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Neurobiology of Disease

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Neurobiology of Disease 29 (2008) 438–445

A microRNA-based gene dysregulation pathway in Huntington's disease

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Received 18 May 2007; revised 7 October 2007; accepted 5 November 2007
Available online 13 November 2007

Huntington's disease (HD) is a dominantly-inherited neurodegenerative disorder which is incurable and ultimately fatal. HD is characterised by widespread mRNA dysregulation, particularly in neurons of the forebrain, by mechanisms which are not fully understood. Such dysregulation has been demonstrated to result, in part, from aberrant nuclear localisation of the transcriptional repressor, REST. Here, we show that expression of a number of neuronal-specific microRNAs is also dysregulated in HD tissues, probably as a result of increased repression by REST. This phenomenon is observed in both murine models of HD and in the brains of human HD sufferers. MicroRNA loss is reflected in increased levels of a number of target messenger RNAs. These data are the first to demonstrate a role for microRNAs in HD, and indicate that the molecular aetiology of HD is reflected in a loss of neuronal identity, caused in part by dysregulation of both transcriptional and post-transcriptional mechanisms.

Keywords: miRNA; REST; Huntington's disease; Neurodegeneration

Introduction

Huntington's disease (HD) is a fatal, incurable neurodegenerative disease caused by a CAG expansion in the gene encoding the protein huntingtin (Htt). The disease manifests in cognitive defects, motor control impairment, and ultimately death, symptoms that result from a neuronal dysfunction characterised by progressive loss of cortical and striatal neurons. This neuronal death appears to be due to a combination of the toxicity of the mutant huntingtin and loss of the neuroprotective effects of the wild type protein (Cattaneo et al., 2005).

However many aspects of HD pathology remain unexplained. In particular, a number of studies have observed widespread differences in mRNA levels between brains of HD sufferers and normal adults with one study reporting at least ~100 mRNAs significantly upregulated, and slightly fewer significantly downregulated (Hodges et al., 2006). The degree of this difference varies with disease severity, both in terms of the disease progression and in the brain region examined. These findings are recapitulated in similar experiments on mouse models (Crocker et al., 2006; Luthi-Carter et al., 2002; Sipione et al., 2002). Htt interacts with several transcriptional regulators including CBP (Steffan et al., 2000; Nucifora et al., 2001), p53 (Steffan et al., 2000; Bae et al., 2005) Sp1 (Dunah et al., 2002), TAFII130 (Dunah et al., 2002) and TBP (Huang et al., 1998) and this interaction is frequently disrupted in the presence of mutant Htt (Rubinsztein, 2003). In previous studies, we have shown that Htt interacts with the essential transcriptional repressor, REST (Repressor Element 1 Silencing Transcription Factor, also known as NRSF, Neuron-Restrictive Silencing Factor) in neurons (Zuccato et al., 2003; Ooi and Wood, 2007). In normal individuals, wild-type huntingtin sequesters REST in the cytoplasm of neurons; in the case of HD, the polyglutamine expansion of mutant huntingtin inhibits this interaction, allowing aberrantly high levels of REST to accumulate in the nucleus of HD neurons and leading to increased transcriptional repression of *BDNF*, a REST target gene (Zuccato et al., 2003). Reduced levels of BDNF consequently lead to reduced survival of striatal neurons.

We have shown that REST can potentially interact with more than 1300 sites in the human and murine genomes and many putative target genes encode proteins regulating neuronal function, survival and differentiation (Bruce et al., 2004; Johnson et al., 2006). Furthermore, we have shown that REST occupancy of

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Available online on ScienceDirect (www.sciencedirect.com).

0969-9961/$ - see front matter © 2007 Elsevier Inc. All rights reserved.
doi:10.1016/j.nbd.2007.11.001

multiple target genes is increased in the presence of mutant Htt and in the absence of wild type Htt and this widespread disruption of the REST regulon may contribute to HD pathology (Zuccato et al., 2007).

Recently, a large class of non-coding regulatory RNAs, microRNAs (miRNAs), have been described in multiple metazoan species including human and mouse (Bartel, 2003). miRNAs play important roles in organogenesis, metabolism and neuronal development through degradation and translational repression of mRNAs. Many miRNAs are selectively expressed in the neurons and glia of the brain (for review, see (Kosik, 2006)) and appear to regulate fundamental neuronal processes, including elaboration of the neuronal transcriptome (Lim et al., 2005) and dendrite growth (Vo et al., 2005). Recently, it was shown that REST can regulate the expression of a number of neuronal miRNAs (Conaco et al., 2006), suggesting that miRNA expression in both development and disease, may be controlled by similar mechanisms as protein-coding genes.

