

RNA metabolism in neurodegenerative disease

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ABSTRACT

Aging-related neurodegenerative diseases are progressive and fatal neurological diseases that are characterized by irreversible neuron loss and gliosis. With a growing population of aging individuals, there is a pressing need to better understand the basic biology underlying these diseases. Although diverse disease mechanisms have been implicated in neurodegeneration, a common theme of altered RNA processing has emerged as a unifying contributing factor to neurodegenerative disease. RNA processing includes a series of distinct processes, including RNA splicing, transport and stability, as

well as the biogenesis of non-coding RNAs. Here, we highlight how some of these mechanisms are altered in neurodegenerative disease, including the mislocalization of RNA-binding proteins and their sequestration induced by microsatellite repeats, microRNA biogenesis alterations and defective tRNA biogenesis, as well as changes to long-intergenic non-coding RNAs. We also highlight potential therapeutic interventions for each of these mechanisms.

KEY WORDS: Disease, RNA binding proteins, Microsatellite repeats, miRNA, tRNA, lncRNA, RNA

Introduction

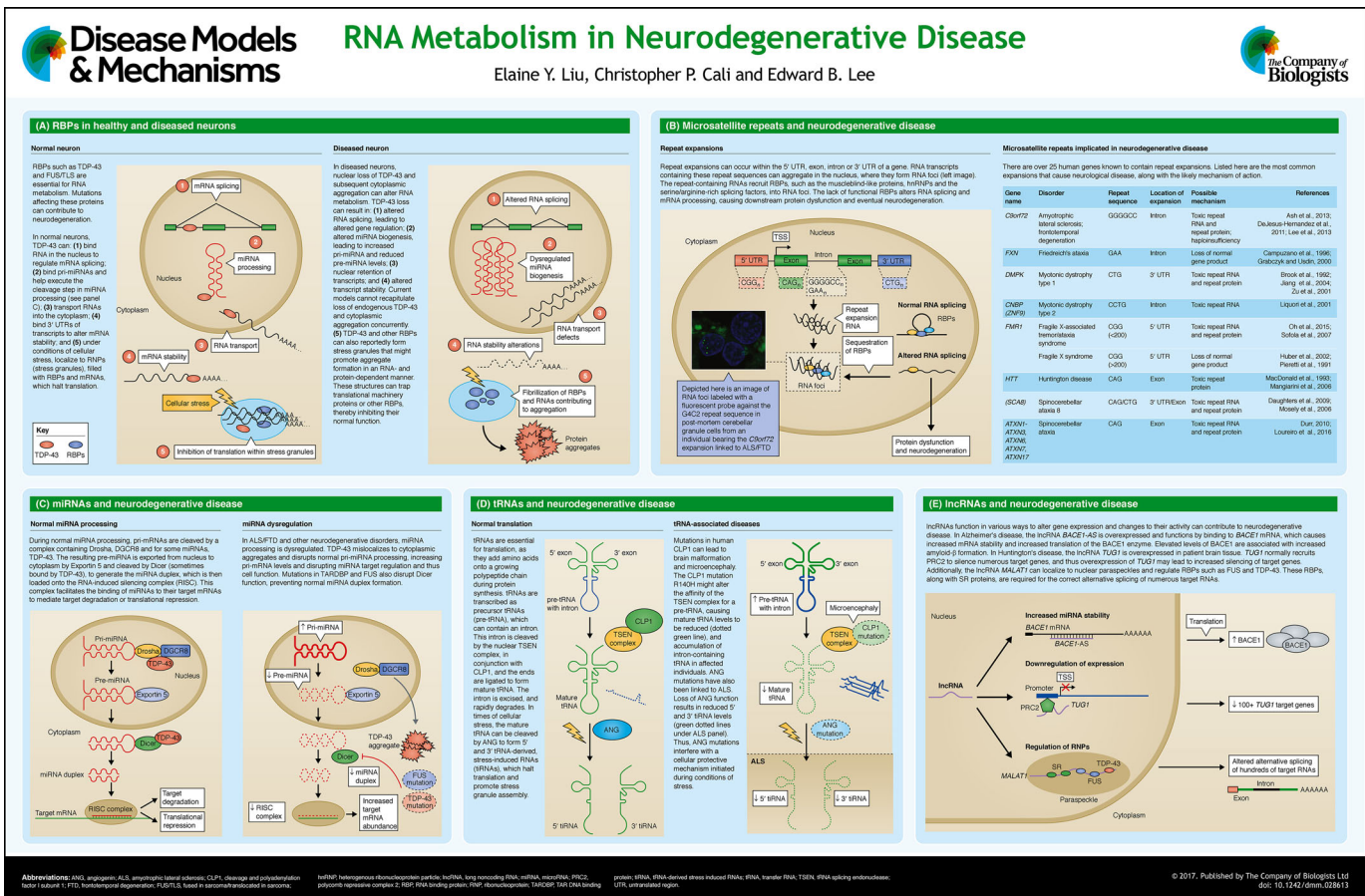
Aging-related neurodegenerative diseases, such as Alzheimer's disease (AD), Parkinson's disease (PD), frontotemporal degeneration (FTD) and amyotrophic lateral sclerosis (ALS), among others, are relentlessly progressive and uniformly fatal neurological diseases that are characterized by irreversible neuron loss and gliosis. Although dementia prevalence as a percentage of the elderly has declined in developed countries, the absolute

