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MicroRNA Pathways Modulate Polyglutamine-Induced Neurodegeneration

Short Article

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Summary

Nine human neurodegenerative diseases are due to expansion of a CAG repeat- encoding glutamine within the open reading frame of the respective genes. Polyglutamine (polyQ) expansion confers dominant toxicity, resulting in neuronal degeneration. MicroRNAs (miRNAs) have been shown to modulate programmed cell death during development. To address whether miRNA pathways play a role in neurodegeneration, we tested whether genes critical for miRNA processing modulated toxicity induced by the spinocerebellar ataxia type 3 (SCA3) protein. These studies revealed a striking enhancement of polyQ toxicity upon reduction of miRNA processing in *Drosophila* and human cells. In parallel genetic screens, we identified the miRNA *bantam* (*ban*) as a potent modulator of both polyQ and tau toxicity in flies. Our studies suggest that *ban* functions downstream of toxicity of the SCA3 protein, to prevent degeneration. These findings indicate that miRNA pathways dramatically modulate polyQ- and tau-induced neurodegeneration, providing the foundation for new insight into therapeutics.

Introduction

Human polyQ diseases are due to the expansion of a CAG repeat within the open reading frame of the respective genes (Zoghbi and Orr, 2000). The polyQ expansion confers dominant toxicity, resulting in progressive neuronal dysfunction and loss. In such diseases, the pathogenic protein accumulates, typically in nuclear inclusions. Although it is unclear how these accumulations contribute to disease pathogenesis, several lines of evidence support the idea that a conformational change occurs in the disease protein associated with reduced solubility, enhanced oligomerization, and toxicity (Muchowski and Wacker, 2005). Studies with animal models and patient tissue indicate that the protein accumulations contain chaperones and components of the ubiquitin-proteasome system, suggesting that the toxic, misfolded protein triggers a stress response to lower the amount of disease protein. A number of suppressors of neurodegeneration in animal models have coupled reduced protein accumulation, or modulation of ability to handle misfolded protein, to mitigation of degeneration (Bilen and Bonini, 2005).

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Although modifiers that implicate protein misfolding pathways have been defined, cellular pathways that link polyQ toxicity and degeneration of the cell are less clear. Morphological analysis of degenerating neurons reveals that they appear to undergo condensation of the nucleus and cytoplasm; however, other hallmarks of programmed cell death, such as apoptotic bodies and fragmentation of nuclear DNA are not observed (Jackson et al., 1998; Turmaine et al., 2000). Studies in *Drosophila* have shown that the baculoviral protein P35, which potently inhibits caspase activity, is largely ineffective at mitigating polyQ-induced degeneration (Jackson et al., 1998; Warrick et al., 1998). Autophagy, which couples reduced protein accumulation with cell survival, is thought to be involved, as are dApaf-1/Dark activities for the Huntington's disease protein (Ravikumar et al., 2004; Sang et al., 2005).

miRNA pathways have been implicated in developmental apoptosis, normal developmental processes, and cancer (Ambros, 2004; Bartel, 2004; Caldas and Brenton, 2005). In *Drosophila*, the miRNAs *ban, mir-14*, and select other miRNAs have been shown to modulate programmed cell-death genes (Brennecke et al., 2003; Leaman et al., 2005; Xu et al., 2003). SCA3 is one of the polyQ diseases and is among the most common dominantly inherited ataxias (Zoghbi and Orr, 2000). To address a possible role for miRNAs in neuronal maintenance, we tested whether compromising miRNA processing modulated polyQ pathogenesis. Our findings extend the processes modulated by miRNAs to human neurodegenerative diseases.

Results

Reduced miRNA Processing Dramatically Enhances Ataxin-3- and tau-Induced Neurodegeneration in *Drosophila*

We tested whether miRNAs modulated polyQ pathogenesis in *Drosophila* by downregulating miRNA processing with the *dicer* mutation and then determining the effect on Ataxin-3-induced neurodegeneration. Dicer activity is essential for miRNA processing in flies and vertebrates (Bernstein et al., 2001; Hutvagner et al., 2001). *Drosophila* has two *dicer* genes: *dicer-1* (*dcr-1*) is critical for maturation of miRNAs, whereas *dcr-2* modulates generation of small interfering RNAs (siRNAs) (Lee et al., 2004). Flies were generated that expressed the pathogenic polyQ protein and were also mutant for *dcr-1* activity in the eye.

