Non-coding RNA in Neurodegeneration

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Abstract Aging-associated chronic diseases, such as neurodegenerative disorders, have a dramatic impact on healthcare systems. Despite progresses in understanding their etiology, unsolved questions still exist. These complex disorders share a common inflammatory *status* and are influenced by common post-transcriptional mechanisms of gene regulation. MicroRNAs, key players in modulating gene expression, and other non-coding RNAs have specific spatial-temporal expression in the brain, and a critical role in neurogenesis and neurodegenerative diseases. Here, we review the emerging impact of non-coding RNAs in their pathogenesis, also performing a computational analysis on microRNAs and target genes. Our findings strengthen the notion of an inflammatory-related component in neurodegenerative disorders, confirming the contribution of microRNA-dependent gene expression regulation in the etiology of such diseases.

Keywords Non-coding RNA · miRNAs ·
Neurodegeneration · Inflammation · Down syndrome ·
Epigenetics mechanisms · RNA-Seq

Introduction

Life expectancy has greatly increased worldwide during the last decades, due to the fact that the elderly population (60 y.o. or over) has tripled over the last 50 years and it is expected to triple again over the next 50 years [1]. Along with the increasing number of elderly persons, aging-associated chronic diseases are assuming a significant relevance. In particular, the prevalence of neurodegenerative disorders (NDs), which rises in step with the aging of the population, is having a broad impact on healthcare systems and is predicted to worsen in the near future [2]. A growing number of studies focusing on NDs are still in progress to understand the pathogenesis and the molecular mechanisms underlying these disorders, with the aim to develop effective programs for prevention and therapy. Nevertheless, many questions remain unanswered and much can still be brought to light.

NDs constitute a wide class of disorders, including Alzheimer's, Parkinson's and Huntington's diseases (AD, PD and HD, respectively), frontotemporal dementia (FTD), amyotrophic lateral sclerosis (ALS), spinocerebellar ataxia (SCA), and Prion diseases. In addition, young individuals with Down syndrome (DS) develop an early impairment of cognitive functions with Alzheimer-like phenotype [3]. These conditions are all - except DS - characterized by a progressive loss of neuronal cells in adulthood [4]. Neurodegeneration is initially limited to a specific cell type in the central nervous system (CNS), in line with the heterogeneity of clinical symptoms. Cortical and hippocampal regions are affected in AD, dopaminergic neurons in the *substantia nigra* in PD, frontal and/or temporal lobes in FTD, motor neurons in the brain and spinal cord in ALS, the basal ganglia in HD [5]. In later stages, neuronal cell death progressively extends to wider CNS regions, gradually leading to a severe physical and cognitive disability, frequently including loss of body control, speech impairment and dementia [6].

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Although their etiology is not fully understood, the presence of intracellular or extracellular aggregates of misfolded proteins in the brain is a common feature. Amyloid plaques, consisting of deposits of β -amyloid protein - a fragment derived from altered processing of amyloid protein precursor (APP) - in extracellular parenchyma, and intracellular neurofibrillary tangles, containing aggregation of hyperphosphorylated tau protein, represent histopathological signatures of AD [7]. Tau aggregates are also observed in FTD [8], whereas TDP-43 inclusions are common in frontotemporal lobar degeneration (FTLD) and in ALS [9]. In PD, dopaminergic neurons are characterized by cytoplasmic inclusions of α -synuclein, known as Lewy bodies [10]. Protein aggregates, due to polyglutamine expansion in huntingtin and ataxin proteins, are detected in HD and SCA, respectively [11, 12]. Moreover, Prion diseases are characterized by the accumulation of conformationally-modified glycoprotein PrP [13]. Since the deposits of abnormal proteins are a distinctive feature of all NDs, it is reasonable to assume that protein misfolding and the aggregation process represent a crucial step in neurodegenerative mechanisms [14]. Although each ND shows a different histological phenotype, the abnormal protein accumulation triggers the activation of inflammatory and oxidative-stress pathways. Thus, a chronic inflammatory state has a relevant impact on neuronal cell death, consistent with the “age-based theory” of neurodegeneration’s pathogenesis, since the levels of oxidative damage increase with aging and, conversely, the anti-oxidative defenses decrease [15].

