Non-coding RNAs and the Deregulation of Ubiquitin-Proteasome Network in Neurodegeneration: A Familia Tria?

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Abstract

Small non-coding RNAs (ncRNAs) represent a diverse group of gene regulatory factors that can posttranscriptionally regulate gene expression in response to various stimuli during brain development and differentiation. Subsets of ncRNAs and miRNAs in particular, are very specifically expressed within the central nervous system and participate in the regulation of important brain functions. miRNAs are essential for the postmitotic survival of neurons, and therefore might play a role in neuroprotection. A number of miRNAs have been reported to be dysregulated in several neurodegenerative diseases implying that they can contribute to pathogenesis. Furthermore, in light of the neuroprotective properties of some miRNAs, these small RNA species may themselves be the focus for drug development. Here, we review recent studies that imply a link between miRNA role in the regulation of ubiquitine-proteasome pathways and neurodegeneration and discuss how increased knowledge of miRNAs might serve the diagnosis and treatment of neurodegenerative diseases.

Introduction

The molecular and structural transformations that shape the human cognitive abilities occur mostly in the period between birth and adulthood, although some developmental processes, such as cortical axon myelinization, extend beyond this time window [1-3]. The primate brain is subjected to dramatic change, both structurally and functionally, during postnatal development [1, 4]. It is quite remarkable that the process of brain aging begins at early adulthood that is manifested by gradual deterioration of the brain capacity to utilize the flow of information. In later life the brain begins to change in a more destructive manner. Such changes include a decrease in brain volume, loss of synapses, cognitive decline, and a rise in the frequency of neurological disorders [2, 5-7].

Multiple cellular and functional transformations take place in the brain during aging. Neural cells may respond to these changes by reprogramming metabolic circuits in order to adapt and maintain its functionality, or they may give in to neurodegenerative cascades that result in disorders such as Alzheimer's, cerebellar ataxias and Parkinson's diseases. A number of mechanisms are employed to maintain the integrity of nerve cell networks and to facilitate responses to external and internal environmental stimuli and maintain neuron integrity and functional capability after damage. The protective machinery includes production of neurotrophic factors and cytokines, expression of various cell survival-promoting proteins (e.g. antioxidant factors, pro-survival and anti-apoptotic proteins, protein chaperones), activation of DNA caretaker cascades to preserve the genomic integrity, and mobilization of neural stem cells to replace damaged neurons and glial cells.

Protein turnover and neurodegeneration

Genetic background and environmental stressors superimposed upon the aging dynamic are the determining factors of the physiological vs pathological brain aging. The importance of genetic predisposition to accelerated aging and neurodegeneration is well documented. The accumulation of toxic proteins transcribed from mutated genes causes inherited forms of Alzheimer's disease (amyloid precursor protein and presenilins), Parkinson's disease (α-synuclein and Parkin), multiple sclerosis (MS), amytrophic lateral sclerosis (ALS), prion disease and trinucleotide repeat disorders (huntingtin, androgen receptor, ataxin, and others) by overcoming the endogenous neuroprotective mechanisms [8].

Many neurodegenerative disorders are associated with the accumulation of protein deposits in the central nervous system (CNS). Neurodegenerative diseases are generally disabling and often fatal. The process is also irreversible, and since neurons cannot be regenerated, it ultimately leads to

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the death and atrophy of neurons in the brain. Thus, health care options are mostly limited to symptomatic treatments. The accumulation of misfolded proteins during brain aging is a well-established phenomenon and is exacerbated in several neurodegenerative diseases, including SCA1, Parkinson's disease and Alzheimer's disease [9-11]. It has been suggested that protein accumulation results from a dysfunction in the ubiquitin-proteasome system (UPS). In fact, there is mounting genetic and biochemical evidence of an involvement of UPS in neurodegenerative disorders [12, 13].

