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RESEARCH HIGHLIGHT

New hope for therapy in neurodegenerative diseases

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The use of genetic screens to define cellular pathways that regulate neurodegenerative disease proteins has emerged as a powerful strategy to identify potential therapeutic targets for these disorders. Using cross-species genetic screens, Park *et al*. recently identified RAS-MAPK-MSK1 as a cellular pathway that modulates levels of the polyglutamine-containing protein ATXN1 and its subsequent toxicity in SCA1.

Neurodegenerative diseases represent a growing public health issue. While many advances in the last 20 years have moved us toward a better understanding of the underlying molecular mechanisms, for most such disorders no treatments exist that can reverse or slow the course of disease. The various inherited neurodegenerative disorders are caused by distinct pathogenic mechanisms triggered by specific mutations, yet a shared aspect to many of them is the accumulation of a "toxic" form of the mutant protein. Thus, in these neurodegenerative proteinopathies, a simple therapeutic strategy is to decrease levels of the mutant gene or encoded protein. Indeed, studies of genetic manipulation in animal models reveal that decreasing levels of neurodegenerative disease-causing transcripts, and consequently their translated proteins, often can reverse the disease phenotype [1-3]. Targeting the mutant protein by stimulating cellular protein quality control systems is another therapeutic approach that has proved effective in reducing levels of certain mutant proteins [4-6]. As part of this latter strategy, unbiased screens to identify genes involved in the degradation of disease-causing proteins has begun to emerge as a potent approach to discover novel therapeutic targets [7, 8].

By screening kinase and kinase-like genes in human cells and Drosophila, Park and colleagues [7] recently identified several components of the RAS-MAPK-MSK1 cellular pathway as modulators of ATXN1, the protein mutated in the neurodegenerative polyglutamine disease Spinocerebellar Ataxia type 1 (SCA1). The authors validated their results in SCA1 mouse models and provided further evidence that pharmacologically targeting the RAS-MAK-MSK1 also reduces levels of mutant ATXN1. The rationale for screening kinase genes as potential modulators of mutant ATXN1 stemmed from the fact that specific phosphorylation events are known to enhance ATXN1 toxicity by stabilizing the protein [9, 10], and many kinases can be pharmacologically targeted (i.e., they are "druggable" genes).

An important strength of the study by Park *et al*. is that they performed two genetic screens using a cross-species strategy: i) a human cell model for SCA1 was used to screen a library of short interfering RNAs (siRNA) targeting 636 human kinase and kinase-like genes; and ii) a *Drosophila* SCA1 model was used to screen 704 alleles (inducible short hairpin RNAs (shRNAs) and loss-of-function mutations) corresponding to 337 kinase-encoding *Drosophila* genes. To differentiate modifiers of transgene expression from modulators of ATXN1 protein levels, the medulloblastoma-derived cell line used in the human kinase screen expressed a transcript encoding monomeric red fluorescent protein (mRFP) fused to expanded ATXN1 (mRFP-ATXN1(82Q)), followed by an internal ribosomal entry site (IRES) and the coding sequence for yellow fluorescent protein (YFP). Using the mRFP/YFP ratio as the readout for ATXN1 levels, 45 genes were identified that reduced ATXN1(82Q) levels in human cells (Figure 1A). In the *Drosophila* genetic screen, fruitflies expressing human ATXN1(82Q) develop a degenerative external eye phenotype as a consequence of mutant ATXN1 toxicity. Through analysis of eye morphology and retinal histology, 51 alleles were found to suppress expanded ATXN1 toxicity in *Drosophila* (Figure 1A).

Comparison of the two complementary genetic screens showed 10 human genes that effectively reduced both ATXN1(82Q) levels in cells and ATXN1(82Q) toxicity in *Drosophila* (Figure 1A). Six of these 10 genes (*ERK1*, *ERK2*, *MEK2*, *MEK3*, *MEK6*, and *MSK1*) proved to be canonical components of the MAPK pathway, and an additional two, IGF1R and WNK4, are known regulators of this pathway. Indeed, reducing levels of *MEK*, *ERK1/2*, and *MSK1* homologs in SCA1 flies led to improvement of motor deficits and lifespan. The authors further showed that genetic suppression of upstream components of the MAPK pathway such as RAS or RAF decreased levels of mutant ATXN1 in human cells, and improved motor performance and reduced eye degeneration in the SCA1 *Drosophila* model.