Most of the molecular causes underlying NDs are still unclear, although in the last three decades the application of molecular cell biology’s techniques to CNS study has improved our knowledge about neurodegenerative processes [16]. In the “pre-genomic era”, the genetic bases of NDs have been investigated by linkage analyses, and a restricted subset of causative genes identified by positional cloning. So, more than 200 disease-causing mutations have been identified in three different genes, *APP* (amyloid precursor protein), *PSEN1* (presenilin1) and *PSEN2* (presenilin2), associated with AD [17], whereas more than 300 pathogenic mutations have been identified in five genes, α -synuclein (*SNCA*), parkin (*PARK2*), PTEN-induced putative kinase 1 (*PINK1*), DJ-1 (*PARK7*), and Leucine-rich repeat kinase 2 (*LRRK2*) in PD families [18]. Moreover, five established genes for Mendelian forms of FTD and thirteen for ALS have been identified [4]. In addition, hereditary forms of Prion diseases, such as Creutzfeld-Jacob disease, have been associated to mutations in the gene encoding PrP (*PRNP*) [5].

Despite the impact of their genetic component, NDs are complex traits and only few cases can be explained by a typical Mendelian inheritance. More than 90 % of cases, defined as “sporadic” or “idiopathic” forms, appear to be regulated by an intricate network of genes and environmental factors. Particularly, the recent application of genome-wide

association studies (GWAS) has led to the identification of many single nucleotide polymorphisms (SNPs) in susceptibility genes, associated with the etiology of NDs, i.e. APOE- ϵ 4 in AD [17]. However, for all human diseases it has been estimated that about 90 % of currently identified disease-associated SNPs are intronic or intergenic, and GWAS explain only a small percentage of NDs cases [19]. Therefore, a significant portion of “missing heritability” remains. Moreover, variation in gene expression has recently been shown to affect susceptibility to complex diseases [20], such as the Alzheimer-like dementia occurring in DS individuals, strictly linked to the presence of an extra copy of critical genes on chromosome 21 [21].

Changes in gene expression occur during aging, and these are accelerated in some forms of neurodegeneration [22•]. Epigenetic mechanisms dynamically regulate gene expression, acting at several levels. In this scenario, other contributors are the non-coding RNAs (ncRNAs) - transcribed from DNA regions considered for a long time to be evolutionary debris and junk sequences - which represent a major portion of transcriptome acquiring an emerging pivotal role as determinants of gene expression regulation, revealing an unexpected involvement in human diseases [23•]. Evidences show the contribution of ncRNAs in complex regulatory networks of differentiation and development, acting through chromatin modification, transcription, RNA modification, splicing, mRNA translation and RNA stability [24]. Moreover, it should also be considered that some classes of ncRNAs have specific spatial-temporal patterns of expression in CNS, suggesting a role in neurogenesis and neuronal functions and, consequently to their deregulation, in neuronal pathological processes [25]. Among them, miRNAs are becoming major players in shaping cell functionality, as well as in determining cell fate. Indeed, they are crucial factors in cell differentiation, and in many other cell processes fundamental for neurons, such as neurotransmission and synaptic plasticity [26•]. In addition, several long non-coding RNAs (lncRNAs) display specific localization limited to restricted brain regions, particular neuronal cell-type or subcellular compartments, suggesting their deregulation might contribute to NDs’ onset [27].

Therefore, given the inability to explain all NDs cases by a typical Mendelian inheritance, and also considering the potential impact of ncRNAs on gene expression and on disease onset/progression, it is reasonable to speculate ncRNAs might be involved in their pathogenesis.

Neurodegeneration and Non-coding RNA

Only 2–4 % of the mammalian genome encodes mRNAs. The ncRNA fraction has been long considered non-functional, with the exception of the common infrastructural

RNAs involved in protein synthesis, transport and splicing [28]. However, researchers have increasingly focused their attention on the part of human genome transcribed and never translated, trying to clarify the genetic information contained therein, and its functions [26•, 29, 30]. In recent years, interesting results have been obtained, challenging the traditional view of RNA as a simple intermediary between DNA and protein, and thus showing that the vast majority of the genome encodes functional RNA species [24].