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number of dementia cases is growing as a result of an increase in the aging population (Langa et al., 2017). Thus, it is important for us to better understand the basic biological mechanisms that contribute to neurodegeneration.

Although much emphasis has been placed on the role of protein aggregates in neurodegenerative diseases, multiple lines of evidence also converge on altered RNA processing as a contributing factor in the pathogenesis of these diseases (Anderson and Ivanov, 2014; Belzil et al., 2013; Bentmann et al., 2013; Halliday et al., 2012; Ling et al., 2013). Defects at all levels of gene regulation, from RNA synthesis, processing, function and degradation, are associated with disease-specific alterations in RNA-binding proteins (RBPs), and in non-coding RNAs, such as microRNAs (miRNA), transfer RNAs (tRNA) and long-noncoding RNAs (lncRNA). Given that these basic processes are essential for normal and properly regulated gene expression, it is increasingly clear that aberrations in these processes can contribute to disease. In this Review and accompanying poster, we highlight several key themes that explain how different classes of RNAs or RBPs can impair gene regulation. We also highlight specific examples with evidence to show that improper RNA metabolism is a critical feature of neurodegeneration.

RNA-binding proteins regulate RNA metabolism

RBPs are essentially required at all levels of RNA processing in both the nucleus and cytoplasm where transcription, splicing, RNA stabilization, and RNA degradation occur (see poster panel A). Two notable examples of RBP defects occur in familial and sporadic cases of ALS and FTD. ALS is a neurodegenerative disease that leads to the loss of upper and lower motor neurons from the motor cortex and spinal cord, respectively, whereas FTD is associated with neuronal loss in the temporal and frontal cortex. Despite different areas of neuronal atrophy, a common link between ALS and FTD patients is a nuclear RBP called TAR DNA-binding protein-43 (TDP-43). Post-mortem brains from human ALS and FTD patients show a characteristic mislocalization of TDP-43 from the nucleus into phosphorylated, ubiquitylated cytoplasmic TDP-43 aggregates (see poster panel A) (Neumann et al., 2006). Indeed, rare disease-causing mutations in *TARDBP*, the gene encoding TDP-43, suggest that TDP-43 dysfunction is sufficient to cause ALS (Van Deerlin et al., 2008; Gitcho et al., 2008; Kabashi et al., 2008; Sreedharan et al., 2008), although the mechanism by which these mutations cause disease is unclear. TDP-43 functions ubiquitously in RNA processing, including splicing (Buratti et al., 2001; Ling et al., 2015; Shiga et al., 2012; Tollervey et al., 2011), stability (Costessi et al., 2014; Liu et al., 2012; Strong et al., 2007) and transport (Alami et al., 2014). Shortly after the discovery of *TARDBP* mutations, mutations in *FUS*, which encodes the nuclear protein fused in sarcoma (FUS) (also known as translated in liposarcoma, TLS), were identified in a subset of individuals with ALS, and FUS was revealed to be mislocalized to the cytoplasm (Kwiatkowski et al., 2009; Vance et al., 2009). Similar to TDP-43, FUS interacts with serine arginine (SR) proteins involved in RNA splicing (Yang et al. 1998) and regulates transcription by recruiting RBPs through non-coding RNAs (Wang et al., 2008). An additional example of abnormally localized nuclear RBPs is evident in individuals with multisystem proteinopathy (MSP), in whom mutations in the gene encoding heterogeneous nucleoriboprotein particle A1 (*HNRNPA1*) and A2B1 (*HNRNPA2B1*) contribute to disease. MSP is characterized by the progressive degeneration of muscle, brain, motor neurons and bone, which sometimes manifests as ALS or FTD (Kim et al., 2013). Mutations in the gene encoding valosin-containing protein (*VCP*), a triple ATPase protein involved in many cellular functions including

endolysosomal degradation, autophagy, and the ubiquitin proteasome system, also causes MSP (Watts et al., 2004).

