MicroRNAs identify shared pathways in Alzheimer's and Parkinson's disease

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Abstract

Despite the identification of several dozens of common genetic variants associated with Alzheimer's disease (AD) and Parkinson's disease (PD), most of the genetic risk remains uncharacterized. It is therefore important to understand the role of regulatory elements such as microRNAs (miRNAs). Dysregulated miRNAs are implicated in AD and PD, with potential value in dissecting the shared pathophysiology between the two disorders. MiRNAs relevant in both neurodegenerative diseases are related to axonal guidance, apoptosis and inflammation, therefore, AD and PD likely arise from similar underlying biological pathway defects. Furthermore, pathways regulated by *APP*, *L1CAM* and genes of the caspase family may represent promising therapeutic miRNA targets in AD and PD since they are targeted by dysregulated miRNAs in both disorders.

Keywords: Neurodegeneration, Dementia, Biomarker, Therapy, Pathophysiology, Diagnosis

Alzheimer's and Parkinson's Disease: Two Disorders Along the Same Continuum?

Despite significant advances in our understanding of key pathomechanisms of neurodegenerative conditions including **Alzheimer's disease** (AD; see Glossary), Parkinson's disease (PD) and dementia with Lewy-bodies (DLB), successful translation of this knowledge to benefits for the affected population has been limited [2]. These disorders show clinical and neuropathologic overlap, which limits diagnostic accuracy and challenges the traditional concept of distinct entities [3-5].

The hypothesis that AD and PD are the extremes of a spectrum, with DLB somewhere between, receives growing support [6]. Cases characterized by pure PD (i.e. α-synuclein aggregation) or pure AD pathology (i.e. amyloid-β [Aβ] and tau aggregation) are not representative of the majority of patients who mostly have mixed pathologies [7]. In PD, spreading of α-synuclein pathology to the limbic system and neocortex is closely associated with emerging dementia. Moreover, sufficient numbers of Aβ plaques to justify diagnosis of AD are found in half of the patients diagnosed with PD dementia; however, it is unknown if and how α-synuclein and Aβ pathologies act synergistically to confer prognosis [8]. Furthermore, an AD-variant of DLB was described, suggesting that Lewy body pathology may be present in individuals who do not show the typical clinical features distinguishing DLB from AD [9].

Increasing evidence supports a causal link between certain microRNAs (miRNAs), short non-coding RNA molecules which modify gene expression post-transcriptionally (see Box 1. MiRNA biogenesis and function), and different neurodegenerative disorders. In AD, miRNAs targeting central elements of the amyloid cascade, such as amyloid precursor protein (APP) [10] and β-site of APP cleaving enzyme (BACE1), have been identified [11, 12]. It has also been suggested that α-synuclein expression, regulated through miRNAs, is

neuron-type specific, possibly explaining the phenotypic heterogeneity of the different α-synucleinopathies [13] (Clinician's Corner).

MicroRNAs in Neurodegeneration

MiRNA expression is controlled by regulatory mechanisms that involve recruitment of specific transcription factors and the miRNA biogenesis machinery [21]. Neurodegeneration affects many of these mechanisms, including miRNA expression (i.e. dysregulation) [22]. Systematic reviews of the published literature for individual neurodegenerative conditions, followed by meta-analyses of miRNA measurements in brain tissue (but also in blood and cerebrospinal fluid [CSF]), indicate robust evidence for dysregulated miRNAs [23, 24] (see Box 3. MicroRNA-based biomarkers). MiRNAs play a major role in neuronal function. Selective experimental depletion of Dicer [see Box 1.] in midbrain dopaminergic neurons is associated with neurodegeneration and motor symptoms mimicking PD in mice [25]. Furthermore, emerging evidence shows that miRNAs are involved in key pathomechanisms shared by different neurodegenerative disorders, such as neuroinflammation and cell death. Important pro-inflammatory, anti-inflammatory and mixed immunomodulatory miRNAs are involved in regulating neuroinflammation in various central nervous system (CNS) pathologies, including AD [26]. Major deficits in human AD brain [27, 28] and murine models of AD and related neurodegenerative diseases [29] are the loss of synaptic contacts and synaptic disorganization. Pathomechanisms related to synaptic dysfunction may link different neurodegenerative diseases [30], as also suggested by the dysregulation of miRNAs enriched in synapses of brain regions affected in early AD, such as the hippocampus [31, 32]. Overall, the emerging knowledge on shared miRNA expression changes in AD and PD can be used to derive information on central biological pathways involved in the pathogenesis of both disorders.

