Neurodegenerative disorders and cancer are severe diseases threatening human health. The glaring differences between neurons and cancer cells mask the processes involved in their pathogenesis. Defects in cell cycle, DNA repair, and cell differentiation can determine unlimited proliferation in cancer, or conversely, compromise neuronal plasticity, leading to cell death and neurodegeneration. Alteration in regulatory networks affecting gene expression contribute to human diseases onset, including neurodegenerative disorders, and deregulation of non-coding RNAs – particularly microRNAs (miRNAs) – is supposed to have a significant impact. Recently, competitive endogenous RNAs (ceRNAs) – acting as sponges – have been identified in cancer, indicating a new and intricate regulatory network. Given that neurodegenerative disorders and cancer share altered genes and pathways, and considering the emerging role of miRNAs in neurogenesis, we hypothesize ceRNAs may be implicated in neurodegenerative diseases. Here we propose, and computationally predict, such regulatory mechanism may be shared between the diseases. It is predictable that similar regulation occurs in other complex diseases, and further investigation is needed.

Keywords: miRNA, pseudogenes, ceRNA, neurodegeneration, cancer

INTRODUCTION

Neurodegenerative diseases (NDs) are assuming a growing relevance in the pathological scenario that jeopardizes human health. Since degenerative processes are closely age-related, NDs incidence is stalking the increase of life expectancy in all industrialized countries. These common and complex disorders are mainly characterized by the selective and progressive death of one or more specific neuronal populations, and an elevated number of cases is represented by Alzheimer’s, Parkinson’s, and Huntington’s diseases (AD, PD, and HD, respectively). Although the increasing interest in exploring neurodegenerative phenomena and mechanisms has led to significant progresses, deciphering the molecular basis of NDs is far from complete. The identification of causative mutations in very rare monogenic Mendelian forms of NDs has provided only clues to interpret their pathological basis. Most of NDs forms rely on the combination of multiple genetic and environmental factors, and the onset and severity are influenced by their complex interactions (Eriksen-Taner, 2011). Thus, exclusively investigating risk factors and mutations in genes responsible of NDs monogenic forms may be reductive.

Regulatory multilayer networks affecting gene expression are emerging as relevant contributors in the etiology of human diseases, including NDs. Particularly, a growing number of studies are showing deregulation of different classes of non-coding RNAs (ncRNAs) – microRNAs (miRNAs), long intergenic (lincRNAs), and long non-coding RNAs (lncRNAs) – suggesting they may have a relevant impact on disease onset/progression (Esteller, 2011). Their involvement in a variety of biological processes related to neurogenesis and neurodegeneration – such as synaptic plasticity – has been demonstrated (Qureshi and Mehler, 2012).