It is thought that the loss of RBPs through their nuclear mislocalization and/or the toxicity caused by their cytoplasmic aggregation can lead to disease, but their relative contributions to disease remain unclear. Mouse models where antisense oligonucleotides (ASOs) against *Tardbp* depleted TDP-43, show altered global RNA expression affecting 601 genes, and specifically neuronal genes with long introns (Polymenidou et al., 2011). To better understand the targets of these RBPs, TDP-43 and FUS have been immunoprecipitated from mice brains and rat primary neuronal cultures, revealing that these proteins bind to non-coding RNA sites, namely introns and 3' untranslated regions (UTRs) of thousands of genes (Lagier-Tourenne et al., 2012; Sephton et al., 2011). Some of these genes include ncRNAs, like metastasis-associated lung adenocarcinoma transcript 1 (*Malat1*) and nuclear paraspeckle assembly transcript 1 (*Neat1*) (Lagier-Tourenne et al., 2012; Polymenidou et al., 2011; Tollervey et al., 2011). Thus, it is possible that the loss of RBPs influences the processing of these non-coding RNAs and contributes to global RNA dysregulation (see poster panel A). Given that the depletion of these RBPs alters the expression of thousands of genes, it is likely that some, or even all of these changes contribute to disease pathogenesis.

The formation of cytoplasmic RNA granules that lead to cytoplasmic aggregates has also been proposed to be pathogenic. When cells are stressed, cytoplasmic RNA granules that contain stalled translational complexes are formed. TDP-43, FUS, and other RBPs, such as hnRNPA1 and A2B1, localize to stress granules (Kim et al., 2013; Li et al., 2013). Indeed, RBPs with low complexity domains (LCD), such as TDP-43, FUS and hnRNPA1, can phase separate to create dynamic membrane-less organelles or liquid droplets that underlie the transient nature of stress granules (Courchaine et al., 2016; Molliex et al., 2015). The liquid properties of these organelles are dependent on their constituents. Namely, the intrinsic properties, type, concentration of the RBP, RNAs that the RBPs are bound to and the concentration of the RNA greatly influence these RNA granules (Kroschwald et al., 2015; Smith et al., 2016). For example, increasing the concentration of RBPs can reduce the liquid-like properties of these RNA granules, thereby promoting the formation of hydrogels and eventually an insoluble amyloid-like aggregate (Guo and Shorter, 2015; Kato et al., 2012; Lin et al., 2015; Molliex et al., 2015; Xiang et al., 2015). Indeed, disease-associated mutations within the LCDs of RBPs can enhance prion-like properties, and accelerate the shift from liquid to solid and disrupt ribonucleoprotein (RNP) granule formation (Murakami et al., 2015). At present, there is little evidence supporting amyloid-like fibrillar aggregates within neurons in ALS/FTD, but the relationship between other biophysical assemblies such as hydrogels, and neuronal aggregates is being investigated (Murakami et al., 2015). Thus, the formation of liquid droplets is another mechanism by which RBP disruption could contribute to disease (see poster).

The formation of cytoplasmic aggregates has also been implicated in neurodegenerative disease. Several different rat and mouse models, in which wild-type or mutant *TARDBP* or mutant *FUS* bearing ALS-associated mutations are overexpressed, develop cytoplasmic aggregation and exhibit features of ALS and FTD, including cortical and hippocampal neuronal loss and motor deficits (Huang et al., 2011; Igaz et al., 2011; Scekkic-Zahirovic et al., 2016; Sharma et al., 2016; Tsai et al., 2010; Wils et al., 2010; Xu et al., 2010). However, none of these models has recapitulated the loss of endogenous nuclear TDP-43 or FUS. Despite the discrepancies

between animal models and human pathology, there is *in vitro* evidence in support of the toxicity of cytoplasmic aggregates. Live tracking of rat primary cortical neurons to assess their survival shows that neurons with cytoplasmic TDP-43 have a greater risk of death, and that this risk depends on the amount of cytoplasmic TDP-43 present (Barmada et al., 2010). This corroborates the finding that overexpression of FUS or TDP-43 in yeast results in cytoplasmic aggregation of these proteins (Johnson et al., 2008, 2009; Sun et al., 2011). TDP-43 toxicity is dependent not only on its RNA-binding ability but also its C-terminus (Elden et al., 2010; Johnson et al., 2009; Voigt et al., 2010), the region where most disease-causing *TARDBP* mutations are found (Gitcho et al., 2008; Kabashi et al., 2008; Sreedharan et al., 2008). Although there is no consensus on which mechanism is more toxic, it is likely that both nuclear clearance and cytoplasmic aggregation of RBPs contribute to disease. Indeed, an effort to parse the effects of nuclear TDP-43 loss and TDP-43 cytoplasmic aggregation in a mouse motor neuron-like hybrid cell line (NSC34) shows that both contribute relatively equally to cellular toxicity (Cascella et al., 2016).