Understanding the complex relationship between the distinct pathologies in the aging brain and their clinical phenotypes is crucial for the development of effective treatments. An approach which considers shared functional networks and pathways seems more promising than strategies only considering certain select biological markers. Here, we focus on miRNAs known to be dysregulated in AD and PD brains, and present evidence to support the thesis of shared biological pathways. We also discuss how therapeutic and diagnostic research could benefit from this knowledge.

Dysregulated MicroRNA Networks Cluster in Shared Biological Pathways

Molecular biology techniques and bioinformatic approaches allow researchers to link differentially expressed miRNAs to specific genes and biological pathways [34]. This provides valuable insights into the molecular alterations driving disease and unveils altered biological pathways common across diseases. Interestingly, some genes involved in the pathogenesis of neurodegenerative diseases are targeted by miRNAs differentially expressed in AD or PD brains, and the *APP* gene provides a striking example. The central role for APP in AD pathogenesis is well established [35], and *APP* gene variants are associated with early onset AD [36]. Although evidence for the role of *APP* in PD is less abundant, one GWAS showed that rare variants in *APP* may drive a dementia phenotype in PD patients [37]Moreover, the mutant form of a key player in PD pathology, the leucine-rich repeat kinase 2 (*LRRK2*) gene, was shown to phosphorylate APP and induce the production of its intracellular domain, resulting in dopaminergic neuron loss in mice [38]. Notably, western blot analysis in rat neuronal cells and reverse transcription quantitative (RT-q)PCR in human embryonic kidney cells overexpressing the *APP* Swedish mutation, reveal that *APP* is targeted by miR-195-5p and miR-497-5p [39-41]; these miRNAs are upregulated in AD [24] and PD brains [23], respectively.

The example of *APP* is one of several found in the literature suggesting pathophysiological connections between AD and PD, mediated by miRNAs. In the following paragraphs, we synthesize evidence from multiple, unrelated studies and provide insights into the possible connections of miRNAs dysregulated in AD and PD, and key CNS biological pathways.

Caspase Signaling Cascade in Neurodegeneration

Neurodegeneration is closely linked to erroneous activation of apoptotic processes in neuronal cells [42, 43]. Increased mRNA levels of caspase-3 and caspase-8 in AD and PD postmortem brain samples demonstrate the importance of caspase genes in controlling apoptosis in these diseases [44, 45]. Dysregulated miRNAs in AD and PD brains, and molecules in the caspase cascade, are linked by evidence from several *in vivo* and *in vitro* studies. For instance, transgenic AD mice overexpressing miR-132-3p [53, 54], a miRNA downregulated in PD brains [23] and knockdown of miR-34a [53, 54], a miRNA upregulated in AD brains [24], reduce caspase-3 activation. Additional evidence can be found in studies conducted in other cell types and conditions. In glioma stem cells, inhibition of miR-138-5p, a miRNA downregulated in AD brains [24], was associated with increased caspase-3 and caspase-7 activity and apoptosis [46]. In hippocampal tissue of a rat model of hypothermic circulatory arrest, inhibition of miR-29c, another miRNA downregulated in AD brains [24] resulted in decreased caspase-3 expression [52]. MiR-363 and miR-34-5p, two miRNAs upregulated in AD brains [24], inhibit caspase-3 and caspase-9 activity in glioblastoma stem cells and upregulate the expression of *CASP3*, *CASP6*, *CASP7*, *CASP8* and *CASP9* in hepatocellular carcinoma [47, 50]. Conversely, miR-146a-5p, which is upregulated in AD brains [24], downregulates caspase-7 in a human neuroblastoma cell line mimicking acute

ischemic injury [51]. These results suggest a common role for caspases in neurodegeneration; it remains to be seen whether they can be extended to AD and PD models.