How does the RAS-MAPK pathway regulate ATXN1 levels? As ATXN1 can be stabilized by phosphorylation at residue S776 [9], Park *et al*. sought to test RAS-MAPK cascade kinases that might be responsible for this protein

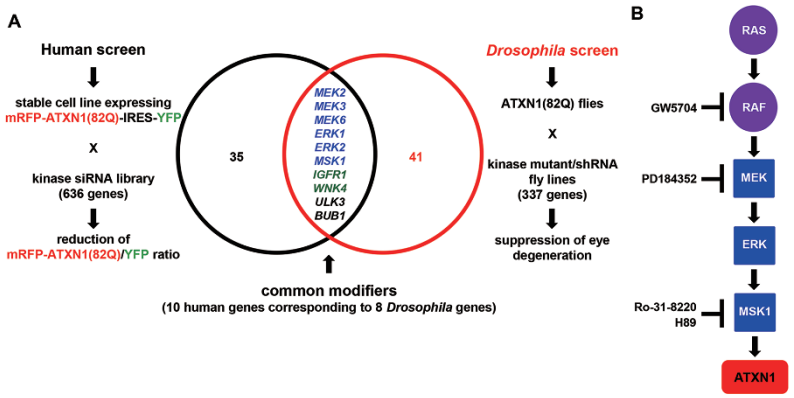

Figure 1 Kinase genetic screens identify components of RAS-MAPK-MSK1 pathway as modulators of levels of ATXN1. **(A)** Diagram of the human cell-based screen for modifiers of ATXN1(82Q) levels by cytometric measurement of mRFP-ATXN1(82Q) to YFP fluorescence ratio, and of the *Drosophila* screen for suppressors of ATXN1(82Q)-induced eye degeneration. Among the 10 human genes identified in both screens, six genes (in blue) are components of the MAPK cascade and two genes (in green) are known to regulate this pathway. **(B)** RAS-MAPK-MSK1 pathway showing the ATXN1 modifiers identified in the screens (blue squares) and chemical inhibitors of this pathway.

modification. Using *in vitro* kinase assays, the authors showed that MSK1 phosphorylates ATXN1 S776, thereby increasing ATXN1 levels. This result was confirmed *in vivo* in mouse Neuro2A cells, by assessing phosphorylation of recombinant ATXN1 with mouse cerebellar extracts immunodepleted of MSK1, and in fruitflies knocked down for the MSK1 homolog. Moreover, knockdown of upstream components of MSK1 on the MAPK pathway also decreased ATXN1 S776 phosphorylation in *Drosophila*, establishing that the mechanism by which the RAS-MAPK-MSK1 pathway regulates ATXN1 stability is through S776 phosphorylation (Figure 1B). In addition, Park *et al*. showed that pharmacological inhibition of the MAPK pathway at the levels of RAF (compound GW5704), MEK1/2 (compound PD184352) or MSK1 (compounds Ro-31-8220 and H89) (Figure 1B) decreased ATXN1 levels in mRFP-ATXN1(82Q) expressing cells and in cerebellar slice cultures from SCA1 (82Q) knock-in mice.

Finally, Park and collaborators provided evidence in mice for genetic interaction between MSK1 and ATXN1: i) *Atxn1* (*154Q*) knock-in and *Msk1* knockout animals ($Atxn1^{154Q/+}Msk1^{-/-}$) show decreased levels of Atxn1 in the cerebellum; ii) $Atxn1^{154Q/+}Msk1^{+/-}$ $Msk2^{+/-}$ animals showed improved motor performance in relation to the $Atxn1^{154Q/+}$ littermates; and iii) knockdown of Msk1 alone or together with Msk2 rescued the Purkinje cell loss observed in SCA1 transgenic mice expressing ATXN1(82Q).

In summary, the successful strategy of complementary genetic screens employed by Park *et al*. to identify modulators of mutant ATXN1 abundance has uncovered several related targets of potential therapeutic intervention in SCA1, perhaps paving the way toward future development of combination therapeutics. As the identified pathway is a cell-signaling cascade that regulates many cellular events, a challenge now will be to develop a viable pharmacological agent targeting this pathway that readily enters the brain and works effectively and safely *in vivo*. This is no small matter, but the results of Park and colleagues suggest that it may be well worth the effort. Moreover, the development of SCA1 therapeutics by targeting the RAS-MAPK-MSK1 pathway might also be applicable to other neurodegenerative diseases in which evidence also implicates this pathway. Finally, the screening approach used in this study, targeting an early event in disease pathogenesis but expanded to a genome-wide scale, might identify additional therapeutic targets in SCA1 and should be considered for other neurodegenerative diseases.

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