At present, potential therapeutic interventions are based on reducing the formation of toxic cytoplasmic aggregates. This is achieved in several ways, for example by: (1) activating the heat shock response; (2) using heat shock protein (Hsp)104 'disaggregases'; or (3) by modulating the ubiquitin proteasome system and autophagy. HSPs function as molecular chaperones and are involved in protein folding, protein trafficking and in coping with denatured proteins (Lindquist and Craig, 1988). Prior work has shown that the overexpression of an HSP, heat shock factor 1 (HSF1), in rat primary neuronal cultures overexpressing wild-type TDP-43 prevents cytoplasmic aggregation of TDP-43 by interacting with other HSPs to enhance refolding. This reduces toxicity in a human bone marrow neuroblast cell line (SH-SY5Y) overexpressing either wild-type or mutant TDP-43 (Chen et al., 2016). Components of the heat shock response have also been engineered to remove aggregated proteins. Modified Hsp104 improves its disaggregation capabilities relative to the wild-type Hsp104, and is able to suppress FUS and TDP-43 toxicity in yeast. This provides a potential intervention to eliminate protein aggregates that contribute to toxicity (Jackrel et al., 2014). Enhancing components of the ubiquitin proteasome system or autophagy can also reduce these aggregates. For example, increasing cAMP levels with forskolin in human embryonic kidney cells (293A) enhances the ubiquitin proteasome system to clear aggregation-prone proteins, such as FUS and TDP-43, in cells overexpressing either wild-type or mutant forms of both proteins (Lokireddy et al., 2015). Furthermore, two different studies show that using autophagy activators rescues motor dysfunction in transgenic FTD mice and also improves survival of neurons and astrocytes derived from human induced pluripotent stem cells from ALS patients with a *TARDBP* mutation (Barmada et al., 2014; Wang et al., 2012b). Until the toxic mechanism that underlies RBP pathology is uncovered, it is difficult to determine which therapeutic intervention will be the most beneficial to patients.

RBP sequestration by microsatellite repeat expansions

Microsatellite repeat expansion disorders are a class of neurodegenerative diseases caused by repetitive DNA elements that form long expansions within gene bodies or in untranslated regions. Over 25 human genes that contain repeat expansions have been identified to date (Loureiro et al., 2016). Some neurodegenerative diseases caused by microsatellite repeat expansion have been linked to the sequestration of RBPs by expanded repeat sequences; these expanded sequences sequester

RBPs away from their target RNAs, thereby altering RNA splicing and metabolism (see poster panel B) (Iwahashi et al., 2006; Jiang et al., 2004; Lee et al., 2013).

One example of altered RNA metabolism in neurological disease comes from the expansion of a CTG triplet in the 3' UTR of the gene *DMPK*, which leads to myotonic dystrophy (DM) (Brook et al., 1992). This expansion is transcribed into repeat RNA that forms aggregates, called RNA foci. These aggregates form within the nuclei of human-derived DM cells (Davis et al., 1997; Taneja et al., 1995) and recruit a class of RBPs that regulate alternative splicing, called the muscleblind-like proteins (MBNLs) (Miller et al., 2000). By sequestering MBNLs into RNA foci, mutant *DMPK* renders MBNLs unable to regulate splicing and the polyadenylation of hundreds of target genes (Batra et al., 2014; Goodwin et al., 2015; Wang et al., 2012a).

Similarly, repeat expansions associated with ALS/FTD and with Fragile X-associated tremor/ataxia syndrome (FXTAS) also sequester RBPs. In FXTAS, a short repeat expansion (<200 repeats) in the untranslated region of the gene *FMR1* is transcribed into RNA and interacts with hnRNPs, MBNL1 and other RBPs (Iwajaszko et al., 2006; Jin et al., 2007; Sofola et al., 2007), thereby altering splicing and microRNA biogenesis in affected individuals (Sellier et al., 2010, 2013).

In the most common inherited form of ALS/FTD, a hexanucleotide (G₄C₂) expansion in the first intron of the gene *C9orf72* is bidirectionally transcribed into mutant RNA that forms aggregates in the nucleus (DeJesus-Hernandez et al., 2011; Gendron et al., 2013; Renton et al., 2011; Zu et al., 2013). Current evidence shows that RBPs involved in splicing, such as hnRNPs and the SR splicing factors that comprise the spliceosome, are sequestered by mutant repeat-containing RNA (Cooper-Knock et al., 2014; Lee et al., 2013) (see poster panel B). Additionally, the repeat expansion, which normally regulates vesicle trafficking and autophagy (Yang et al., 2016; Aoki et al., 2017), can interfere with transcription of the *C9orf72* gene, resulting in haploinsufficiency of the protein product (Burberry et al., 2016; Ciura et al., 2013; DeJesus-Hernandez et al., 2011). Reduced transcription is due, in part, to hypermethylation of the mutant *C9orf72* promoter. Hypermethylation is observed in about one third of *C9orf72* mutation carriers and is associated with reduced mutant RNA accumulation and an attenuated clinical phenotype, suggesting that reduced transcription of mutant *C9orf72* is actually protective against disease (Liu et al., 2014; McMillan et al., 2015; Russ et al., 2015). Thus, altered RNA metabolism is clearly implicated in neurodegenerative diseases caused by repeat expansions.