Caspase signaling involves a complex interplay of molecular mediators. For instance, binding of Fas-ligand to FAS, a transmembrane protein, leads to the oligomerization of caspase-8 and subsequently activates a series of downstream caspases resulting in cell death [60]. MiRNAs reportedly dysregulated in AD and PD brains regulate mediators of the caspase cascade. In human neural stem cells, FAS is downregulated by miR-146a [24, 55], a miRNA upregulated in AD brains. Similarly, miR-133b, which is downregulated in PD brains [23], reduces the expression of FAS in rat cardiomyocytes [56], possibly due to decrease of *FAIM* (FAS Apoptotic Inhibitory Molecule) mRNA by miR-133b as reported in HeLa cells [57]. These results hold promise for exploring the effect of miR-133b in PD models or neuronal cells. Interestingly, reduced FAIM expression was noted in AD post-mortem hippocampal specimens [58]. MiR-133b might also participate in AD pathogenesis, given that miR-133b upregulation was reported in AD patients' frontal cortex [61].

Another member of the caspase family attracting attention is *CASP7*; it has a missense variant associated with familial late-onset AD, supporting earlier findings for a role in APP cleavage [59]. Caspase-7 mediates apoptosis by regulating caspase-1 activity, according to a study in neuroblastoma cells and PD mouse model [62]. Interestingly, overexpression of miR-132-3p, a miRNA reduced in PD brains [23], decrease *CASP7* expression in pancreatic cancer cells [49]. Further studies into the link between miR-132-3p, caspase-7 and apoptosis are needed in PD models to confirm these findings.

In summary, there is a broad base of experimental evidence from neuronal and non-neuronal cells that particular miRNAs target mediators of the caspase activation cascade. Notably, several of the miRNAs identified are significantly dysregulated in AD and PD brains. This observation is relevant to the debate about the role of caspases as therapeutic

targets in neurodegenerative diseases [63]. However, given the complex nature of the caspase signaling cascade regulatory mechanisms, the effect of individual miRNAs on particular mediators remains to be determined. Replication of these observations in neuronal cells and disease-specific animal models is an essential objective to be met.

Neuroinflammation and Dysregulated MicroRNAs

The role of inflammation in neurodegeneration has been investigated intensively [64]. While several pathways contribute to inflammation-driven neurodegeneration, *p38/MAPK1* is key to the vicious cycle of inflammation and neurodegeneration [65, 66]. Aβ, α-synuclein and inflammatory cytokines released by microglia can activate *p38/MAPK1* in neuronal and glial cells [67], which in turn elicits multiple signaling cascades, including the NF-κB pathway regulating cytokine and chemokine production. This fuels microglial activation, aberrant protein accumulation and mitochondrial dysfunction and leads to neurodegeneration [71]. The role of p38/MAPK1 has been studied extensively in patients and disease mouse models. Increased levels of phosphorylated p38/MAPK were reported in lymphocytes of AD and PD patients compared to healthy controls [72]. Moreover, administration of a selective p38/MAPK1 inhibitor reduced pro-inflammatory cytokine production, tau accumulation and synaptic dysfunction in an AD mouse model [68, 69]]. Similarly, in a PD mouse model, activation of p38/MAPK was associated with increased production of pro-inflammatory cytokines and dopaminergic neuron degeneration [70]. Notably, several studies with non-neuronal cells show that *p38/MAPK1* mRNA is targeted by miRNAs downregulated in AD and PD brains (i.e. miR-132-3p, miR-129-5p, miR-769-5p), and upregulated in AD brains (i.e. miR-152-3p, miR-195-5p and miR-454-3p) [23, 24, 41, 48, 73-79]. A rat model of chronic brain hypoperfusion (CBH) which exemplifies vascular disease, a known risk factor for AD [81], had increased microglial activation and Aβ accumulation [82, 83]. In addition,