Ubiquitination of proteins and their degradation within the proteasome is the main proteolytic mechanism used by mammalian cells to regulate cytosolic and nuclear protein levels. The ubiquitin-proteasome pathway eliminates a variety of normal and abnormal proteins, and the ubiquitin-proteasome system (UPS) plays a central role in most cellular functions. Ubiquitination is carried out by a series of enzymatic steps that accomplish the covalent attachment of multiple ubiquitin residues to the protein designated for degradation. This process includes the ATP-dependent activation of ubiquitin by the ubiquitin-activating enzyme (E1), transfer of ubiquitin to a member of the ubiquitin-conjugating enzyme (E2) family, and the linkage of the 76-amino acid ubiquitin to a lysin residue of the target protein, which is then catalyzed by an E3 ubiquitin ligase [14]. E3s confer the specificity in the ubiquitination process. Proteins labeled with ubiquitin chains of four or more ubiquitin molecules are recognized by the 26S proteasome. The proteasome is located in the cytosol and in the nucleus of eukaryotic cells. The 20S core is attached at both sides to 11S (PA28) or 19S (PA700) cap complexes consisting of multiple proteins that participate in recognition and unfolding of the polyubiquitinated proteins. The target protein is then unfolded and cleaved at the carboxy-terminal end of large hydrophobic, basic, or acidic residues. Monomeric ubiquitin is recycled through ubiquitin carboxy-terminal hydrolase, and the degradation products (short peptide fragments and amino acids) re-enter protein synthesis chains.

Neurodegenerative disorders like Alzheimer's disease and dementia with are characterized by the accumulation of intraneuronal inclusions, which may be regarded as neuropathological hallmark of a number of chronic neurodegenerative disorders as well. The protein insoluble aggregates, or inclusion bodies, are found in the neuronal cytoplasm, axoplasm, or neuronal nuclei and may be encapsulated in the form of filamentous deposits. The importance of the UPS in inclusion body formation was elucidated when a variety of molecules involved in ubiquitin-mediated protein degradation, among them proteasomal subunits and other components of the UPS, were detected in inclusion bodies. Increasing evidence suggests that proteins forming aggregates either resist or inhibit proteolysis via the UPS. Mutations that impair normal ubiquitination (or deubiquitination) lead to protein accumulation, resulting in proteasomal inhibition [15]. Neurofibrillary tangles in Alzheimer's disease or Lewy bodies in Parkinson's disease represent examples of cytoplasmic inclusions. Nuclear inclusions are observed in expanded polyglutamine protein disorders, e.g., spinocerebellar ataxia type 1 (SCA1). Ataxin-1, the disease-causing gene of SCA1, is ubiquitinated like normal ataxin-1 but resists proteasomal degradation. The HECT ubiquitin ligase E6-AP is apparently necessary for inclusion formation, because mice exhibiting expanded polyglutamine ataxin-1 and simultaneously lacking E6-AP have significantly fewer nuclear inclusions [16]. Other neurodegenerative disorders reveal similar neuropathological findings, for example, Lewy bodies contain ubiquitin and other proteins, which have been identified as the cause of familial forms of Parkinson's disease.

Mutations in E3 ligases reduce the formation of ubiquitin-positive inclusions, suggesting that segregation of abnormal proteins requires ubiquitination and serves as a natural defense mechanism against the cytotoxic effects of abnormal proteins that resist proteasomal degradation. This hypothesis is supported by the frequent occurrence of Lewy bodies in late-onset sporadic Parkinson's disease, whereas they are rarely found in patients with the young-onset familial form of Parkinson's disease that is often manifested by severe neurodegeneration. Thus, it appears that UPS promotes inclusion body formation to reduce the toxicity of misfolded mutants, or otherwise damaged proteins in their soluble form [17].

Recently, HECTD1 and RNF8 E3 ubiquitin-protein ligases have been identified as targets of ncRNA in the cortex and cerebellum of individuals diagnosed with spinocerebellar ataxia type 1 and Alzheimer disease [18]. The HECT family of protein ligases have non-redundant functions in regulating protein turnover and ubiquitinate proteins that are subsequently degraded by the 26S proteosome protein complex [19, 20]. As such, deregulation of HECT ligases can severely perturb

neuronal structure and function and may lead to functional collapse of the postmitotic neurons and withdrawal from the brain circuitry.

miRNA metabolism in the brain

miRNAs comprise a large family of ~21-nucleotide-long RNAs that have emerged as key post-transcriptional regulators of gene expression in metazoans and plants, and have revolutionized our understanding of the gene regulatory all protein-coding genes. Functional studies indicate that miRNAs participate in the regulation of almost every cellular process investigated so far and that changes in their expression are associated with many human pathologies.

