News & views

Neurodegeneration

Selective clearance of mutant huntingtin protein

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Compounds have been found that reduce levels of the harmful protein present in Huntington's disease, without affecting the normal version. The compounds interact with the mutated protein and the cell's protein-clearance machinery. **See p.203**

Several neurodegenerative diseases involve the slow accumulation of a misfolded protein in neurons over many years. The proteins involved in these diseases might differ, but the result is similar – eventually, the neurons die from the build-up of toxic misfolded proteins. Scientists have long been searching for ways to reduce the levels of the disease-driving proteins without also clearing their wild-type counterparts, which typically have myriad crucial functions. On page 203, Li *et al.*[1] show that this can be accomplished using compounds that interact specifically with both the misfolded part of the protein and the neuron's protein-clearance machinery.

Li and colleagues chose to focus on Huntington's disease, which is caused by an abnormally long stretch of glutamine amino-acid residues in the huntingtin (HTT) protein. This expanded polyglutamine tract causes HTT to misfold. Affected individuals typically carry one copy (one allele) of the *HTT* gene that encodes the mutant protein, and one allele that encodes a protein with the normal-length glutamine tract.

Cells are able to degrade the mutant huntingtin (mHTT) through autophagy[2] – a clearance mechanism that involves engulfment of proteins by a vesicle called the autophagosome. Li *et al.* hypothesized that compounds that bind to both the mutant polyglutamine tract and the protein LC3B, which resides in the autophagosome, would lead to engulfment and enhanced clearance of mHTT (Fig. 1). But no such compounds had been reported. The authors therefore conducted small-molecule screens to identify candidate compounds, and used wild-type HTT in a counter-screen to rule out compounds that bind to the normal version of the protein.

Li and colleagues initially identified two candidates, dubbed 10O5 and 8F20. These compounds had been shown[3,4] to inhibit, respectively, the activity of the cancer-associated protein c-Raf and kinesin spindle protein (KSP), which has a key role in the cell cycle. The team found that 10O5 and 8F20 were able to clear mHTT independently of their effects on these other proteins.

The researchers showed that the regions of 10O5 and 8F20 that interacted with mHTT and LC3B in the screen shared structural similarities. Next, they screened for compounds that shared these structural properties but were structurally distinct from other c-Raf and KSP inhibitors (a compound that acts on mHTT without altering these proteins would be desirable for clinical treatment). This led them to discover two more compounds, AN1 and AN2, that link mHTT to LC3B and thereby selectively reduce levels of mHTT.

Li and colleagues validated their exciting discovery by showing that the four compounds reduced levels of the full-length mHTT protein (not just the protein fragment used in the screen). The compounds lowered levels of mHTT both *in vitro* – in mouse neurons and neurons derived from the biopsied skin cells of people with Huntington's disease – and *in vivo*, in mouse and fly models of the disease.

A key strength of the compounds identified by Li and co-workers is that they leave levels of wild-type HTT unchanged. This is crucial because HTT has multiple neuronal functions, both during embryonic development and after birth[5]. Existing mHTT-lowering strategies typically affect both *HTT* alleles[6,7], which is not ideal. Equally, the compounds found by Li *et al.* did not affect other proteins that contain polyglutamine tracts of variable, but not disease-causing, length. These proteins often have many roles in the brain.