regulation of *APP* and *BACE1* expression via the NF-κB pathway was partially-dependent on miR-195 expression [24, 80], a miRNA upregulated in AD [24, 80]; therefore, this animal model provides evidence linking these miRNAs with p38/MAPK1. In PD, a mouse model study showed that Nurr1, a transcription factor regulated by p38/MAPK1, inhibits the activity of NF-κB [84].

Taken together, these findings offer useful insights into p38/MAPK1 as a common mediator of AD and PD pathogenesis; the role of miR-132-3p, miR-129-5p, miR-769-5p, miR-152-3p, miR-195-5p and miR-454-3p on p38/MAPK1 will benefit from new investigations, e.g. in disease animal models. These miRNAs likely target other genes involved in AD and PD pathogenesis and possibly also the *p38/MAPK1* signaling pathway (influencing its role in neuroinflammation or other biological pathways) and need to be explored further. Despite the observations that dysregulated miRNAs in AD and PD target common mediators involved in neuroinflammation, the precise role of inflammation in neurodegeneration – as a friend or foe – remains ambiguous, and to be further clarified by additional experiments [85].

Dysregulated microRNAs Affect Axonal Growth and Guidance

Axonal development plays a critical role in maintaining and restoring disrupted neuronal networks [88]. The mRNA of several genes involved in axonal guidance, such as *CDK5R1* and *p38/MAPK1*, are targeted by miRNAs dysregulated in AD and PD, corroborating the theory of impaired guidance in neurodegenerative diseases. MiR-195, an upregulated miRNA in AD [24], led to dendrite degeneration and neuronal loss in CBH rat hippocampi, via activation of the MAPK/DR6 pathway Similarly, in a rat model of neurodegeneration, overexpression of miR-132, a miRNA downregulated in PD brain [23], was associated with decreased dopaminergic neuron development and differentiation via decreased levels of

Nurr1 [90, 91]. In this study Nurr1 downregulated BDNF, a growth factor inducing axonal growth in AD and PD models [92].

The central role of *p38/MAPK1* in regulating axonal guidance is also evidenced by its involvement in signaling pathways mediated by L1CAM and NCAM, two transmembrane proteins involved in cell adhesion, neurogenesis and synaptic plasticity [93]. *L1CAM* and *NCAM* downregulation may affect cell adhesion and axonal guidance in AD and PD. Moreover, proteolytic cleavage fragments of L1CAM and NCAM may inhibit axonal development by competing with other trophic substances, as indicated by increased levels of L1CAM and NCAM proteolytic fragments in AD patients. Interestingly, L1CAM is cleaved by ADAM10 and BACE1, similar to APP, indicating a possible intersection between APP processing and cell adhesion molecules, potentially opening new therapeutic avenues [96]..

Finally, the mRNA of *CDK5R1*, another gene involved in axonal growth, is targeted by the top miRNAs for dysregulated expression in brain; miR-133b downregulated in PD and miR-363-3p upregulated in AD [23, 24, 41, 75]. CDK5R1 is involved in the Semaphorin 3 (Sema3) signaling pathway; Sema3 may be implicated in AD and PD synaptic dysfunction [104]. However, it is important to stress that transcripts differ across cell types, with particular genes expressed in one cell type but not another. The ability of one miRNA to target a specific mRNA depends on the affinity of the miRNA for that particular target, on the availability of that target in the cell and on the pool of other possible targets that will compete with that particular target. Therefore, the above described mechanisms need to be confirmed in brain-related cell types.