In mammals, miRNAs generally bind imperfectly to consensus elements in the 3'-untranslated region (3'-UTR) of mRNAs and repress protein synthesis, either by inhibiting translation or causing destabilization of the targeted transcripts [21, 22]. miRNAs are implicated in the control of a number of fundamental processes and most miRNAs are expressed in a development- or tissue-specific manner [23, 24]. Subsets of miRNAs are specifically expressed or enriched in neuronal cells in a temporal and spatial manner [25], consistent with the growing evidence of miRNAs role in brain development and function [26-28].

The mechanisms that control miRNAs turnover has received only limited attention to date, but recent findings appear to indicate that the rate of miRNA degradation is cell-specific and play an essential role in neuronal gene regulation [29]. In general, miRNAs are highly stable molecules and experiments using transcription inhibitors or depletion of miRNA processing enzymes, have indicated that the half-lives of miRNAs in many cell lines and organs is hours or even days [30, 31]. However, such slow turnover is unlikely to be a universal feature of the miRNAs that often are temporally and spatially restricted. Rapid and regulated decay of many miRNAs occurs in different types of neurons. Importantly, it has been reported recently that miRNAs in retinal, hippocampal and cortical neurons undergo faster turnover than miRNAs in nonneuronal cells. High turnover of several miRNAs (miR-124, miR-128, miR-134 or miR-138) also occurs in primary dissociated rodent neurons and neurons differentiated from mouse eSCs, and in human primary neural cultured cells and post-mortem brain tissues. Also, miRNA metabolism in neurons was found to be dependent on neuronal activity [29]. However, the significance of rapid and activity-dependent turnover of many other neuronal miRNAs remains unknown. Nevertheless, these findings emphasize that fundamental differences exist in the miRNA metabolism in the brain, which is likely to underlay the specificity of miRNA regulatory networks in the central nervous system.

miRNA-mediated regulation of ubiquitin-proteasome pathway and neuronal dysfunction

An important question concerning the role of miRNAs in neurodegeneration is whether their deregulated expression contributes directly to disease development or is consequence of the disease. miRNA expression is regulated by similar mechanisms that control the transcription of mRNAs. This includes genetic background, epigenetic modifications, transcription factor interactions, feedback loops, and the maturation process of precursor molecules. Moreover, miRNAs are influenced by the commonly known risk factors for neurodegenerative diseases, such as age, gender, environmental factors, and genetic predisposition.

Posttranscriptional modifications of the ubiquitine-proteasome pathway are likely to play a role in impaired proteasome function. As discussed above, degradation of proteins via the ubiquitin/proteasome pathway involves two successive steps: 1) conjugation of multiple ubiquitin moieties to the substrate and 2) degradation of the tagged protein by the downstream 26S proteasome complex. Ubiquitination is a protein post-translational modification process that consists of three steps driven by E1, E2 and E3 enzymes and involves hundreds of proteins. In the ubiquitination cascade, E1 can bind with dozens of E2s, which can bind with hundreds of E3s in a hierarchical way. The final step of the ubiquitylation cascade creates an isopeptide bond between a lysine of the target protein and the C-terminal glycine of ubiquitin and requires the activity of one of the numerous E3 ubiquitin-protein ligases.

Many cellular pathways are affected by the regulatory function of miRNAs; the most prominent of these pathways control developmental and oncogenic processes. The combined alterations in gene expression regulation of multiple ubiquitine-proteasome system (UPS) genes as a

result of miRNA dysregulation and unique polymorphisms within the respective 3'-untranslated regions (3'UTRs) that affects miRNA binding might be determining factor for the progression and individual differences in gene expression pattern and predisposition to neurodegeneration. In a recent study, we functionally annotated the target genes of the small non-coding RNAs (ncRNAs) that were selectively activated in the affected brain compartments of SCA1 patients. The primary targets of these RNAs, which exhibited a significant enrichment in the cerebellum and cortex of SCA1 patients, were identified to be members of the UPS [18]. Thus, the spatial-temporal changes of miRNA expression levels in conjunction with target gene accessibility might be a reliable marker for the responsiveness of neurodeneration patients to therapeutic interventions.