Expression of truncated pathogenic Ataxin-3 induces degeneration, which is characterized by partial loss of pigmentation and retinal structure (Figure 1A; Warrick et al., 1998). *dcr-1* mutation dramatically enhanced degeneration due to the pathogenic protein, such that the eye was now severely degenerate with complete loss of pigmentation (Figure 1B). Because loss of *dcr-1* alone affects eye morphology (Figure 1C; Lee et al., 2004), we also examined the effects of a second gene critical for miRNA maturation, *R3D1/loquacious*. R3D1 is a dsRNA binding protein that is required for the

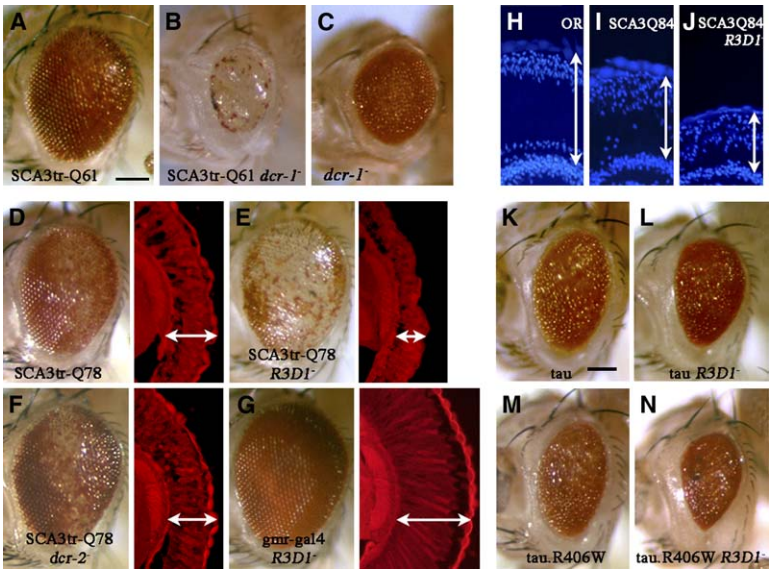

Figure 1. Reducing miRNA Processing Enhances polyQ and tau Toxicity in *Drosophila*

(A–C) External eyes of 1 day flies. (A) SCA3tr-Q61 normally shows weak degeneration. Eye genotype: *ey-FLP; gmr-gal4 UAS-SCA3tr-Q61/+; FRT82B*. (B) SCA3tr-Q61 degeneration in *dcr-1* is dramatically enhanced. Eye genotype: *ey-FLP; gmr-gal4 UAS-SCA3tr-Q61/+; FRT82B dcr-1*^{Q11147X}. (C) Loss of *dcr-1* activity alone. Eye genotype: *ey-FLP;; FRT82B dcr-1*^{Q11147X}.

(D–G) External eye and retinal sections. Arrow highlights retinal thickness, which reflects severity of degeneration. (D) SCA3tr-Q78 causes partial degeneration. Genotype *gmr-gal4 UAS-SCA3tr-Q78/+*. (E) Loss of *R3D1* enhances degeneration, with less pigmentation and little retinal tissue compared to control (D). Genotype: *R3D1*^{f00791}*/R3D1*^{f00791}*; gmr-gal4 UAS-SCA3tr-Q78/+*. (F) Loss of *dcr-2*, which modulates siRNA production, has no effect on polyQ toxicity. Eye genotype: *FRT42D dcr-2*^{L811fsX}*; gmr-gal4 UAS-SCA3tr-Q78/ey-gal4 UAS-FLP*. (G) Control flies bearing driver line alone with loss of *R3D1* have normal retinal morphology. Genotype: *R3D1*^{f00791}*/R3D1*^{f00791}*; gmr-gal4/+*.