Recently, and rather unexpectedly, NDs are displaying similarities at different levels with cancer. Epidemiological studies suggest an association between NDs’ incidence and a reduced (or increased) risk of specific cancers, although conflicting results have been reported (Pluim-Faveeuw et al., 2010). Cancer cells go through uncontrolled divisions and show unlimited proliferative potential, whereas neurons degeneration implies progressive loss of synaptic structure or function and substantial cell death. At first glance it might seem paradoxical that a plethora of molecules may be common to both diseases, even though dramatic changes in transcriptional and post-transcriptional regulation similarly occur in both cancer cells and degenerating neurons. Accordingly, miRNAs have been reported in both conditions as key regulators, exerting their inhibiting roles either on common genes involved in cancer and neurodegeneration, either on different genes belonging to common pathways. Moreover, the same pool of miRNAs is predicted to regulate up to hundreds of targets, altered expression of such transcripts – named competitive endogenous RNAs (ceRNAs) – may be implicated in NDs.
gene-dosage effect" disorders: AD could be caused by gene dosage (Kocerha, 2012; Geekiyanage et al., 2012). In the past few years, a growing number of reports have shown that precursor and mature miRNA expression modifies the abundance of mRNAs (Poliseno et al., 2010; Ta y et al., 2011) or repressing their translation (Bartel, 2009). Since each miRNA can target thousand of genes and, vice versa, each gene can be targeted by several miRNAs (Rajewsky and Socci, 2006; Rajewsky, 2006), such molecules are crucially implied in the fine-tuned regulation of gene expression. The proven involvement of miRNAs both in physiological and pathological processes has rapidly exposed the cell has been established in cancer, and their altered expression levels and epigenetic changes, are emerging as new contributors to neurodegenerative disorders. Indeed, AD and PD can be seen as "gene-dosage effect" disorders: AD could be caused by gene duplication of APP precursor protein (APP; Podlissy et al., 1987; Roevek-Lecriux et al., 2006), likewise a-synuclein locus duplication or triplication causes PD (Singleton et al., 2003; Chartier-Harlin et al., 2004). Thus, it is reasonable to speculate that altered levels of some crucial transcripts may have a dramatic impact on neurons functionality. Specific patterns of miRNAs expression in restricted areas have been documented in brain development and senescence (Miska et al., 2004; Kapsimali et al., 2007). In the past few years, a growing number of reports have shown that precursor and mature miRNA transcripts and miRNA processing machinery itself (Drosa and Dicer) are disrupted during ND progression (Hébert et al., 2009; Ghose et al., 2011; Schofield et al., 2011). In particular, gene expression analyses of sporadic PD (Kim et al., 2007) and AD (Lukow, 2007; Cogswell et al., 2008) revealed that miRNA deregulation is associated to neurodegeneration, and that some miRNAs repress APP expression (Long and Lahiri, 2011; Liu et al., 2012), although discordant results suggest that some experimental and technical concerns still exist (discussed in Costa et al., 2010, 2012).

Nonetheless, the hypothesis that miRNAs are involved in ND etiology is intriguing, and understanding how, and at what extent, they contribute to neurodegenerative processes remains a crucial endpoint.

miRNA THEORY

Competition among different classes of RNAs for a pool of miRNAs has been first suggested, then demonstrated, by both theoretical and experimental studies (Seitz, 2009; Poliseno et al., 2010; Karreth et al., 2011; Tay et al., 2011). Seitz (2009) proposed that many computationally identified miRNA target genes might represent some "non-legitimate targets," or low-affinity miRNAs "pseudotargets." Therefore, such miRNAs would act as competitive inhibitors of miRNAs, by preventing their binding to legitimate targets.

In the wake of such hypothesis, the "competing endogenous RNAs" theory (Salmena et al., 2011) has proposed the existence of legitimate bona fide miRNA competitors, such as demonstrated for the gene/pseudogene pairs PTEN/PTENP1 and ERAS/ERASIP (Karreth et al., 2011; Tay et al., 2011). miRNAs can talk each other through their 3′ UTRs, and the "indirect interactions" can regulate their expression levels. Such transcribed – but untranslated – regions contain MREs which can regulate in cis the transcript levels itself and in trans can alter the levels of different pools of miRNAs, consequently affecting the levels of other miRNAs. Such theory, experimentally confirmed in a mouse model of melanoma (Karreth et al., 2011; Tay et al., 2011), proposes that virtually all types of RNA can communicate each other through a new fascinating "biological alphabet," in which MREs are the "letters" whose different combinations may form an entire universe of "words" (Licatalosi et al., 2008; Chi et al., 2009).

PSEUDOGENES IN NEURODEGENERATIVE DISEASES

The contribution of ceRNAs to the availability of miRNAs in the cell has been established in cancer, and their altered expression modulates the abundance of miRNAs (Poliseno et al., 2010; Tay et al., 2011). Thus, understanding the contribution of ceRNAs on gene expression deregulation is particularly relevant not only in different tumors but also in other human complex diseases. In particular, since recent evidences show NDs share common altered genes, pathological mechanisms, and cellular processes with cancer, we decided to address whether ceRNAs may contribute also to NDs pathogenesis.