One question that naturally arises is whether

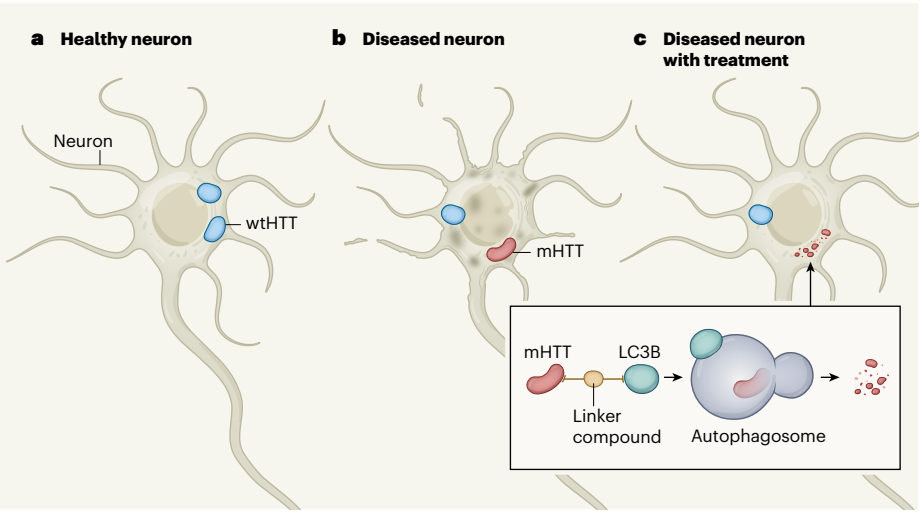

Figure 1 | Lowering levels of mutant huntingtin protein. a, Healthy neurons typically carry two copies of the gene that encodes the wild-type version of huntingtin protein (wtHTT). Only two proteins are shown, for simplicity, although many are produced from each gene copy. **b**, Huntington's disease involves the expansion of a tract of glutamine amino-acid residues in one copy of HTT protein, producing a mutant version (mHTT) that accumulates in neurons, causing them to shrink and eventually die. Any strategy to decrease levels of mHTT in these cells must not affect wtHTT, which has key functions in the brain. **c**, Li *et al.*[1] have identified four linker compounds that fulfil this role. Treatment with these compounds inhibits neuronal degeneration in various models of Huntington's disease. The compounds bind to both mHTT and the protein LC3B – a key component of a protein-clearance pathway called autophagy. This enables selective engulfment of mHTT by a vesicle called the autophagosome, leading to the mutant protein's degradation.

treating cells with the compounds led to enhanced autophagic clearance of proteins other than mHTT. Li *et al.* assessed the levels of the repertoire of proteins in the cortices of mice that carried an *mHtt* allele. They found changes in the abundance of a small percentage of proteins in mice treated with the compounds, compared with untreated animals. What remains unclear is whether the levels of some proteins decreased because mHTT levels were diminished, or because of autophagy. Modest changes in protein-expression level (in the 20–30% range for some wild-type proteins) can cause neurological deficits[8], so pinpointing any off-target effects of the compounds will be a crucial next step. Even effects that initially seem inconsequential might build up over the course of long-term therapy, becoming as problematic decades later as the original toxic protein.

Despite these concerns, the authors found encouraging evidence that the compounds could produce functional improvements in models of Huntington's disease across three species. First, patient-derived neurons treated with each of the compounds showed significantly less shrinkage, degeneration of neuronal projections and cell death than was seen in untreated neurons. Second, flies that model Huntington's disease and were treated with the compounds recovered climbing ability and survived longer than did untreated counterparts. Third, treated mice that model Huntington's disease showed improvements in three motor tests, compared with untreated mice. That said, preclinical trials in mice will be necessary to ascertain that the benefit is sustained and robust over the course of long-term therapy.

Finally, Li *et al.* analysed mutant ataxin-3, a protein that is involved in a neurodegenerative disorder called spino-cerebellar ataxia type 3. The researchers found that the compounds targeted the long polyglutamine tract of mutant ataxin-3 and lowered protein levels. We already know that small reductions in the levels of mutant ataxin-1, ataxin-2 and ataxin-3 can reduce the severity of spino-cerebellar ataxia types 1, 2 and 3, respectively, in mouse models[9–11]. Thus, this therapeutic strategy might be useful not only for Huntington's disease, but also for other diseases involving expanded polyglutamine tracts.

Moving forwards, there are three major research paths to pursue. The first involves establishing the mechanism by which Li and colleagues' compounds recognize proteins with expanded polyglutamine tracts but spare normal proteins. Perhaps the compounds recognize a particular structural conformation that arises only after the polyglutamine tract exceeds a specific length. The second involves testing the compounds in other models of polyglutamine disorders and assessing their effects.