The profound importance of miRNAs in gene regulation, and the large numbers of genes they probably regulate (predicted to be >30% of all mRNAs) have led many to speculate on the potential role of miRNAs in human disease. Consistent with this, miRNAs have highly specific expression patterns in certain cancers (Esquela-Kerscher and Slack, 2006). Nevertheless, the role of miRNAs in disease, including neurodegenerative disorders is only now coming to light. Bilen et al. have shown that the miRNA processing pathway has a broadly protective effect against the toxicity of the Ataxin-3 polyglutamine-expansion mutant (Bilen et al., 2006). This effect was observed in both flies and human cell lines, suggesting that one or more microRNAs have conserved, neuroprotective functions. The authors identified at least one of these to be the miRNA, *ban*. More recently, a regulatory network containing the miRNA mir-133b and the transcription factor Pitx3 was shown to control the development and identity of midbrain dopaminergic neurons (Kim et al., 2007). Importantly, the same study found that mir-133b is amongst a small set of miRNAs which display significantly reduced expression in the brains of Parkinson's Disease sufferers. These studies suggest that loss of expression of certain miRNAs may be a general feature in the pathogenesis of both polyglutamine and non-polyglutamine neurodegenerative diseases.

In the present study, we show that REST can regulate a number of brain-specific miRNAs in vivo and further, we show that a number of these miRNAs have significantly altered expression levels in HD. Finally, we demonstrate that the reduction in expression of two miRNAs, mir-124a and mir-132, leads to increases in the levels of their target mRNAs. This study is the first indication of the potential involvement of miRNAs in HD, and suggests that REST may play an important role in this process.

Results

Identification of miRNA targets of REST

We hypothesised that REST might regulate the expression of brain-restricted miRNA genes. Therefore, the position data from our previous search of the human genome for binding sites of REST (known as Repressor Element-1 sites, or RE1s) (Johnson et al., 2006) was compared to the positions of known miRNA genes (Griffiths-Jones, 2004), with the search criteria that either the miRNA gene resides within 100 kb of an RE1, or that the miRNA resides in the intron of a gene within 100 kb of an RE1. This analysis uncovered 17 likely miRNA targets of REST, 13 of which have an orthologous miRNA-RE1 pair in mouse (see Table 1). Most have a neuron-specific or brain-specific expression pattern, while mir-1d and mir-133a expression is restricted to heart and skeletal muscle, patterns consistent with REST's known role of regulating gene expression in neural and cardiovascular tissue (Palm et al., 1998; Cheong et al., 2005; Bingham et al., 2007). Some miRNAs are encoded by more

Table 1
Identification of REST–target miRNAs in the human genome

miRNA	Expression	Host gene	RE1 ID	RE1 PSSM score (Johnson et al., 2006)	RE1 distance (kb)	Conserved in mouse?
1-d	Heart/muscle (C)	*C20orf166*	hum42172	0.9398	35	Yes
133a-2	Heart/muscle (C)	*C20orf166*	hum42172	0.9398	35	Yes
9-1	Brain (S)	*CROC4*	hum2610	0.9628	5	Yes
9-3	Brain (S)		hum33584	0.9426	3	Yes
29a	Brain (S)		hum18483	0.9364	20	Yes
29b-1	Brain (S)		hum18483	0.9364	20	Yes
124a-1	Brain (S)		hum19259	0.9237	20	IG
124a-2	Brain (S)		hum19995	0.981	0.8	No
124a-3	Brain (S)		hum42331	0.9583	0.5	Yes
132	Brain (S)		hum35996	0.9574	0.2	Yes
135b	Brain (S)		hum3303	0.9721	10	Yes
139	Brain (S)	*PDE2A*	hum27012	0.9507	2	Yes
203			hum32236	0.9506	15	No
204		*Q9HOX2*	hum21795	0.913	600	Yes
212			hum35996	0.9574	200	Yes
330	Brain (S)	*EML2*	hum40445	0.9134	40	IG
346	Brain (S)	*GRID1*	hum24546	0.9337	40	Yes

A bioinformatic search for RE1 sites in the human genome identified a number of miRNA genes as likely targets of REST (Johnson et al., 2006). The RE1 ID can be used to access relevant data from the RE1 Database [http://www.bioinformatics.leeds.ac.uk/RE1db_mkII/]. RE1 Position-Specific Scoring Matrix (PSSM) score reflects the similarity of the sequence to the RE1 motif, where a score >0.91 indicates that the sequence is likely to be a REST binding site. RE1 distance refers to the genomic distance between the RE1 and corresponding miRNA gene in human. Instances where the orthologous miRNA gene in mouse is also proximal to an RE1, are defined to be conserved targets in mouse. IG: intervening gene, i.e. in mouse another annotated gene lies between the orthologous miRNA/RE1 pair. C: Expression data from Chen et al. (2006); S: Expression data from Sempere et al. (2004).