Therefore, we first identified the subset of genes differentially expressed in AD, PD, and HD, retrieving datasets from Gene Expression Atlas database2 (accession n. E-MTAB-62,

1www.miR23a/b.org

2http://www.ebi.ac.uk/ena/
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FIGURE 1 | (A) Venn diagram showing intersections of DE genes in all three NDs. Colored circles contain the number of genes per disease. (B) Bar graph showing the distribution of all the miRNAs binding sites (y-axis) identified in IncRNA DE in HD (x-axis) which bind the same miRNA. Matrices built with E-GEOD-3790, E-GEOD-1751, E-AFMX-6, E-GEOD-7621, E-GEOD-7307, E-GEOD-20295, E-GEOD-20168, E-MEXP-2280, E-GEOD-6613). Particularly, only genes with a statistical significance of differential expression inferred from at least two independent experiments were used. As shown in Figure 1A, these datasets consisted of 17,1082, and 5361 genes, for AD, PD, and HD, respectively. Interestingly, by using a bootstrap resampling procedure (10^5 iterations), a significant overlap (563 genes; p < 0.01) was disclosed between genes DE in PD and HD (Figure 1A), showing they may represent crucial genes in the etiology of neurodegeneration. Moreover, in line with the notion that common genes with proven involvement in cancer and in NDs are deregulated in both conditions (Morris et al., 2010; Plun-Favreau et al., 2010; Du and Pertsemlidis, 2011), pathway analysis (performed using PANTHER; Thomas et al., 2003) revealed a significant overlap with cancer hallmarks, including apoptosis, p53, Ras, PDGF, FGF, EGF, and MAPK signaling pathways (data not shown).

Since pseudogenes have been shown to act as miRNAs sponges in cancer we evaluated such finding also in NDs. Thus, we intersected the above-described datasets of DE genes in NDs with a full list of human pseudogenes retrieved from HUGO Gene Nomenclature Committee (HGNC) database. The intersection revealed that 49, 1, and 10 pseudogenes are DE in HD, AD, and PD, respectively. Thus, 3’ UTR sequences of pseudogenes and their parent genes were downloaded from University of California Santa Cruz (UCSC) and aligned by using BLAT algorithm to assess sequence identity. Only pseudogenes’ sequences showing high homology (95–99%) were used as described below. The 3’ UTRs of some parent genes aligned outside the boundaries of their annotated cognate pseudogenes, indicated the need to revise annotations. In such cases, we used for further computational analysis the matching genomic sequences. Therefore, FASTA sequences of selected pseudogenes and the 3’ UTRs of parent genes were independently scanned for the presence of miRNA binding sites using a TargetScan perl script (Lewis et al., 2005). Pseudogenes with only one miRNA binding site were excluded from further analyses. Analyzed pseudogene/gene pairs – in each ND – are listed in Table 1.
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Table 1 | Pseudogenes deregulated in HD, PD and AD sharing common miRNA binding sites with their parent genes.

