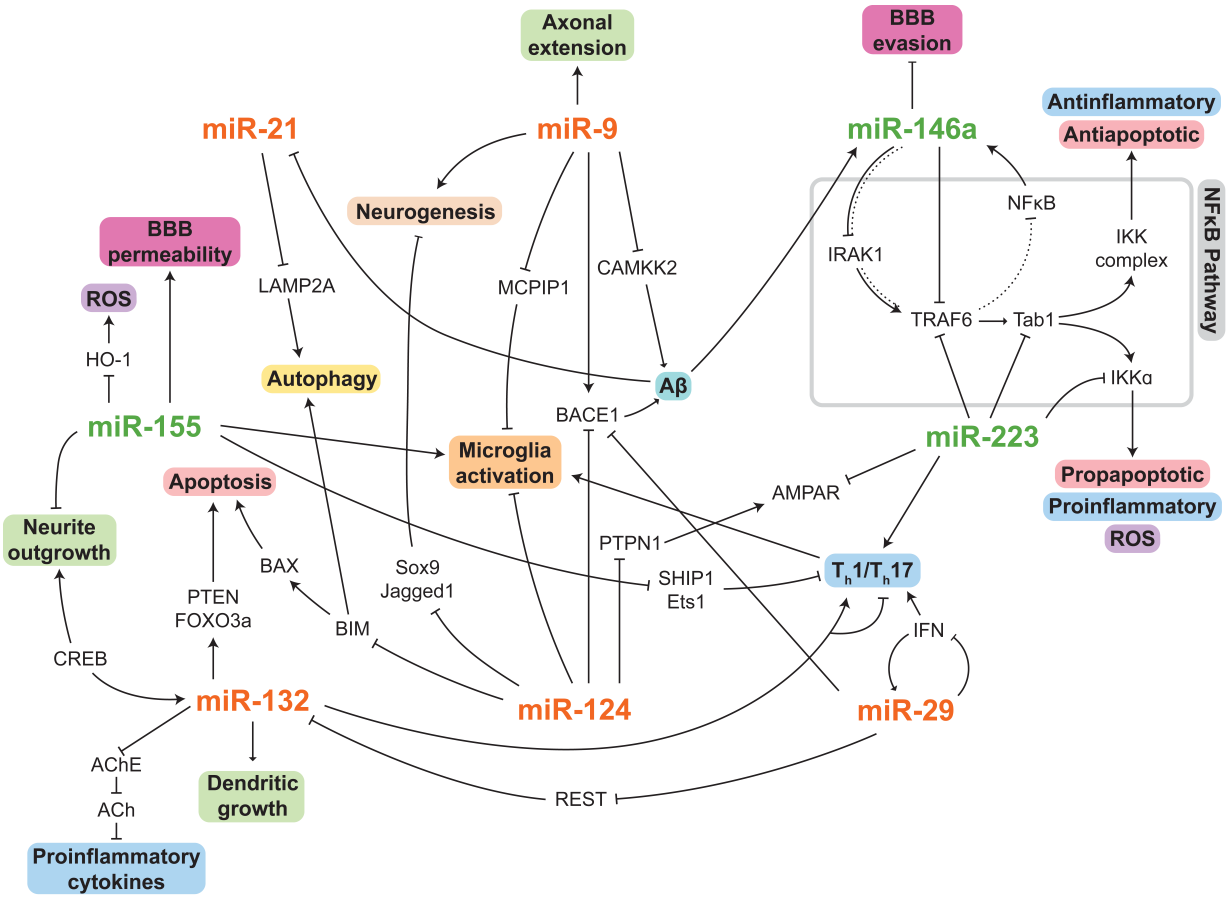


**Figure 4. Shared pathways between commonly regulated miRNAs during neurodegenerative disease and their animal models.** Overlaps include shared molecular pathways or differential pathways leading to similar end phenotypes, indicated by similarly coloured squares. miRNAs colour coded in green represent those that are generally upregulated during neurodegenerative disease; and orange represent those miRNAs with mixed regulation. Dotted arrows represent indirect interactions.

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