Mini-review

Non-coding RNAs in polyglutamine disorders: friend or foe?

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Polyglutamine (polyQ) disorders constitute a family of devastating, dominantly inherited neurodegenerative conditions caused due to dynamic mutation involving the expansion of CAG triplet repeats in the coding region. A common feature of polyQ disorders is that they become clinically evident only late in life – sometimes in the fourth decade or later. Subsequent to the onset, the symptoms of the disorder may worsen over the next 10–20 years as the affected neurons degenerate and finally die (Zoghbi and Orr 2000; Siwach and Ganesh 2008). Till date, ten such neurodegenerative disorders known to be caused by expansion of the CAG repeat in the coding region of the respective genes have been identified (Siwach and Ganesh 2008). PolyQ disorders include Huntington disease (HD), six distinct forms of spinocerebellar ataxia (SCA-1, 2, 3, 6, 7 and 17), dentatorubropallidoluysian atrophy (DRPLA) and spinobulbar muscular atrophy (SBMA) (reviewed in Bates 2005; Gatchel and Zoghbi 2005). Barring SBMA, which is X-linked, all other polyQ disorders are autosomal dominant in inheritance (*see* Gatchel and Zoghbi 2005; Siwach and Ganesh 2008). A pathological hallmark of polyQ disorders is the presence of intracellular protein aggregates in the neurons of affected patients, in cell culture models and in mouse and fly models (DiFiglia *et al* 1997; Davies *et al* 1997; Warrick *et al* 1998; Kim *et al* 2002). In the affected neuron the protein aggregates, also called as polyQ inclusion bodies, are found in the cytoplasm, perinuclear space and nucleus (*see* figure 1). A direct correlation between inclusion bodies in affected tissue and their toxicity, however, remains unresolved. PolyQ inclusion bodies contain chaperone, ubiquitin and components of proteasome, indicating that such protein aggregates trigger a stress response in the cell. The expanded polyQ tract is thought to prevent the protein from folding into its native conformation but instead favours the formation hydrophobic aggregates (reviewed in Muchowski and Wacker 2005; Siwach and Ganesh 2008). Thus, an increase in the cellular load of such non-native misfolded proteins could saturate the chaperone and ubiquitin–proteasome systems (UPS) and, over time, lead to neuronal dysfunction (McClellan *et al* 2005; Bukau *et al* 2006). In addition to aggregate-induced toxicity, polyQ proteins are also known to bind to various transcription factors such as cAMP response element-binding protein, TATA box-binding protein and associated

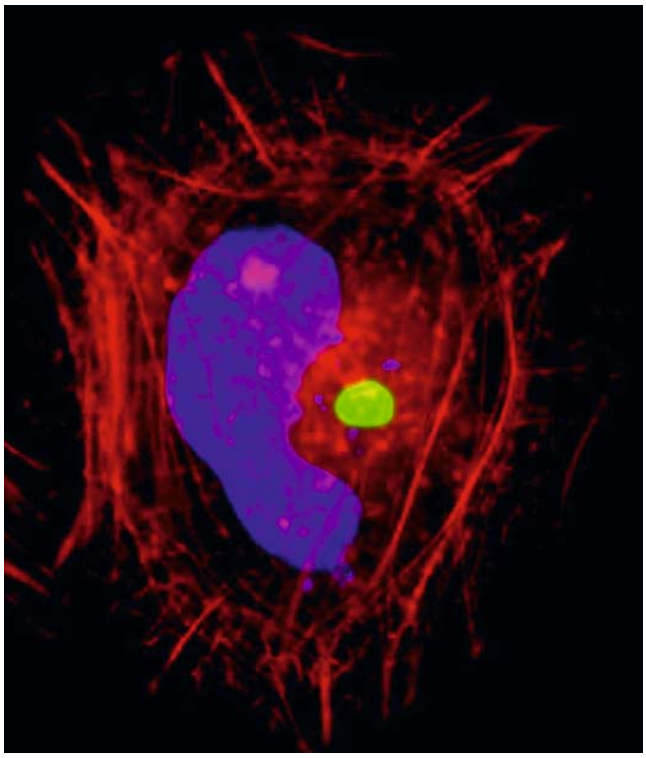

Figure 1. HeLa cell expressing expanded huntingtin protein with 97 glutamine repeats, co-stained for actin filaments (red) and nucleus (blue). The image reveals the aggregation of mutant huntingtin (green) in the perinuclear region of the cell.