In conclusion, the current knowledge linking miRNAs dysregulated in AD and PD to inflammation, apoptosis and axonal guidance, supports the hypothesis that AD and PD may result from a similar interplay of altered pathways. Magnitude of change, direction of dysregulation and cell-type affected, are key determinants of the clinicopathological outcome,

along the AD - PD continuum (Figure 1, Key Figure). So far, most studies have been conducted in animal models, or cell-cultures from tissues not directly affected by disease pathology. Thorough characterization of these miRNA-gene interactions in disease-relevant human tissues and cells would strengthen the current findings of overlapping pathologies and set the path for developing new biomarkers and therapeutic targets (Box 3).

MicroRNA-based Treatment of Neurodegenerative Disorders

Dysregulated miRNAs in AD and PD reveal alterations in shared biological processes such as inflammation, apoptosis and axonal growth hence nourishing hopes for the development of miRNA-based therapies common to both diseases. Acting as targets or therapeutic agents, miRNAs could modulate the expression of genes involved in pathways driving both diseases. For a mouse model of stroke, delivery of a caspase 9 specific-inhibitor, XBir3, to the cytoplasm of neuronal cells, led to apoptosis reduction [63]. Using a different target, the administration of the L1CAM mimetic trimebutine, in mice with spinal cord injury, lead to increased concentrations of phosphorylated MAPK, resulting in increased regrowth of axons [109].

Targeting pathways known to be altered in unrelated pathologies is not a new approach. In oncology, chemotherapies based on tyrosine kinase receptors are approved for different types of malignancies. Building on this experience, a liposomal mimic of miR-34a, known to downregulate more than 30 oncogenes involved in pathways common to several malignancies, has been developed recently [111]. Lessons learned from drug development in oncology can stimulate the development of miRNA-based neurodegeneration treatments. However, it is striking that no clinical trials to investigate miRNA-based treatments in AD or PD have been launched so far, in contrast to a number of clinical trials in diseases such as

hepatitis C or type-II diabetes. This is possibly explained by the challenges associated with the development of miRNA-based treatments for brain disorders.

MiRNA-based therapeutics rely on two general approaches, either antagonising a miRNA using an anti-miR or restoring a downregulated miRNA via miRNA mimics. The miRNA target should be able to modulate the harmful activity of some cells while enhancing the protective role of others. This represents a first challenge as a given cell type may have different functions during acute and chronic stages of AD and PD; for instance, while microglia may play a protective role in early stages of AD by removing Aβ aggregates, they may increase Aβ plaque formation and neurodegeneration at later disease stages. Once the optimal cell target and timing of drug administration have been identified, ways to deliver the drug to the brain need to be defined. MiRNA-based drugs should be protected from serum endonuclease activity. Also, the hurdle of the blood brain barrier (BBB) needs to be overcome, and so far, no optimal non-invasive solution has been found. As AD and PD progress, changes in cell population and composition of the microenvironment surrounding neurodegeneration may further alter the diffusion of the drug to the target [117, 118]. Besides these CNS specific hurdles, the development of miRNA therapeutics is also challenged by overactivation of the immune system following miRNA-based drug administration.

In summary, miRNAs represent a promising opportunity for the yet unmet need of disease-modifying treatments in AD and PD. However, questions regarding optimal choice of target and delivery method to the targeted site need first to be answered.

Concluding Remarks

Here, we reviewed the current knowledge of exemplary miRNA targets based on cumulative data that demonstrate commonly dysregulated miRNA in AD and PD physiology. Although these disorders may arise from similar pathway defects, the clinicopathological outcome is

most likely determined by key factors, such as the extent of miRNA dysregulation. In the absence of specific evidence, further experimental validation in appropriate systems would help to confirm whether the dysregulation of selected miRNAs indeed impacts these pathways in the diseased brain.