A large variety of ncRNAs has been progressively identified in the CNS, and their roles in neurogenesis, neural stem cell maintenance, synaptic and neural network connectivity and plasticity have been highlighted [25]. These evidences suggest a strong connection between the regulatory potential of ncRNAs and CNS complexity [31]. Consequently, it is intuitive that altered expression and function of ncRNAs may be directly linked to the onset of CNS disorders.

The most widely studied class of small-ncRNAs (<400 nucleotides) is microRNAs (miRNAs), highly conserved molecules of 20–22 nucleotides in length, found in almost all eukaryotic cells [32•]. Mature miRNAs are generated through a multi-step process. They are post-transcriptional regulators of gene expression which, binding to complementary sequences, downregulate target mRNAs' levels, causing the degradation of related transcripts or the inhibition of their translation [23•, 33–35]. Whereas some miRNAs regulate specific individual targets, others can function as master regulators of a process, or through the simultaneous regulation of hundreds of genes, or acting cooperatively on target genes [31, 36].

miRNAs are involved in numerous biological processes, including cell proliferation, development, stress responses and apoptosis [36]. In mammals, they are predicted to control the activity of more than 50 % of all protein-coding genes [33]. About 70 % of miRNAs are expressed in specific brain regions, and experimental evidences have shown their role in terminal differentiation and maintenance of many neuronal types, suggesting a relevant functional role in brain activities [31, 36, 37]. A growing number of reports have shown that miRNA deregulation is associated with the pathogenesis of human NDs [38]. For instance, altered miRNA levels have been directly linked to AD pathogenesis [39•]. Analyses performed on the brains of AD patients, with deregulated levels of β -site APP cleaving enzyme (encoded by *BACE1*), have displayed a significant alteration in miRNA expression. *BACE1* contributes to the accumulation of toxic A β fragment, and is a target of several miRNAs [40–43].

In addition, it is reasonable to speculate that miRNAs may represent a crucial regulatory mechanism also linking AD and DS pathogenesis. Indeed, both the overexpression of specific genes located on chromosome 21 (HSA21) - such as *APP* - and HSA21 miRNAs have been reported in DS patients. The

simultaneous downregulation of their target genes has suggested a relevant role for HSA21 miRNAs on their expression during DS pathological processes. Therefore, these molecules may represent good candidates for the onset of the Alzheimer's-like dementia occurring in young DS individuals, as well as for other NDs [44, 45]. Interestingly, accumulating evidences of miRNAs' role have been reported also in PD. Two miRNAs (miR-7 and miR-153) have been demonstrated to inhibit *SNCA* - encoding α -synuclein - crucially related to PD pathogenesis [46]. Furthermore, expression profiling of miRNAs in adult PD vs. normal midbrain, revealed the downregulation of miR133b - normally enriched in the midbrain - possibly acting as negative regulator in dopaminergic neuron maturation, targeting the transcription factor (TF) Pitx3 [39•].

The evidence of direct and indirect roles of miRNAs has also been progressively suggested in other NDs, such as in HD onset. Patients affected by HD show a pathogenic polyglutamine expansion in huntingtin, responsible for disrupted interaction with the transcription factor REST that, in normal neurons, is sequestered in the cytoplasm by the protein huntingtin. In HD, its aberrant nuclear-cytoplasmic trafficking is promoted, and the expression of its target genes is deregulated. REST target genes include not only protein-coding, but also miRNAs and other ncRNAs [39•]. Interestingly, REST regulates the expression levels of miR9 and miR9*, which in turn can regulate REST itself, suggesting a negative feedback loop during HD development [47, 48]. ALS is another member of NDs in which miRNAs play a critical role. Notably, mutations in the components of Drosha microprocessor complex - involved in biogenesis of miRNAs - cause up to 50 % of familiar cases of ALS [49].