Keywords. hsrω miRNA; non-coding RNA; poly-Q

factor, SP1 transcription factor and p53, suggesting that such abnormal interactions could alter transcriptional regulation in the affected neurons (Cha 2000; Nucifora *et al* 2001; Steffan *et al* 2001). These defects, either independently or collectively, are known to activate caspases that promote cell death through the apoptotic and/or non-apoptotic pathways (Tobin and Singer 2000; Ross *et al* 2003; Bossy-Wetzel *et al* 2004). In order to identify and develop possible routes for the application of therapeutic strategies, scientists have discovered several modifiers of the polyQ pathology. These modifiers, primarily identified using model systems, include heat shock proteins, components of the UPS pathway and autophagy, transcription factors, detoxifying enzymes, several RNA-binding proteins and RNA species, among other examples (Celloto and Palladino 2005). Among these groups of modifiers, the promising therapeutic targets are molecular chaperones and components of the UPS, as activation of cellular pathways that target misfolded proteins may be beneficial to the neuron. Indeed, pharmacological induction of molecular chaperones has been shown to rescue cellular toxicity and aggregation of polyglutamine proteins in animal models (Hay *et al* 2004; Katsuno *et al* 2005). Similarly, administration of mTOR, a stimulator of autophagy, was found to be beneficial in cellular and animal models of polyQ disease (Ravikumar *et al* 2004). As mentioned above, transcriptional dysregulation is also implicated in polyQ disorders. For example, polyQ aggregates interact with several transcription factors and disrupt their normal functions. Studies have demonstrated that the histone acetyl transferase activity of various transcription factors and histone H3 are diminished in animal models of polyQ disorders, suggesting that global gene repression is an underlying mechanism for neurodegeneration in polyQ expansion disorders (Ying *et al* 2006; Steffan *et al* 2001). Indeed, the use of inhibitors of histone deacetylases slowed down progression of the HD phenotype in animal models and is currently undergoing phase I clinical trials in humans (Hockly *et al* 2003; Steffan *et al* 2001). A unique group of modifiers currently under investigation are the non-coding transcripts that were shown to modulate polyQ toxicity in human cell lines and in fly models. These include the hsr*ω*−n RNA in *Drosophila* (Fernandez-Funez *et al* 2000; Sengupta and Lakhotia 2006) and the miRNA species in both humans and flies (Bilen *et al* 2006). This commentary attempts to provide an update on the rather unexpected link between non-coding transcripts and polyQ pathology.

In a genetic screen using an SCA-1 fly model, Fernandez-Funez *et al* (2000) identified the non-coding *hsrω* gene as one of the modifiers of polyQ pathology. Intriguingly, overexpression of hsr*ω*–n RNA was found to enhance polyQ protein-induced toxicity in fly models expressing the expanded polyQ (127Q) or the mutant huntingtin protein (htt exon1 with 93Q) (Sengupta and Lakhotia 2006). The *hsrω* gene in *Drosophila* is developmentally regulated and is also induced by a variety of stressors. The hsr*ω* transcripts are of different sizes and none of them code for any protein (Lakhotia 2003). Among these, the large, nuclear-limited transcript (hsr*ω*–n) is localized as small speckles in the nucleoplasm in close proximity to the perichromatin fibrils (Prasanth *et al* 2000). Cellular stressors such as heat shock lead to the reorganization of nucleoplasmic omega speckles into larger aggregates inside the nucleus (Prasanth *et al* 2000). Such clustering of omega speckles under stress could ensure tight binding of heterogeneous nuclear ribonucleoproteins (hnRNPs) and several RNA-binding proteins to the hsr*ω*-nuclear transcripts. It is a well known fact that heat shock leads to reduced transcription and RNA processing, possibly by disengaging the hnRNPs and other critical players from their site of action. Under these conditions, the nucleoplasmic speckles of the hsr*ω*–n transcripts are thought to serve as a dynamic sink for hnRNPs and protect them from thermal denaturation (Prasanth *et al* 2000; Lakhotia 2003). Several hnRNPs bind to the hsr*ω*−n transcripts. Intriguingly, a null allele for one of the hnRNP proteins, HRB87F, also enhances polyQ-induced neurodegeneration (Sengupta and Lakhotia 2006). Because hnRNPs are involved in mRNA metabolism, disruption of cellular mRNA processing by the mutant alleles of *hsrω* or *Hrb87F* appears to enhance the toxic effects of expanded polyQ proteins (Sengupta and Lakhotia 2006).