Given that repeat expansions cause widespread disruption to RNA metabolism, it will be challenging to target downstream processes for therapeutic intervention. Therefore, the most promising therapeutic approaches are those that work upstream to reduce the amount of repeat-containing transcripts. Several studies have used ASOs to target *C9orf72* and DM expansions for degradation via an RNase H-mediated pathway (Donnelly et al., 2013; Jiang et al., 2016; Lagier-Tourenne et al., 2013; Sareen et al., 2013; Wheeler et al., 2012). In fact, ASOs are already in clinical trial for the treatment of Huntington's disease (HD) (Kordasiewicz et al., 2012) and have been approved for the treatment of spinal muscular atrophy (SMA) (FDA, 2016, <http://www.fda.gov/NewsEvents/Newsroom/PressAnnouncements/ucm534611.htm>). An alternative to targeting the repeat-containing transcript for degradation is to target proteins that are responsible for transcribing the repeat expansion. For instance, knockdown of the transcription elongation factor *SUPT4H1* selectively decreases the repeat-containing RNA

in *C9orf72* expansion fibroblasts derived from human carriers (Kramer et al., 2016). This strategy is attractive because transcription of the repeat is blocked in both the sense and antisense directions (Jiang and Cleveland, 2016; Kramer et al., 2016). A final therapeutic approach involves small molecules that target the expanded RNA to prevent their interactions with RBPs. Several of these compounds have been identified but are still in the early stages of development (Disney et al., 2012; Luu et al., 2016; Su et al., 2014). Although the repeat-expansion disorders offer a clear therapeutic target, reversing alterations in RNA metabolism will be challenging in neurodegenerative diseases that lack a clear genetic etiology.

MicroRNA dysregulation in neurodegeneration

Genes can also be regulated post-transcriptionally via miRNAs, a class of small non-coding RNAs (18-25 nucleotides). miRNAs are initially transcribed by RNA polymerase II and then undergo sequential cleavage, first by Drosha and DGCR8 in the nucleus – to generate pre-miRNAs from pri-miRNAs – and then by Dicer after being exported into the cytoplasm, to generate a miRNA duplex (Lee et al., 2003). The miRNA duplex is unwound and one of the strands is incorporated into the RNA-induced silencing complex (RISC), where it binds to Argonaute (Agos) proteins (Schwarz et al., 2003). Agos then cleave the mRNA complementary to the miRNA or inhibit cap-dependent mRNA translation, both of which lead to translational repression (Chendrimada et al., 2005; Kiriakidou et al., 2007).

There are two ways in which miRNA dysregulation contributes to neurodegeneration: alterations to miRNA biogenesis or to miRNA expression, both of which can affect disease-associated genes. A notable example of altered miRNA processing is evident in ALS/FTD through TDP-43 function. In normal neurons, TDP-43 binds to Drosha in the nucleus to cleave select pri-miRNAs and to Dicer in the cytoplasm to cleave some pre-miRNAs (Ling et al., 2010). In mouse neuroblastoma cells (Neuro2a), TDP-43 regulates neuronal outgrowth by modulating pri-miRNA-132 production (Kawahara and Mieda-Sato, 2012). Using lysates from NSC-34 motor neuron cells overexpressing ALS-causing mutations in *TARDBP* and *FUS* in a cell-free dicing activity assay, Dicer function was shown to be altered resulting in an inhibition of miRNA biogenesis (Emde et al., 2015) (see poster panel C). Conversely, activation of Dicer with enoxacin in an ALS mouse model that carries a mutation in the gene Cu/Zn superoxide dismutase 1 (*SOD1*) reverses miRNA downregulation and neuromuscular defects (Emde et al., 2015). This finding indicates that impairment of Dicer activity and miRNA downregulation likely contributes to ALS pathogenesis. Similarly, extensive miRNA dysregulation is seen in FXTAS patients, where FXTAS-associated CGG repeats sequester DGCR8 and prevent proper miRNA processing. In primary mouse cortical neuron cultures that express a plasmid containing toxic 60 CGG repeats, overexpression of DGCR8 is sufficient to reverse cellular toxicity (Sellier et al., 2013). These examples demonstrate that improper miRNA processing leads to global miRNA dysregulation and contributes to different neurodegenerative diseases.