Although experimental evidences have shown the relevant role of miRNAs in ND pathogenesis, further studies have gradually highlighted the involvement of other ncRNAs in their onset. Among them, lncRNAs (>400 nucleotides) represent one of the most abundant classes [26•, 50]. They derive from genomic *loci* proximal to protein-coding genes, and are usually regulated by the same transcriptional and epigenetic mechanisms. These transcripts are fundamental in a plethora of subcellular processes, including formation of cellular structural compartments, neuronal differentiation, hippocampal development and oligodendrocyte myelination [51•]. Indeed, their expression is largely reported in the brain and, interestingly, some of them have been shown to interact with promoter elements and TFs, critically modulating the transcriptional activity. For instance, the Sox2OT lncRNA may have a regulatory effect on Sox2, a TF required for neural induction and maintenance of neural stemness [52]. Similarly, Nkx2.2AS lncRNA modulates the expression of Nkx2 and other factors involved in oligodendrocyte lineage specification [53].

Furthermore, lncRNAs can regulate post-transcriptional mRNA processing and translation by interacting with specific sequences [54] and modulating the epigenetic *status* of protein-coding genes through *cis* and *trans* mechanisms. In addition, lncRNAs may be the precursor of small ncRNAs, such as miRNAs [55]. Interestingly, a large number of studies have revealed a very dynamic profile of expression - and functions - for lncRNAs during the development of adult tissues, including brain and several neural cell subtypes [51•]. Deregulation of lncRNAs has been associated with the pathogenesis of different NDs, including *ATXN80S* in SCA8 [56–58], *BACE1-AS* and *BC200* in AD [59, 60]. Moreover, further findings have suggested that some lncRNAs might be involved in molecular mechanisms underlying ALS and HD [51•].

Our recent transcriptome analysis in DS has revealed lncRNAs' deregulation in trisomic vs. euploid cells, suggesting their involvement in some pathological features typical of DS [61]. Finally, it is reasonable to assume many disease-associated SNPs, identified in intronic and intergenic regions in patients affected by NDs, are likely to fall in previously unannotated non-coding transcripts, such as lncRNA, possibly contributing to the disease.

It should be mentioned that another class of ncRNAs, the small nucleolar RNAs (snoRNAs), has been directly linked to Prader-Willi syndrome characterized by neonatal muscular hypotonia, obesity, hypogonadism, behavioral problems and mental retardation. In particular, HBII-52 snoRNA, which regulates alternative splicing of 5-HT_{2C}R mRNA, is silenced in patients, leading to the production of different isoforms compared to healthy individuals [62].

In light of these evidences, studying the function, spatial-temporal localization and regulation of ncRNAs in neurons is becoming essential to understanding physiological neurogenesis and neural plasticity, as well as to disclose the molecular bases of neurodegenerative processes occurring in patients' brains.

miRNAs in Neurodegeneration

As mentioned above, miRNAs represent the major and better-studied class of ncRNAs involved in neurodegenerative processes. Given their relevant contribution to ND onset and severity, and also considering the large amount of molecular data available from gene expression studies, and consequently, the growing number of well-curated miRNA databases, we focused our analysis on this ncRNA species.

In particular, after browsing commonly used databases, we collected from miRWalk [63] and mir2disease [64] all miRNAs with a proven involvement in NDs and neurodegenerative processes (listed in Table 1). miRBase was also used to retrieve additional information about the selected miRNAs [65].