(H–J) Hoechst staining of retinal sections of 1 day flies. (I) Expression of full-length pathogenic Ataxin-3 causes mild retinal degeneration, seen as reduced retinal depth compared to (H) normal. (J) Reducing miRNA processing by mutation of *R3D1* enhances degeneration, seen as the dramatically reduced retinal thickness. Genotypes: (H) Oregon-R, (I) *gmr-GAL4/ UAS-SCA3-Q84*, and (J) *R3D1*^{f00791}*/R3D1*^{f00791}*; gmr-GAL4/ UAS-SCA3-Q84*.

(K–N) Normal or mutant (R406W) human tau causes degeneration that is enhanced by loss of *R3D1*. Genotypes: *gmr-gal4* in *trans* to *UAS-tau* or *UAS-tau.R406W* with or without *R3D1*^{f00791}*/R3D1*^{f00791}.

Bar in (A), 100 μm for eyes in (A)–(G); bar in (K), 100 μm for (K)–(N).

activity of Dcr-1 in miRNA processing (Forstemann et al., 2005; Jiang et al., 2005). We confirmed that flies homozygous for the hypomorphic allele *R3D1*^{f00791} had reduced miRNA processing and that *R3D1* mutation had no effect on transcription from the Gal4-UAS system (Figures S1A and S1B in the Supplemental Data available with this article online). Loss of *R3D1* alone had minimal effects on the eye (Figure 1G). However, reduction of *R3D1* activity dramatically enhanced SCA3tr-Q78-induced neural degeneration (Figures 1D and 1E). This confirmed that miRNA pathways normally play a protective role in polyQ-induced neurodegeneration.

We considered whether reduction of miRNA processing enhanced SCA3 pathogenesis through the same type of degeneration pathways that normally occur upon pathogenic polyQ protein expression or whether reduction of miRNA processing modulated programmed cell death, which then enhanced the phenotype. To address this, we determined whether we could detect features of programmed cell death when expressing the disease protein alone or upon enhanced degeneration. TUNEL assays and western immunoblots for activated caspase failed to reveal an effect in polyQ pathology or with reduced *R3D1* activity, and coexpression of the baculoviral anticaspase protein P35 had minimal effect (Figure S2). These data support the idea that reduction of miRNA activity enhances degeneration by modulating pathways that normally contribute to polyQ toxicity.

To further test the specificity of miRNA pathways in neurodegeneration, we determined whether reduction of siRNA activity affects polyQ toxicity. In contrast to loss of *dcr-1* and reduction of *R3D1*, loss of *dcr-2*, which reduces siRNAs, had little or no effect on polyQ-induced neurodegeneration (Figures 1D and 1F). These data indicated that siRNA-dependent pathways do not modulate polyQ toxicity; rather, protective activity appears specific to miRNA-dependent pathways.

We then addressed the broader role of miRNA pathways in neurodegeneration. We first determined that reduction of *R3D1* activity enhanced not only pathogenicity of truncated forms of Ataxin-3 but also of the full-length pathogenic protein (Figures 1H–1J). To extend these findings beyond polyQ disease, we then examined the effect on tau. Abnormal tau accumulation is associated with Alzheimer's disease and frontotemperal dementia (Lee et al., 2001). In *Drosophila*, expression of normal or pathogenic tau induces severe neurodegeneration, reflected by a severely disrupted eye phenotype (Figures 1K and 1M; Wittmann et al., 2001). Reduction of *R3D1* activity dramatically enhanced tau toxicity (Figures 1L and 1N). These data indicate that the miRNA pathway not only modulates polyQ toxicity but also toxicity of other proteins associated with human neurodegenerative disease.

Blocking miRNA Processing Dramatically Enhances Ataxin-3 Toxicity in Human Cells

Given these observations in flies, we addressed whether these findings extended to human cells. To do this, we expressed normal and pathogenic full-length Ataxin-3 protein in human HeLa cells in culture, in the presence of normal or reduced Dicer activity. HeLa cells were treated with siRNA directed to the *dicer* sequence; this treatment lowered *dicer* activity, as shown by reduced expression and processing of endogenous Dicer targets

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