Hunting for the mutant without the MAP(K)

Cell Research (2017) 27:1403–1404. doi:10.1038/cr.2017.140; published online 14 November 2017

In a paper recently published in *Cell Research*, Yu *et al.* identify two MAPK-related kinases, MAPK11 and HIPK3, as positive regulators of levels of mutant huntingtin protein, a toxic species highly involved in Huntington's disease (HD) pathology. The identification and validation of these kinases as therapeutic targets for knockdown in multiple relevant experimental model systems reveal novel potential approaches for treatment of HD.

The neurodegenerative disorders are a class of diseases that collectively affect millions of individuals worldwide. Although there have been extensive efforts towards understanding the pathological mechanisms of each disorder, development of effective disease-modifying therapeutics has proven unsuccessful, owing to a multitude of factors including the overall complexity of each disease, the extended asymptomatic prodromal disease period before treatment administration, and many others. Currently, treatment of neurodegenerative diseases is limited almost entirely to symptom management, aiding with particular motor, cognitive, and psychiatric aspects of each disease [1]. As the prevalence of neurodegenerative disorders continues to rise, a better understanding of the underlying disease mechanisms is essential to guide disease-modifying therapy development.

While there are specific genetic, cellular, and environmental underpinnings that are unique to each of the neurodegenerative disorders, there are several commonalities across these disorders. Two hallmark features of the

neurodegenerative disorders include the progressive degeneration of a particular, selectively vulnerable population of neurons, and the anomalous accumulation of toxic aggregated protein species. The latter has been the subject of much discussion as an entry point for therapy development, as these aberrantly accumulating toxic proteins represent an entity that, if removed, could substantially ameliorate disease pathogenesis and subsequent progression.

Huntington's disease (HD) is a monogenetic neurodegenerative disorder caused by expansion of the (CAG) repeat tract of the *Huntingtin* (*HTT*) gene. This expansion produces a mutant protein containing a polyglutamine (polyQ) expansion with a high propensity to aggregate and form nuclear inclusions. The expanded mutant Huntingtin protein (mHTT) is thought to underlie the etiology of HD by altering many fundamental neuronal processes such as axonal transport, transcriptional regulation and protein degradation [2]. A long-standing idea in HD therapy development has centered around the clearance of mHTT, and previous studies have shown attenuated HD phenotypes upon genetic reduction of *mHTT* [3]. Recently, substantial progress has been made using anti-sense oligonucleotides (ASOs) to reduce *mHTT* in pre-clinical models of HD [4], however, the challenge of delivering ASOs clinically has precluded its widespread use and application. Therefore, pharmacological interventions that can be more easily delivered while similarly reducing mHTT levels are especially appealing.

To date, several groups have per-

formed unbiased RNA interference (RNAi) screens to identify candidate molecules whose modulation may alter the development and progression of HD, however, these screens have been primarily performed in models that rely on the exogenous overexpression of polyQ expanded mHTT [5–9]. Although these previous reports have successfully identified a diverse set of pharmacologically targetable molecules capable of reducing mHTT aggregation and its associated toxicity in cellular and animal models of HD, these target pathways may not directly apply to the endogenous expression of pathological mHTT, especially in the context of HD-relevant human cell types.

In a paper recently published in *Cell Research* [10], Yu *et al.* have expanded upon previous RNAi screens for HD by examining the effects of siRNA knockdown of regulome genes with a rigorous, multiple-step, multiple-platform pipeline to identify candidate genes whose reduction can mitigate two key phenotypes central to HD pathology — elevated levels of mHTT and neuronal toxicity. Importantly, this study is the first of its kind to conduct its primary screening in human cells endogenously expressing *mHTT*, HD patient-derived fibroblasts, which more accurately recapitulate the mutant protein expression seen in HD patients compared to previously utilized transgenic overexpression models. By initially screening for siRNAs capable of reducing mHTT levels in HD patient primary fibroblasts and subsequently assessing the preliminary candidates for their effects on neuronal toxicity in stem cell-derived neurons,

Yu *et al.* present a narrow list of 11 prioritized putative candidate genes whose reduction may prove beneficial for HD, warranting further exploration of therapeutic possibilities.

Excitingly, several candidates identified in their screen belong to the class of MAPK-related genes, and this convergence increases confidence that modulation at various steps along this specific pathway may provide an avenue for therapy development. Following their screen, Yu and colleagues extensively assessed the genetic interactions of two of the identified genes, *HIPK3* and *MAPK11*, with mHTT in multiple different HD models, including *in vitro* cultures of patient induced pluripotent stem cell (iPSC)-derived neurons and mouse striatal cells, as well as in a HD knock-in mouse model *in vivo*. Importantly, mHTT expression in these models is driven by the endogenous promoter, thus mimicking tissue-specific, physiological expression. These experiments revealed a positive regulation of HTT by HIPK3 and MAPK11, which is dependent on each of their kinase activities functioning in two distinct pathways, autophagy and *HTT* mRNA stability, respectively. A more comprehensive assessment of these kinases' functions in healthy and diseased states must be performed to ascertain the mechanisms

by which these kinases regulate mHTT, as well as the potential adverse off-target effects on other pathways through inhibiting these kinases.

Interestingly, the genetic interactions of these kinases with HTT are dependent on the presence of mHTT, as there is no modulation in cells and mice expressing wtHTT exclusively upon MAPK11 or HIPK3 reduction. These findings prompt several key questions. What mediates this dependency on mHTT? In other words, how does polyQ expansion of HTT alter HIPK3 and MAPK11 activity and/or levels, initiating the early step in the positive feedback loop reported by Yu *et al.*? Given its polyQ dependence, understanding the precise nature of this genetic interaction and how it changes in the context of variable polyQ lengths and number of mHTT copies will be essential for application in HD.

These findings represent an exciting step forward in HD therapy development, as the authors present compelling evidence of substantial benefit of modulating these two kinases on the cellular changes observed in HD. Whether alterations in these two kinases translate into significant behavioral recovery in other HD mouse models, as well as in HD patients, and whether these treatments can sufficiently and

specifically produce similar results as the genetic perturbations demonstrated here, remain to be determined.

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References

- McColgan P, Tabrizi SJ. *Eur J Neurol* 2017 Aug 17. doi:10.1111/ene.13413
- Bates G. *The Lancet* 2003; **361**:1642-1644.
- Yamamoto A, Lucas JJ, Hen R. *Cell* 2000; **101**:57-66.
- Kordasiewicz HB, Stanek LM, Wancewicz EV, *et al.* *Neuron* 2012; **74**:1031-1044.
- Miller JP, Yates BE, Al-Ramahi I, *et al.* *PLoS Genet* 2012; **8**:e1003042.
- Dietrich P, Johnson IM, Alli S, *et al.* *PLoS Genet* 2017; **13**:e1006846.
- Zhang S, Binari R, Zhou R, *et al.* *Genetics* 2010; **184**:1165-1179.
- Doumanis J, Wada K, Kino Y, *et al.* *PLoS One* 2009; **4**:e7275.
- Lu B, Al-Ramahi I, Valencia A, *et al.* *Nat Neurosci* 2013; **16**:562-570.
- Yu M, Fu Y, Liang Y, *et al.* *Cell Res* 2017; **27**:1441-1465.