| Pseudogene/gene pair | miRNA family |
|----------------------|-------------|
| Huntington's disease | Pseudogene | Gene | n | common (%) |
| BCRP2/BCR* | 41 | 71 | 38 (92.7) |
| BZW1P2/BZW1* | 4 | 5 | 2 (50.0) |
| CES1P1/CESE1 | 6 | 6 | (100.0) |
| CHCHD2P2/CHCHD2 | 4 | 5 | 2 (50.0) |
| CKX7A2P2/CKX7A2* | 3 | 5 | 2 (86.7) |
| DKG2P2/DKG2 | 14 | 16 | 10 (66.7) |
| EEF1A1P5/EEF1A1* | 9 | 64 | 5 (88.9) |
| EIF2S2P4/EIF2S2 | 6 | 33 | 6 (100.0) |
| ETF1P1/ETF1 | 24 | 59 | 19 (79.2) |
| FABP5P1/FABP5 | 3 | 2 | 1 (66.7) |
| FAM108A3P/FAM108A1 | 6 | 7 | 6 (100.0) |
| FAM115B/FAM115A* | 76 | 76 | 76 (100.0) |
| GBP1P1/GBP1* | 39 | 33 | 24 (61.5) |
| HIGD1AP14/HIGD1A* | 19 | 56 | 14 (73.7) |
| HMGB1P1/HMGA1* | 42 | 36 | 29 (80.6) |
| HMGB1P10/HMGB1 | 15 | 48 | 13 (69.4) |
| HMGB1P5/HMGB1 | 14 | 48 | 13 (92.9) |
| HMGN1P36/HMGN1 | 1 | 1 | 1 (100.0) |
| HMGN2P3/HMGN2 | 12 | 24 | 12 (100.0) |
| HNRNPA3P1/HNRNPA3 | 53 | 118 | 5 (9.4) |
| HSD17B7P2/HSD17B7 | 7 | 9 | 6 (85.7) |
| HIP3/HER7 | 35 | 43 | 17 (48.6) |
| MYJ1PMT1/MT1* | 2 | 4 | 2 (100.0) |
| MYJ2PMT1/MT1* | 2 | 5 | 2 (100.0) |
| NAD3P/NAD3 | 18 | 17 | 17 (94.4) |
| NUSNP1/NSUN5 | 15 | 39 | 12 (80.0) |
| PAAK2/PAAK2 | 7 | 7 | 5 (71.4) |
| PIK1/PIK1 | 7 | 9 | 6 (85.7) |
| PP5P2/PP5P2 | 58 | 55 | 38 (68.5) |
| PTPN1/PTPN1 | 23 | 91 | 21 (91.9) |
| RBBP4/RBBP4 | 13 | 147 | 11 (78.4) |
| RBMS1P1/RBMS1* | 15 | 52 | 15 (100.0) |
| RHQ2/RHQ2 | 52 | 93 | 48 (93.3) |
| RPP7P9 | 16 | 19 | 15 (93.8) |
| RPL16NP/RPL16P0 | 2 | 2 | 1 (50.0) |
| S100A11P1/S100A11 | 6 | 5 | 3 (60.0) |
| SKP1P1/SKP1* | 25 | 35 | 18 (72.0) |
| TAC3/TAC3 | 30 | 64 | 19 (59.4) |
| VDAC1/VDAC1* | 21 | 26 | 20 (92.3) |
| VEZ2P1/VEZ2* | 64 | 85 | 53 (62.4) |
| YWHAZP3/YWHAZ2 | 11 | 54 | 8 (72.7) |
| ZFAND6P1/ZFAND6 | 15 | 16 | 14 (93.3) |

(Continued)
In cancer, recent evidences show that untranslabeled pseudogenes, and presumably lncRNAs, compete for a pool of miRNAs acting as endogenous sponges and regulating parent genes and other miRNAs (Poliseno et al., 2010; Karreth et al., 2011; Salmena et al., 2011; Tay et al., 2011). Such findings are likely to have broader implications for other diseases and cellular processes, largely beyond the regulation of few genes in cancer.

Therefore, given that NDs and cancer share common causative genes and altered signaling molecular pathways, even considering the crucial role of miRNAs in neurogenesis- and cancerogenesis-related processes, we have proposed and computationally predicted both pseudogenes and lncRNAs may be involved in the etiology of AD, HD, and PD, acting as ceRNAs.

In such NDs, independent analysis of DE lncRNAs, pseudogenes, and parent genes, revealed they contain a huge number of shared MREs, potentially representing miRNAs sponges. It suggests that ceRNAs may represent the rule, rather than the exception, also in the etiology of NDs. Our observations indicate that a ceRNA-based regulatory mechanism might be shared between neurodegenerative and cancerous processes, and we cannot exclude that similar complex regulatory networks may also underlie other human complex diseases. However, studying pseudogenes is challenging due to the high sequence identity with their parent genes, and genome-wide expression studies may report conflicting results about pseudogenes expression. The introduction of NGS, particularly of RNA sequencing, is substantially contributing to overcome some technological challenges for the transcriptome analysis (Cloonan et al., 2008; Mortazavi et al., 2008; Costa et al., 2010, 2011) also for studying expressed pseudogenes. We believe this technology will increasingly help researchers to encrypt the novel ceRNAs code, giving an incredible boost to understand this new “language.”

Finally, targeted functional studies are clearly needed to validate and confirm this predictive study, even though we believe that ceRNAs have traced a novel revolutionary route in the landscape of human genetics.

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