The newly developed miRWalk algorithm was used to predict miRNA binding sites within the complete set of annotated human genes, particularly those implicated in crucial biological pathways. Unlike other algorithms producing prediction of miRNAs binding sites within 3'UTRs of target genes, the "Validated Targets module" of miRWalk provides information on experimentally validated miRNA-gene interactions, dramatically reducing the rate of false positive sites. Such analysis was performed by pooling - for each disease - miRNAs retrieved by both miRWalk and mir2disease databases. The number of identified target genes is also indicated in Table 1. The PANTHER (Protein ANalysis THrough Evolutionary Relationships) tool was used to classify target gene lists according to their molecular function as well as their involvement in specific cellular pathways [66]. Notably, as shown in the bar graphs (Fig. 1), the most recurrent pathways for almost all examined NDs were inflammation, integrin, interleukin and Wnt signaling, angiogenesis and apoptosis pathways. These findings are of particular interest since chronic inflammatory state is a common feature in NDs (as discussed in detail in "Neurodegeneration and Inflammation"). Intersection among the lists of miRNAs' target genes for each disease was performed with a custom Matlab script in order to explore all possible combinations (schematically shown in Fig. 1). A significant overlap of genes among some NDs was disclosed, as indicated by the heat map in Fig. 1. In addition, by using the Genetic Association Database [67], an archive of human genetic association studies for complex diseases, we identified - as expected - that a significant fraction of common genes is associated with aging, neurological and psychiatric disorders (pie charts in Fig. 1).

Neurodegeneration and Inflammation

Neurodegenerative process is characterized by unremitting activation of the inflammatory response, which might trigger neuronal cell death [68•]. Enduring inflammation implies the persistence of the inflammatory *stimulus*, which may consist of environmental (exogenous) factors or endogenous molecules perceived by the immune system as "stranger" or "danger" signals. Therefore, it is reasonable that in each ND, misfolded proteins accumulated in cytoplasm or parenchyma of specific brain regions are recognized as "non-self" by the immune system [69••]. Thus, a chronic inflammatory state might be a direct consequence of the inflammatory response triggered by abnormal protein deposits, a common feature of NDs. Moreover, the age advancement is associated with increased levels of oxidative stress and reduced effectiveness of antioxidative defense mechanisms, in line with the late onset of such diseases [70]. Both evidences could explain the typical alterations in inflammatory *status* observed during neurodegenerative

Table 1 Deregulated miRNAs in neurodegenerative diseases

Disease	miR2Disease db	miRWalk db	N° target genes	Ref
AD	↑ miR125b, miR-128a, miR-146, miR-146a, miR-197, miR-320, miR-511, miR-9 ↓ let-7i, miR-101, miR-106b, miR-107, miR-124a, miR15a, miR-181c, miR-210, miR-22, miR-26b, miR-298, miR-29a, miR-29b-1, miR-328, miR-34a, miR-363, miR-9, miR-93	N/A miR-101, miR-146a, miR-146a*, miR-298, miR-29a, miR-29a*, miR-29b, miR-29b-1*, miR-29b-2*, miR-29c, miR-29c*, miR-328, miR-338-3p, miR-34a, miR-34a*	1025	[40–43] [88–91]
PD	↑ miR-433 ↓ miR-133b, miR-64, miR-65, miR-7	N/A miR-30a, miR-30a*, miR-30b, miR-30b*, miR-30c, miR-30c-1*, miR-30c-2*, miR-30d, miR-30d*, miR-30e, miR-30e*, miR-433	622	[37] [92–94]
HD	↑ miR-29a, miR-330 ↓ miR-128, miR-22, miR-132, miR-9, miR-9*		320	[99] [100] [47]
FTLD		N/A let-7b, let-7b*, miR-663	628	
FTD	↑ miR-659	N/A miR-107, miR-29b, miR-29b-1*, miR-29b-2*, miR-659	153	[96]
ALS		N/A let-7b, let-7b*, miR-206, miR-29a*, miR-29a, miR-29b, miR-338-3p, miR-663	804	
SCA	↑ miR-144, miR-144* ↓ miR-101, miR-130, miR-19	N/A miR-144, miR-144*	112	[97, 98]
DS	↑ let-7c, miR-125b-2, miR-155, miR-802, miR-99a	N/A let-7c, miR-125b-2, miR-155, miR-802, miR-99a let7c*, miR-125b, miR-125b-2*, miR-155*, miR-99a*	967	[45]
Prion	↑ miR-342-3p	N/A miR-342-3, miR-494	40	[95]