miRNAs have been widely reported to modulate the cell death cascades in the nervous system [32]. In this respect, decreased proteasome activity has been suggested as a cause of aggregation in neurodegeneration and ultimately as a contributing factor to neuronal death. The involvement of the proteasome in neuron apoptosis is suggested by both the increase in protein ubiquitination and of E1, E2, and E3 enzymes that is observed in several cell types well in advance of apoptosis [33, 34]. The inhibition of proteasome activity appears to have differential effects on apoptosis induction in different cell types. In general, the proteasome is not required for apoptosis in some cell lines [35], but certain type of neurons appears to enter programmed cell death pathway following exposure to proteasome inhibitors [36, 37]. The mechanism that prevents apoptosis after proteasome inhibition remains unclear, but is hypothesized to be specific to cells in quiescent states, or to result from the differential activity of the pro-apoptotic kinase JNK [35]. Thus, it is feasible to speculate that miRNA influence on UPS genes will affect the neuronal death mechanisms that operate in the aging brain and during the progress of neurodegeneration.

Future directions

Despite intensive research, the unknown still exceeds what we currently know on intracellular protein degradation, and major key questions have remained unsolved. Among these are the modes of specific and timed recognition for the degradation of the many substrates and the mechanisms that underlie aberrations in the system that lead to pathogenesis of diseases.

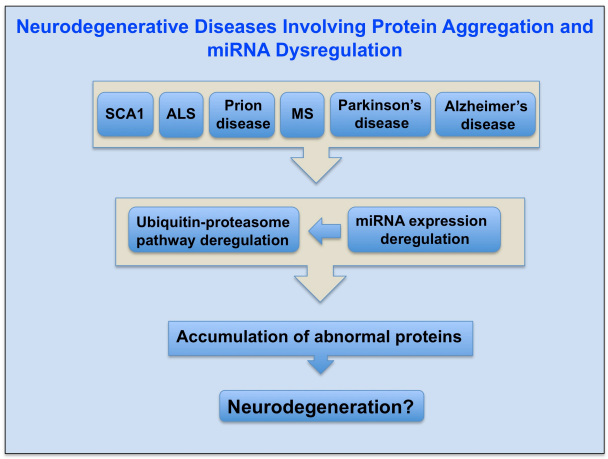

Figure 1. Schematic depicting the interactions between ubiquitine-proteasome system (UPS) and miRNAs in neurodegeneration. The model predicts that altered miRNA expression observed during aging, especially the upregulation of certain brain-specific miRNAs, would have negative impact on UBS and will exacerbate the accumulation of protein aggregates. The outcome will be increased neuronal cell death.

In addition to its role in the intracellular protein degradation machinery, the UPS has been identified as an important regulator of transcription regulation, protein activation and endocytosis. In the nervous system, the physiological significance of this pathway is just beginning to be explored. Dissecting the role of the UPS during neuronal differentiation and brain neuronal apoptosis in cell culture models has laid the groundwork for our current understanding of ubiquitination and

proteasomal protein degradation in the brain. The UPS is clearly involved in the pathogenesis of various neurodegenerative disorders. The high sensitivity of postmitotic, fully differentiated cells to oxidized or misfolded proteins, which need to be eliminated rapidly to maintain neuronal metabolism, makes neurons particularly vulnerable to defects in ubiquitin-mediated protein degradation. The UPS presents miRNAs with multiple miRNA targets that are likely to play significant role in the regulation/deregulation of target gene expression (**Figure 1**). In this regard, the effects of miRNAs on gene expression is predictable and might lead to deregulation of genes that contribute to the pathogenesis of neurodegenerative disorders. Moreover, the rule that dictates the outcome of small RNA–mRNA interaction according to the target sites complementarity, together with the modular structure of miRNA target sites, also permits combinatorial usage of miRNAs to modulate and eventually correct gene expression patterns with widespread applications for the gene therapy. In this context, alterations in the pattern of miRNA-regulated gene expression combined with existing single nucleotide variants within the 3'UTRs of target genes might be responsible for the individual differences in gene expression patterns and as such might influence the pathogenesis and therapeutic response of neurodegenerative diseases.

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