In addition to altered miRNA processing, specific miRNAs that affect certain disease-linked genes are also associated with neurodegenerative diseases (see poster). There are notable examples in Alzheimer's disease (AD) and ALS. AD is the most common form of dementia and is characterized by progressive memory loss, impaired cognitive function, and the inability to perform daily tasks. Pathologically, AD is defined by the presence of extracellular amyloid- β (A β) plaques and of intracellular hyper-

phosphorylated neurofibrillary tangles composed of tau (Hardy and Selkoe, 2002). Beta-site APP-cleaving enzyme 1 (BACE1) and γ -secretase cleave amyloid precursor protein (APP), resulting in A β peptides that accumulate into plaques (Vassar et al., 1999). Multiple miRNAs have been implicated in A β production via BACE1 modulation and in tau phosphorylation that leads to hyperphosphorylated neurofibrillary tangle formation. Additionally, multiple miRNAs have also been implicated in ALS pathogenesis or as biomarkers of disease. For example, miR-23a is overexpressed in skeletal muscle biopsies from ALS patients. This miR-23a has been shown to regulate peroxisome proliferator-activated receptor γ coactivator-1 α , a regulator of mitochondrial biogenesis and function (Russell et al., 2013), which is important for skeletal muscle function. Using spinal cord from individuals with ALS, miR-155-5p and miR-142-5p are upregulated whereas let-7e, miR-148-5p, miR-133b, miR-140-3p and miR-577 are downregulated. These miRNAs regulate neuronal homeostasis, pathogenesis of ALS and other neurodegeneration-related transcripts ranging from ubiquilin, RNA-binding protein fox-1 and reelin among others (Figuroa-Romero et al., 2016) (see Table 1). Indeed, miRNA dysregulation can affect a range of disease-associated targets, which can contribute to neurodegeneration.

Altered miRNA signatures can also indicate potential diagnostic biomarkers. Indeed, various miRNA studies have identified differentially expressed miRNAs in post-mortem tissue or blood and in cerebrospinal fluid (CSF) that differ by disease stage (see Table 1) (Cogswell et al., 2008; Lau et al., 2013; Wang et al., 2011). Furthermore, miRNA-based therapeutics, such as miRNA mimics or miRNA antagonists (antagomirs), have been designed to either reverse the downregulation or upregulation of disease-associated miRNAs, respectively. These have been investigated for the treatment of cancer and cardiovascular disease (Broderick and Zamore, 2011; Thum, 2012; Wu et al., 2007b) but few have been used to treat neurodegeneration. One example of an antagomir in neurodegeneration comes from an AD mouse model (Tg2576) characterized by elevated levels of A β and the presence of amyloid plaques. The treatment of Tg2576 with an antagomir against miR-206, which targets brain-derived neurotrophic factor (*Bdnf*), increases BDNF levels and improves memory function (Lee et al., 2012). Another example is based on the finding that miR-155 is increased in spinal cord from ALS patients and in an ALS mouse model with a mutation in *SOD1*. A locked nucleic acid (modified RNA nucleotide) anti-miR-155 reduces miR-155 levels in this mouse (Butovsky et al., 2015; Figuroa-Romero et al., 2016), thereby increasing survival and restoring the abnormal microglia and monocyte inflammatory signature (Butovsky et al., 2015). Because of the burgeoning importance of miRNAs in disease, it seems important to first investigate and develop an miRNA signature that is validated as a biomarker of disease by independent studies. Then, therapeutic interventions can be designed to target these specific miRNAs.

Other non-coding RNAs in neurodegeneration

With the discovery that other classes of non-coding (nc)RNAs are important for gene expression (Cech and Steitz, 2014), it is perhaps unsurprising that alterations to some of these other ncRNAs, such as tRNAs and lncRNAs, can also lead to neurodegeneration. tRNAs are essential for translation; they use aminoacyl-tRNA synthetase to attach amino acids to the tRNA molecule, which then transfer the appropriate amino acid to a growing polypeptide chain during protein synthesis. tRNA biogenesis entails the transcription and