HD, Huntington's disease; PD, Parkinson's disease; AD, Alzheimer's disease; FTLD, Frontotemporal Lobar Degeneration; FTD, Frontotemporal dementia; SCA, spinocerebellar ataxia; DS, Down Syndrome; ALS, Amyotrophic Lateral Sclerosis; Prion, Prion diseases. ↑, ncRNAs upregulated and ↓, downregulated

processes. Indeed, a wide spectrum of studies has highlighted the involvement of activated microglia and reactive astrocytes in NDs [71]. Inflammatory response activation implies the synthesis of a wide range of proinflammatory mediators and the production of free radicals and oxygen/nitrogen reactive species (ROS and RNS), increasing the oxidative damage and determining a positive feedback [72]. In addition, dysfunctions of mitochondria, involved in the defense mechanisms against oxidative stress, occur in all major NDs [73]. Notably, mutations in *SOD1* gene - encoding for the superoxide dismutase enzyme responsible for destroying toxic superoxide radicals - is associated with ALS [74] and evidence of altered expression of the *SOD1* gene has been reported in DS for several cells/tissues [75, 76]. Definitively, the immune response to chronic inflammatory state might

play a pivotal role in synaptic dysfunction and selective neuronal loss, strongly contributing to the onset and progression of neurodegeneration.

Recent evidence shows that miRNAs have a determinant function in modulating immune response in NDs. For instance, miR-125b, miR-146a and miR-155 downregulate the Complement Factor H encoding gene (*CFH*) in AD e DS [77]. In addition, miR-146a overexpression in Prion diseases is involved in microglia activation [78], whereas miR-101 contributes to the regulation of the *APP* gene in response to the proinflammatory cytokine interleukin-1β (IL-1β) [79].

Such findings stimulate further research aimed at the comprehension of the connection among the three elements of the triad ncRNAs-inflammation-neurodegeneration.

Fig. 1 Graphical representation of target genes of miRNAs involved in all examined NDs. The matrix, shown on a blue background, was built considering “disease-specific” target genes and all combinations of those genes shared by two or more NDs. Color intensity of vertical blocks indicates the number of common genes, according to the heat map (on the left). NDs are indicated

in the rows and, on the right, the bar graphs show the most recurrent pathways in each disease. For the most numerous sets of shared target genes, pie charts (on the bottom) display the percent of common genes associated with aging, neurological and psychiatric disorders according to the Genetic Association Database

Inflammation-Related miRNAs and Genes: A Bridge to Neurodegeneration?

NDs display different histological phenotypes and clinical symptoms, although the production of abnormal proteins and their pathological accumulation into cellular aggregates are common features [12]. Such events, as above described, have been correlated with a chronic inflammatory state, and consequently to neuronal cell death [15]. The finding that a significant fraction of genes targeted by ND-related miRNAs belongs to inflammatory-related pathways (described in “miRNAs in Neurodegeneration”; Fig. 1) further suggests a direct, and perhaps strong, impact of inflammation in the etiology of NDs. Thus, to observe the extent of such overlap we first catalogued annotated human genes involved in inflammatory-related pathways (retrieved by PANTHER) and then identified the validated miRNAs that regulate them (mirWalk).

Interestingly, as shown in Table 2, the examined NDs - except DS - share a very significant fraction of “disease-associated” miRNAs with analyzed inflammatory-related genes, with, on average, 84 % of common miRNAs. These data further support the notion that an altered expression of

inflammation-related genes, possibly due to miRNA deregulation and thus a chronic activation of the inflammatory cascade, may significantly contribute to neurodegenerative

Table 2 Disease- vs. Inflammation- specific miRNAs

Disease	Validated miRNAs		
	Disease-specific	Common	Inflammation-specific
AD	5	29 (85,3 %)	351
PD	2	14 (87,5 %)	366
ALS	0	8 (100,0 %)	372
FTD	1	4 (80,0 %)	376
FTLD	0	3 (100,0 %)	377
SCA	2	3 (60,0 %)	377
HD	1	6 (85,7 %)	374
Prion	1	2 (66,7 %)	378
DS	8	2 (20,0 %)	378

AD, Alzheimer’s Disease; PD, Parkinson’s Disease; ALS, Amyotrophic Lateral Sclerosis; FTD, Frontotemporal Dementia; FTLT, Frontotemporal Lobar Degeneration; SCA, Spinocerebellar Ataxia; HD, Huntington’s disease; Prion, Prion Diseases; DS, Down Syndrome

processes, possibly triggering the onset (or determining the severity) of NDs, in line with previous findings [68•].