However, questions remain (see "Outstanding Questions") and while new miRNA-based diagnostic and therapeutic options may be on the horizon, experimental validation of hypothesis and thorough evaluation will be required. Deciphering the role and relevance of dysregulated miRNAs in different brain tissue may yield further answers and offer potential treatment strategies.

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Box 1. MiRNA Biogenesis and Function

MiRNAs are short non-coding RNA molecules which modify gene expression post-transcriptionally via recognition of binding sites located in the 3′-untranslated region of their target messenger RNAs (mRNAs) [16]. Upregulated miRNA expression may therefore lead to translational repression and reduced protein levels. Most of the miRNA families follow a canonical biogenesis leading to the stepwise conversion of a primary miRNA into a mature miRNA of approximatively 18-22 nucleotides length (Figure I). Briefly, the process begins in the cell nucleus, where RNA polymerase II or less frequently RNA polymerase III transcribes a primary miRNA transcript (pri-miRNA) from miRNA genes. Once transcribed, a 5' cap and a 3' poly-A tail are added to the pri-miRNA. Following this, a complex composed of Drosha, an enzyme and DGCR8, a RNA binding protein, anchors to the pri-miRNA and cleaves it into a stem-loop precursor miRNA (pre-miRNA). The pre-miRNA is then exported into the cytoplasm via Exportin 5, a nuclease export protein. In the cytoplasm, the miRNA precursor binds to an endonuclease called Dicer. The RNA binding protein TRBP within the Dicer protein complex cleaves the stem-loop of the precursor and releases a double stranded RNA molecule. Only one strand, either the 5' end or the 3' end strand (referred to as "-5p" and "-3p" strand), is loaded on a RNA induced silencing complex (RISC). The other strand may be cleaved by Argonaute proteins located on the RISC. On the RISC, the guide strand miRNA interacts with different mRNAs depending on the complementarity between the two structures. In most cases, the 3' untranslated region of the mRNA interacts with a seed region located at the 5' end of the miRNA. This region consists of 6-8 nucleotides and is thought to be the most important factor regulating the miRNA-mRNA interaction. Enzymes with endonuclease activity like Argonaute protein 2 and GW182 then cleave the mRNA. Finally, complexes such as CCR4-NOT and PAN2-PAN3 induce the de-adenylation of the mRNA leading to its degradation. In recent years, alternative (noncanonical) pathways – though rare

– in the biogenesis of miRNA have been described. For example, miRNA targeted mRNA can be sequestered into P-bodies where they can either undergo degradation or be stored for later use [121]. Also, repression of mRNA can occur independently of RISC [122]. Finally, while most of the miRNA genes are dispersed in intergenic regions of the genome, recent evidence suggests that some miRNA genes are within introns of protein coding genes.

Box 2. Clinicians' Corner

- Cases of "pure" Alzheimer's disease and Parkinson's disease without concomitant other neuropathologic changes are relatively rare and many cases show diverse pathologies, including deposition of different pathologic proteins and damage related to vascular disease.
- Alzheimer's disease and Parkinson's disease are increasingly considered as spectrum disorders. These two neurodegenerative conditions are seen to be positioned at the two extremes of the spectrum, with Parkinson's disease dementia and dementia with Lewy bodies positioned somewhere in the middle.
- MicroRNAs are small, non-coding molecules, which critically affect gene expression by binding to their target messenger RNAs, thereby reducing the levels of the associated proteins. Several microRNAs have been repeatedly associated with Alzheimer's disease and Parkinson's disease pathogenesis by targeting key pathological pathways such as amyloid-β and α-synuclein accumulation.
- MicroRNAs significantly associated with Alzheimer's disease and Parkinson's disease cluster in key biological pathways and there is a functional overlap in inflammation, axonal guidance and apoptosis.
- In the future, it may be possible to use microRNAs in peripheral body fluids such as blood and cerebrospinal fluid as pathophysiological biomarkers to aid diagnosis and prognosis of different late-onset neurodegenerative disorders. Efforts in other areas of medicine, such as cancer, show that a better understanding of microRNA dysregulation can also lead to more effective targeted, personalised therapies.