Table 1. MicroRNAs implicated in neurodegenerative disease

Disease	MicroRNA	Regulation in human brain	Mechanism	References
Alzheimer's disease	miR-107	Downregulated in AD cortex in stage-dependent manner	Increased BACE1 expression	Nelson and Wang, 2010; Wang et al., 2008
	miR-29a/b-1	Downregulated in AD cortex	Increased BACE1/ β -secretase expression	Hébert et al., 2008; Shioya et al., 2010
	miR-146a	Upregulated in AD neocortex and hippocampus	Represses inflammatory response via downregulation of CFH, IRAK1	Cui et al., 2010; Iyer et al., 2012; Lukiw and Alexandrov, 2012
	let-7	Upregulated in CSF of AD patients	TLR7 activation; causes neurodegeneration	Lehmann et al., 2012
	miR-181c	Downregulated in CSF of AD patients	Regulates SIRT1	Cogswell et al., 2008; Schonrock et al., 2012
	miR-34c	Upregulated in AD hippocampi	Regulates cognitive decline and reduced memory function	Zovoilis et al., 2011
	miR-206	Upregulated in AD temporal cortex	Reduction of BDNF	Lee et al., 2012
Parkinson's disease	miR-34b/c	Downregulated in affected brain regions	Reduction in cell viability by altering mitochondrial function, promotes oxidative stress and total ATP content	Miñones-Moyano et al., 2011
	miR-126	Upregulated in dopaminergic neurons	Reduction of IGF1/PI3K/AKT signaling	Kim et al., 2014
	miR-133b	Upregulated in midbrain of PD patients	Downregulation of PITX3 by suppressing dopaminergic neuron maturation and function	Kim et al., 2007
ALS/FTD	miR-132/212	Downregulated in frontal cortex of FTLTDP patients	Increases TMEM106B expression resulting in endolysosomal dysfunction	Chen-Plotkin et al., 2012
	miR-206	Upregulated in serum samples	Preferentially affects fast-twitch muscles of neuromuscular connections	Toivonen et al., 2014
	miR-155-5p	Upregulated in spinal cord of fALS and sALS patients	Impairs microglia, phagocytic function in ALS mouse model	Butovsky et al., 2015; Figueroa-Romero et al., 2016
	miR-23a	Upregulated in skeletal muscle biopsies from ALS patients	Regulates PPRC1 α	Russell et al., 2013
	miR-338-3p	Upregulated in blood leukocytes, CSF, serum, and spinal cord of sALS patients	Predicted to affect apoptosis and/or glutamate clearance	De Felice et al., 2014
	miR-142-5p	Upregulated in spinal cord of sALS patients	Regulates neuronal homeostasis and neurodegeneration-related transcripts	Figueroa-Romero et al., 2016
	miR-140-3p, miR-577, let-7e, miR-148-5p, miR-133b, miR-140-3p, miR-577	Downregulated in spinal cord of sALS patients	Regulates neuronal homeostasis and neurodegeneration-related transcripts	Figueroa-Romero et al., 2016
Huntington's disease	miR-9, miR-9*	Downregulated in HD patient cortex	Affects REST and CoREST binding	Packer et al., 2008
	miR-10b-5p	Upregulated in HD prefrontal cortex	Predicted to affect nervous system development and transcriptional regulation pathways	Hoss et al., 2015
	miR-22	Downregulated in HD brains	Predicted regulation of <i>HDAC4</i> , <i>RCOR1</i> , <i>RGS2</i>	Jovicic et al., 2013
	miR-34b	Upregulated in plasma of pre-symptomatic HD	Mediates protective effect on mutant HTT	Gaughwin et al., 2011
	miR-132	Downregulated in HD frontal cortex	Predicted to affect neurite growth and neuronal connectivity via p250GAP (<i>ARHGAP32</i>) mRNA	Johnson and Buckley, 2009

There are many dysregulated miRNAs in different neurodegenerative diseases. Listed here are the dysregulated miRNAs that are dysregulated as identified in human tissue, along with the mechanisms of action. sALS and fALS, sporadic and familial ALS, respectively. miR-9 and miR-9* are two different miRNAs.

splicing of tRNAs by complexes that involve the proteins CLP1 (cleavage and polyadenylation factor I subunit 1) and TSEN (the tRNA-splicing endonuclease complex) (Paushkin et al., 2004; Trotta et al., 2006) (see poster panel D). Additionally, during cellular stress, angiogenin (ANG) cleaves tRNAs into fragments that might inhibit translation or target specific mRNAs for degradation as a cellular protective mechanism (Fu et al., 2009; Ivanov et al., 2011; Yamasaki et al., 2009). Indeed, mutations in tRNA biogenesis components lead to neurodegeneration. Mutations in aminoacyl-tRNA synthetases are found in individuals with

Charcot-Marie-Tooth, a disease of peripheral neuropathy (Antonellis et al., 2003; Nangle et al., 2007; Xie et al., 2007). It is unclear why abnormalities in tRNA biogenesis result in different peripheral neuropathies but various mechanisms have been proposed, including: (1) loss of function of tRNA loading and subsequent protein translation inhibition; (2) a dominant-negative effect whereby mutant protein interferes with the wild-type protein activity; and (3) impaired axonal transport due to tRNA synthetase mutations, among others (Motley et al., 2010; Niehues et al., 2015). A mutation in *CLP1* (R140H) is a cause of progressive brain atrophy

and microcephaly in humans (Karaca et al., 2014; Schaffer et al., 2014). *CLPI* R140H renders CLP1 unable to interact with TSEN, resulting in altered tRNA processing (see poster panel D). Mutations in *ANG* that might contribute to the loss of ANG function also occur in ALS (Greenway et al., 2004, 2006; Wu et al., 2007b). Altered translation can also contribute to neurodegenerative disease. Specifically, a mutation in *GTPBP2*, which encodes a ribosome rescue factor, leads to global ribosomal stalling by epistatically interacting with an isodecoder of nuclear-encoded tRNAs, called n-Tra20. This aberrant interaction ultimately leads to neurodegeneration via altered translation (Ishimura et al., 2014). These studies only provide a snapshot of how aberrations in tRNA processing can lead to neurodegeneration.