The third path involves conducting similar small-molecule screens for compounds that can clear polyglutamine proteins using other types of protein-clearance machinery. For instance, small molecules dubbed proteolysis-targeting chimaeras (PROTACs) link a ubiquitin ligase enzyme to a protein of interest. The enzyme tags the protein with ubiquitin groups, leading to the protein's degradation by a cellular machine called the proteasome[12]. PROTACs have yet to be applied to a polyglutamine-expanded protein. But given that some of these proteins are degraded by the proteasome, the strategy could well prove viable – as long as it targets only the abnormally long polyglutamine tract.

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1. Li, Z. *et al. Nature* **575**, 203–209 (2019).
2. Ravikumar, B. & Rubinsztein, D. C. *Mol. Aspects Med.* **27**, 520–527 (2006).
3. Lackey, K. *et al. Bioorgan. Med. Chem. Lett.* **10**, 223–226 (2000).
4. Reddy, K., D'Orazio, A., Nadler, E. & Jain, V. K. *Clin. Genitourin. Cancer* **4**, 156–159 (2005).
5. Liu, J. P. & Zeitlin, S. O. *J. Huntington's Dis.* **6**, 1–17 (2017).
6. Kordasiewicz, H. B. *et al. Neuron* **74**, 1031–1044 (2012).
7. Spronck, E. A. *et al. Mol. Ther. Methods Clin. Dev.* **13**, 334–343 (2019).
8. Gennarino, V. A. *et al. Cell* **160**, 1087–1098 (2015).
9. Friedrich, J. *et al. JCI Insight* **3**, e123193 (2018).
10. Scoles, D. R. & Pulst, S. M. *RNA Biol.* **15**, 707–714 (2018).
11. McLoughlin, H. S. *et al. Ann. Neurol.* **84**, 64–77 (2018).
12. Sakamoto, K. M. *et al. Proc. Natl Acad. Sci. USA* **98**, 8554–8559 (2001).

This article was published online on 30 October 2019.

Engineering

Soft microbots controlled by nanomagnets

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Arrays of nanoscale magnets have been constructed to form the magnetized panels of microscopic robots – thus allowing magnetic fields to be used to control the robots' shape and movement. **See p.164**

In science-fiction films, robots are often depicted as human-sized or larger machines made of rigid materials. However, robots made of soft materials or with flexible structures, and that can be much smaller than the human body, have attracted great interest in the past few years because they have the potential to interact with humans more safely than can rigid machines. Indeed, sufficiently small soft robots could even be used for biomedical applications in the human body. Various options are available to power these robots, but magnetic fields offer a safe and effective means of wireless operation in confined spaces in the body. On page 164, Cui *et al.*[1] report a key step towards the fabrication of micrometre-scale robots that, in a programmable manner, can quickly morph into different shapes in applied magnetic fields.

The ability of minerals known as lodestones to align with Earth's magnetic field was first reported in the ancient Chinese manuscripts *Gui Gu Zi* and *Han Fei Zi*, and was later used in early magnetic compasses[2]. A similar principle has been used in the past few years in magnetic soft robots[3–10], in which magnets of varying sizes (nanometres to millimetres) are integrated into flexible structures or soft materials. The tendency of the magnets to orient in externally applied magnetic fields provides a way of quickly moving or changing the shape of these untethered robots remotely. This actuation mechanism allows much flexibility in the design of the robots' structures, magnetization patterns and strengths, and in when and where magnetic fields are applied to control the robots. In addition, because the forces and torques exerted on magnets by external magnetic fields can be accurately calculated, models have been developed to quantitatively describe the actuation of specific robot designs[11].

Magnetic soft robots have been developed for various uses, especially in biomedical applications in which they interact closely with the human body. For example, self-folding 'origami' robots have been reported that can crawl through the gut, patch wounds and dislodge swallowed objects[4]; and capsule-shaped robots have been made that roll along the inner surface of the stomach and can perform biopsies and deliver medicine[3]. Magnetically steerable robotic catheters have also been developed, which can perform minimally