RNA-Seq: A Promising Tools for Transcriptome Profiling in NDs

NDs have been widely studied from a “DNA perspective.” The search for causative mutations in few disease-causing genes or for SNPs and *loci* associated with disease susceptibility have only partially explained NDs inheritance and the molecular causes of such diseases. In the last decade, gene expression studies have gradually been employed to investigate many human genetic disorders, including NDs [80••]. Transgenic animal models, *post-mortem* brains and patient-derived cell lines have been frequently used for gene expression analyses [81•].

Most of the gene expression studies performed so far, mainly on microarray platforms, have focused on PD, AD and HD, although conflicting results have been reported. Technological drawbacks [82], the variable quality/integrity of RNAs and the fragile nature of RNA samples from brains have been reported as the main causes of such discrepancies [81•].

Sequencing-based assays, such as Serial and Cap Analysis of Gene Expression have been successfully used in PD, AD and DS [83]. The introduction of NGS platforms for high-resolution analyses, and particularly of accurate methods for massive-scale transcriptomics (RNA-Seq), allows exploration of such NDs from a different perspective [84]. Indeed, RNA-Seq has been revealed to be a promising tool to study human diseases and to analyze transcriptomics’ changes, also in NDs’ patients [85]. Nonetheless, to date, only one group has employed RNA-Seq on AD patients’ brains [86••]. In particular, they observed significant differences in the expression, and promoter usage, for different *APOE* splice isoforms in normal vs. AD brains, indicating that alternative splicing plays a crucial role in the progression of neurodegeneration in AD patients.

Of note, another group has profiled human neurons derived from induced pluripotent stem cells proposing a model to study defective neurogenesis [87]. In addition, our group has recently analyzed by RNA-Seq the transcriptome of endothelial progenitor cells - impaired in DS - showing that coupling a protocol of rRNA depletion to RNA-Seq allows investigation of transcriptome in pathological conditions [61].

Conclusions

In recent years, the increased prevalence of NDs is having a dramatic impact on healthcare systems worldwide. Despite steps taken toward an understanding of the etiology behind neurodegenerative processes, there are still many molecular aspects to clarify. Studies aimed at identifying disease-causing

mutations in the Mendelian forms of NDs and large-scale GWAS have only partially explained their pathogenesis, showing a substantial lack of knowledge. Gene expression studies, particularly those investigating the role of ncRNAs, have started to highlight the contribution of these molecules to ND pathogenesis. Particularly, miRNAs and lncRNAs are showing relevant roles in neural cell plasticity and in neurodegenerative processes [39••].

Moreover, it has been recognized that despite having different histological phenotypes, distinction in brain areas affected, and varying clinical symptoms, there are distinctive common features among NDs. Pathological deposits of misfolded proteins are the first step toward formation of cell aggregates [14], and activation of inflammatory and oxidative-stress pathways, possibly leading to neuronal cell death, are common characteristics of these diseases [16].

Our analysis of miRNAs and their target genes retrieved interesting results, confirming that recurrent pathways for all examined NDs are inflammation-mediated by chemokines and cytokines, integrin and Wnt signaling, angiogenesis and apoptosis pathways. Moreover, a significant fraction of miRNA target genes common to more NDs was associated to aging, neurological and psychiatric disorders according to the Genetic Association Database.

Such observations further strengthen the evidence of an inflammatory-related component in NDs, confirming the relevant contribution of miRNA-dependent gene expression regulation in the etiology of such diseases.

However, functional studies are needed to validate such findings, and large-scale expression analyses of ncRNAs in these diseases will surely provide a significant contribution. Despite the fact that NGS application to NDs is still in its infancy, and few groups have currently employed RNA-Seq to profile the transcriptome of neuronal cells [87, 88], we expect it will rapidly reveal its great potential, generating a major improvement in the knowledge of the molecular etiology of many neurodegenerative disorders.

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