lncRNAs have also been implicated in neurodegenerative diseases (see poster panel E). These RNAs are longer than 200 nucleotides and fulfill various functions, including acting as scaffolds for chromatin modifiers and nuclear paraspeckles, as transcriptional co-regulators, and even as decoys for other RNAs (Prensner and Chinnaiyan, 2011). Alterations in lncRNAs can affect any one of these processes, thereby contributing to neurodegeneration. lncRNAs associated with disease can post-transcriptionally increase gene expression, as seen with the lncRNAs, *BACE1*-antisense (AS) and lnc-SCA7 (official symbol ATXN7L3B). *BACE1*-AS is increased in AD brains and competes with miRNA-545-5p binding to stabilize *BACE1* mRNA. This is associated with increased *BACE1* expression and with the formation of the A β peptides that contribute to AD pathology (see poster panel E) (Faghihi et al., 2008). In spinocerebellar ataxia type 7 (SCA7), mutant CAG repeats in the *ATXN7* gene contribute to cerebellar neuronal death. Normally, *ATXN7L3B* regulates *ATXN7*, which is loaded into a transcriptional activator complex, called STAGA. STAGA promotes miR-124 biogenesis, which in turn, represses *ATXN7L3B* expression. In SCA7, mutant CAG repeats promote mutant *ATXN7* protein levels, which reduce STAGA activity. This consequently reduces miR-124 biogenesis, increases *ATXN7L3B* expression and promotes more mutant *ATXN7* production (Tan et al., 2014). Other examples of genes with altered lncRNAs include *MALAT1* and *NEAT1*, which are important for splicing and synapse formation (Bernard et al., 2010; Tripathi et al., 2010). Both of these RNAs are bound by TDP-43 and FUS (Lagier-Tourenne et al., 2012; Polymenidou et al., 2011; Tollervey et al., 2011). *MALAT1* and *NEAT1* have been shown to colocalize in nuclear paraspeckles, sites where RNA is retained to control gene expression during different cell processes (see poster panel E) (Fox and Lamond, 2010). Additionally, *NEAT1* is upregulated in the HD brain, which is thought to make cells susceptible to oxidative stress (Johnson, 2012; Sunwoo et al., 2016). Other HD-associated genes with lncRNAs include *TUG1*, which is increased in HD and which normally associates with polycomb repressive complex 2 (PRC2) to repress gene expression (Johnson, 2012; Khalil et al., 2009) (see poster). Thus, lncRNAs are likely to influence gene expression post-transcriptionally to contribute to neurodegenerative disease.

Therapies designed to target lncRNAs involve inhibiting the function of lncRNAs usually by: (1) blocking the interaction of the antisense and sense mRNA by degrading the antisense strand, which leads to the transcriptional repression of the gene; (2) using aptamers to bind and inhibit lncRNA structures and prevent their activity; and (3) employing small molecules that inhibit lncRNA interactions (Fatemi et al., 2014; Sullenger and Nair, 2016). Although it has been shown that treating a mutant APP-expressing human HEK-SW cell line with siRNA against *BACE1*-AS leads to reduced A β (Faghihi et al., 2008), this finding has not

been validated in an AD mouse model or human patients. A better understanding of how specific lncRNAs contribute to the disease phenotype is integral to designing better-targeted therapeutics for these diseases.

Conclusions

Neurodegenerative diseases occur by different means and present with various pathologies. However, it is becoming increasingly clear that altered or defective RNA metabolism, including mislocalized RBPs and aberrant ncRNA biogenesis or expression, can contribute to neurodegeneration. Because numerous disease-associated pathways are perturbed in these neurodegenerative diseases (as summarized in the accompanying poster), it is unlikely that targeting only one of these modalities will lead to a complete cure. Nonetheless, reversing some of these RNA aberrations could prove to be effective in modifying the incessantly downward disease trajectory associated with these diseases. Perhaps by modifying disease progression, such approaches could provide a significant benefit for those afflicted by neurodegenerative disease.

This article is part of a special subject collection 'Neurodegeneration: from Models to Mechanisms to Therapies', which was launched in a dedicated issue guest edited by Aaron Gitler and James Shorter. See related articles in this collection at <http://dmm.biologists.org/collection/neurodegenerative-disorders>.

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Competing interests

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At a glance

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