Box 3. MicroRNA-based Biomarkers

Different platforms are used to determine relative microRNA (miRNA) abundance in biological samples. Among these, there are technologies and techniques with narrow assay focus and high sample throughput (e.g. quantitative polymerase chain reaction, qPCR) on the one end, and broad assay focus and low sample throughput on the other end (e.g. microarrays and sequencing).

The potential of circulating miRNAs as biomarkers for early disease detection was demonstrated in studies of cancer patients (e.g. colorectal cancer). Notably, circulating miRNAs were also shown to predict lung cancer incidence several years before the onset of disease [125]. Elsewhere, when miRNAs in blood samples from patients with one of 14 different diseases were analysed, including autoimmune conditions and cancers, the disease was correctly identified in about seven out of ten patients [126].

In the context of the Alzheimer's disease (AD)-Parkinson's disease (PD) continuum, miRNA-based biomarkers incorporated in the diagnostic process would be useful in different ways: 1) they could be sensitive and specific to distinguish disorders with overlapping symptoms and pathology, especially at early disease stages, to enable targeted treatment, and 2) they could be indicative of biological pathways affected, which may be shared between different disorders, availing healthcare professionals of the opportunity for pathway-specific treatment options for their patients. On the one hand, miRNAs that are differentially expressed in PD but not in AD (e.g. miR-221-3p, down; miR-214-3p, down) [23, 24], may be used to distinguish between PD and AD, and *vice versa*. On the other hand, miRNAs that are differentially expressed both in PD or AD cases, versus controls, may be indicative of shared disease pathway(s); for example, miR-29c-3p and miR-146a-5p, which are downregulated in PD and AD [23, 24], are implicated in apoptosis through regulation of caspase genes.

Interestingly, changes in miRNA concentrations in a bodily fluid and in an organ involved in pathology are not always concordant and sometimes change in opposite directions. For instance, miR-501-3p was down-regulated in AD patient serum, but it was up-regulated in the post-mortem brains of the same donors [127]. Therefore, it is unclear whether differential miRNA expression is a cause or effect of the disease process, particularly in blood.

In the future, a blood-based test incorporating miRNA biomarkers may be available, facilitating diagnosis on the AD-PD continuum; a qPCR-based approach would provide the required sensitivity and accuracy to enable reliable quantitative measurements, in line with findings from a systematic comparison of 12 (two sequencing, three hybridization, seven qPCR) commercially available platforms for miRNA expression analysis [128].

Glossary

α-synuclein: a presynaptic neuronal protein that is linked genetically and neuropathologically to Parkinson's disease.

α-synucleinopathies: central nervous system disorders characterized by the presence of aggregated α-synuclein intracellularly, including Parkinson's disease, dementia with Lewy bodies and multiple system atrophy.

Alzheimer's disease (AD): slowly progressive, late-onset neurodegenerative disorder, which affects cognitive performance, daily activities and behaviour and which is the most frequent cause of dementia.

Alzheimer's disease dementia: dementia syndrome caused by the Alzheimer's disease pathophysiological process.

Amyloid cascade: the series of events triggered by the proteolysis of amyloid precursor protein, which results in the production and deposition of harmful amyloid-β.

Amyloid precursor protein (APP): a ubiquitously expressed transmembrane protein, which serves as the precursor molecule whose proteolysis generates amyloid-β.

Amyloid-β protein (Aβ): a protein fragment processed from amyloid precursor protein, which is a major component of senile plaques. Cerebrospinal fluid concentrations are typically reduced in Alzheimer's disease.

β-site amyloid precursor protein cleaving enzyme 1 (BACE1): a transmembrane aspartyl protease with β-secretase activity, responsible for the rate limiting amyloid precursor protein cleavage step.