A second example for a functional link between non-coding RNAs and polyQ pathology is micro RNAs (miRNA) (Bilen *et al* 2006). The miRNAs represent an evolutionary, well-conserved class of small RNA species, usually 21–25 nucleotides in length, which are known to modulate the translational efficiency of mRNAs to impart several tissue-specific functions (Lau *et al* 2001; Lagos-Quintana *et al* 2001). For example, in *Drosophila,* the miRNAs ban and mir-14 are involved in cell growth, proliferation and cell death (Brennecke *et al* 2003; Xu *et al* 2003). miRNAs are excised by the dicer RNase complex (a ribonuclease complex that cleaves the double-stranded RNA) from longer precursor RNAs that form imperfect hairpin structures. Therefore, dicer activity is required for the maturation of miRNAs. *Drosophila* has two dicer genes – *dcr1* and *dcr2*. The *dcr1* gene is involved in the maturation of miRNAs whereas *dcr2* modulates the formation of small interfering RNAs (siRNAs) (Lee *et al* 2004). Similar to the *hsrω* transcripts, miRNAs were shown to play modulatory roles in polyQ pathogenesis as reduction in miRNA levels correlated with enhanced toxicity to ataxin-3 proteins with expanded polyQ repeats in a fly model of SCA-3 (Bilen *et al* 2006). Intriguingly, flies that are mutant for *dcr1* and express ataxin-3 had aggravated neurotoxicity as compared with flies that express ataxin-3 but are wild-type for the *dcr1* gene.

A mutation in *R3D1*, a gene critical for miRNA maturation, also enhanced ataxin-3 induced toxicity (Jiang *et al* 2005; Bilen *et al* 2006). The suppression of miRNA processing also enhanced toxicity in human cell lines expressing the polyQ protein (Bilen *et al* 2006). Taken together, these findings highlight the importance of miRNA in neuronal functions, and that defects in this process might enhance the toxicity caused by polyQ proteins. Interestingly, in the fly model, suppression of one of the miRNA, ban, alone enhanced the polyQ-associated toxicity, suggesting that the *dcr1*-mediated effect of polyQ pathology could be limited to a few miRNAs and ban is one among them (Bilen *et al* 2006). Corroborating this view, overexpression of ban rescued the polyQ-mediated toxicity in flies. Ban was also able to reduce the toxicity of abnormal tau – a protein associated with Alzheimer disease in humans, suggesting that miRNAs are also involved in protein quality-control processes (Bilen *et al* 2006). Intriguingly, the suppression of toxicity by ban miRNA was neither associated with a reduction in the levels of polyQ protein nor affected the aggregation properties of polyQ protein. Moreover, ban does not seem to act through the stress response pathway because the level of Hsp70, a chaperone, remained unaltered in the ban mutants. Thus, the ban miRNA appears to modulate polyQ pathogenicity downstream of its aggregation (Bilen *et al* 2006). The miRNA ban is coded by a gene named *bantam* and is known to negatively regulate a proapoptic gene, *Hid,* in *Drosophila* (Brennecke *et al* 2003). Thus, ban upregulation suppresses *Hid*-mediated cell-death (Brennecke *et al* 2003). It is therefore expected that, in the SCA-3 fly model, ban rescues polyQ toxicity through suppression of Hid. However, Hid expression was found to be at its normal level in the SCA-3 flies overexpressing ban, suggesting that ban-mediated rescue could utilize an unknown pathway (Bilen *et al* 2006). Therefore, targeted overexpression of miRNA appears to be an attractive therapeutic option for the treatment of polyQ and other neurodegenerative disorders. In this regard, it would be important to check whether the specific response to overexpression of miRNAs would be restricted to the cell or cell type, or whether a systemic response would be evoked. The latter concern is important while considering miRNAs as therapeutic targets because our understanding of the controls of miRNA expression and their cellular functions are rather limited.

Until recently, the non-coding RNA fraction was considered to be "junk material" with the exception of infrastructural RNAs such as rRNA, tRNA, snRNA and snoRNAs, which are involved in protein synthesis, transport and splicing (Lakhotia 1996; Mattick and Makunin 2005). With emerging knowledge on the role of non-coding RNAs in human diseases such as cancers and neurodegenerative disorders, the next few years should witness a significant improvement in our understanding of the biology of non-coding RNAs and in the development of technologies for therapeutic modulation of fatal disorders.

Acknowledgements

Financial support from the Department of Biotechnology, New Delhi, in the form of a postdoctoral fellowship to SS, is gratefully acknowledged.

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MS received 20 October 2007; accepted 4 April 2008

*e*Publication: 22 April 2008

Corresponding editor: VIDITA A VAIDYA