Dementia: a decline in global deterioration of intellectual function that is severe enough to interfere with daily life.

Dementia with Lewy bodies (DLB): second most common type of progressive dementia after Alzheimer's disease dementia. People with DLB may experience visual hallucinations

and changes in alertness and attention early during the disease course, in addition to locomotor symptoms of Parkinson's disease.

Lewy bodies: circular clumps of α-synuclein (and other proteins) that are found in the brains of people with Parkinson's disease. They are abundant in areas of the brain that have suffered cell loss, such as the region containing dopamine neurons.

Parkinson's disease (PD): slowly progressive, late-onset neurodegenerative disorder, which affects the motor system with shaking, rigidity, slowness of movement and difficulty walking.

Parkinson's disease dementia: dementia syndrome caused by the Parkinson's disease pathophysiological process.

Tau protein: an intracellular protein that stabilises microtubules. Alzheimer's disease and other tauopathies are associated with a hyperphosphorylation of tau. Cerebrospinal fluid concentrations are typically increased in Alzheimer's disease.

Figure 1. Network of selected genes targeted by dysregulated microRNAs in Alzheimer's disease and Parkinson's disease

Panel A. The same gene (square) can be targeted by multiple microRNAs dysregulated in Alzheimer's disease (AD, red circle) and Parkinson's disease brain (PD, blue circle). Some genes (square yellow) are key actors in altered biological processes participating in the pathogenesis of both disorders [41, 50, 75, 129-132]. Symbols: circle, microRNA; square, gene. Color: red, microRNA dysregulated in AD; blue, microRNA dysregulated in PD; yellow, crossing points between pathways.

Panel B. Impact of dysregulated genes on apoptosis, inflammation, and axonal guidance in AD and PD. **(1)** Aβ and α synuclein can activate or upregulate FAS [133, 134]. Activation of FAS stimulates the caspase signalling cascade resulting in apoptosis. **(2)** Aβ binds to Tumor Necrosis Factor (TNF) receptor and Toll-like receptors (TLR) leading to cytokine and chemokine production via activation of the NF κB and p38/MAPK1 pathways [136]. TNF receptor associated protein 6 (TRAF6) is involved in TLR signalling and has been linked to ubiquitination of α-synuclein and tau resulting in intracellular protein accumulation in AD and PD [137, 138]. **(3)** The neuron adhesion molecules N1CAM and L1CAM regulate actin cytoskeleton via CDK5R1 and MAPK1 pathways and induce axonal growth [93, 94]. Semaphorin3 and Slit2 stimulate axonal growth by binding to L1CAM/NRP1 and Robo2 respectively [97, 98, 104]. The nature of interaction between soluble fragments of L1CAM, α-synuclein and Amyloid-β on L1CAM, N1CAM and Robo2 has yet to be further investigated.

Figure 2. Potential microRNA-based drug targets

MicroRNA (miRNA)-based therapeutics rely on either antagonising a miRNA using an anti-miR or restoring a downregulated miRNA via miRNA-mimics. The miRNA target should be able to modulate the harmful activity of some cells while retaining the protective role of others. However, miRNA-based drug development is associated with challenges: miRNAs need to survive serum endonuclease activity (A), blood brain barrier needs to be overcome (B), the compound needs to diffuse across the brain parenchyma (C), immunogenicity of miRNA-based drugs on microglia is unclear (D) and finally, ways to deliver miRNA-based drugs to the target cell (E) and methods to induce the desired repression (F) need to be clearly identified. Pathways regulated by caspase genes (Option 1), *APP* (Option 2) and *L1CAM* (Option 3) represent potential therapeutic miRNA targets in Alzheimer's disease and Parkinson's disease as they involve genes targeted by dysregulated microRNAs in both disorders. Moreover, experimental studies based on XIAP, a caspase-9 inhibitor and L1-mimics have reported promising results on their ability to inhibit neuronal death and stimulate axonal growth.