Pore, Pore Huntingtin!

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Mutant huntingtin disrupts the nuclear pore complex in Huntington's Disease. The current study conveys obscure cellular mechanisms and provides a basis for developing treatment.

Stop looking to see whether your skin is clear, because you may be looking at the wrong pores! Huntington's Disease (HD) is a neurodegenerative disorder that is characterized by rapid, involuntary successions of muscular contraction and relaxation. The disease is caused by the abnormal replication of CAG, a 3-base sequence within the genetic code (DNA) of the huntingtin gene (HTT). HTT genes include a range of 9-35 CAG sequences, but any number that exceeds that range results in Huntington's Disease. The time of HD onset depends on the number of CAG sequences, as indicated by juvenile HD (JHD) patients carrying at least 75 CAG repeats (Saudou and Humbert, 2016). Consequently, the amino acid sequence, which is the protein's structural code, develops extensive chains of the amino acid glutamine that are characteristic of the mutant huntingtin protein (mHTT). mHTT is cut into fragments which then form toxic bundles called aggregates, which cause the destruction of countless cellular components. Damage by mHTT aggregates primarily takes place in the basal ganglia (BG), a set of brain structures that encode the decision to move. One of the structures of the BG, the striatum, consists of neural cells called spiny projection neurons (SPNs). SPNs exhibit patterns in which they fire in one striatal region but cancel their firing in the other. Such patterns are implicated in the selection of a motor sequence to induce specific movements (Purves et al., 2018). SPNs sustain the most damage by mHTT, and their degeneration corresponds to the emergence of unwanted involuntary movements in HD.

Transport between the nucleus and cytoplasm (nucleocytoplasmic transport or N/C transport) allows for the entry of ions and small proteins that are necessary for protein synthesis and the regulation of gene expression (Nigg, 1997). Nuclear pore complexes (NPCs) serve as the main sources of nucleocytoplasmic transport. They span the outer layer of the nucleus and consist of proteins called nucleoporins (NUPs), which each carry out distinct functions. NUPs that consist of phenylalanine and glycine within their amino acid sequence (FG NUPs) act as docking sites for the NPC at which cellular cargo is transported (Alber et al., 2007). It is known that mHTT disrupts nucleocytoplasmic transport within the NPC and adds to HD pathology. Grima et al. (2017) examines NPC defects caused by mHTT, and their work may contribute to the development of an HD treatment.

Initially, the researchers examined the brains of two genetic mouse models of HD: R6/2 and Q175, which consist of 150 and 175 CAG repeats, respectively (Cepeda et al., 2010). They also utilized immunofluorescence staining, a method that increases the visibility of specific molecules within tissue by tagging them with a fluorescent substance (Donaldson, 2015). Immunofluorescence stains of NUPs indicate that both NUP aggregates and mHTT aggregates are present within the nucleus of SPNs (Figure 1). Nuclear interactions between NUPs and mHTT may contribute to SPN degeneration.

Subsequently, the Grima and colleagues inspected brain tissue postmortem from the brains of human HD and JHD patients. They used immunohistochemistry, a subset of immunofluorescence staining that examines frozen tissue (Renshaw, 2017), to display NUP pathology. They learned through this technique that NUP aggregation occurs within the NPC, and NUP concentrations shift from the nucleus to the cytoplasm. Of course, NUP aggregates cause injury to SPNs, but the mislocalization of NUPs indicates the absence of docking sites that are integral to nucleocytoplasmic transport through the NPC. Therefore, protein synthesis and SPN function decline.

During nuclear export, contents are released into the cytoplasm when a protein called RanGAP1 breaks down another protein called Ran-GTP (or Ran) with water. This process, called hydrolysis, separates Ran-GTP into smaller components to fuel nucleocytoplasmic transport and establish its direction. Ran-GTP and RanGAP1 have corresponding functions, so lower levels of either protein will alter the efficacy of N/C transport. To evaluate the capacity of N/C transport, the researchers examined induced pluripotent stem cell-derived neurons (iPSNs). Pluripotent stem cells can be transformed into another type of cells, such as a blood cell. Although postmortem studies already provided some insight into human neuronal pathology, pluripotent stem cells made it possible to study live human neurons (hence iPSNs), allowing for a better comprehension of neurodegeneration (Dolmetsch and Geschwind, 2011). Grima and colleagues produced immunofluorescence stains of iPSNs from HD patients to determine the presence of Ran and RanGAP1. They found that high concentrations of both proteins were mislocalized to the cytoplasm, indicating the impairment of nuclear export and the destruction of striatal neurons (Figure 1).

To find sources of treatment, Grima and colleagues utilized a protein that combined RanGAP1 and a green fluorescent protein (GFP) to visualize RanGAP1 concentrations. They overexpressed the RanGAP1-GFP fusion protein to create its abundance within the neurons of mice transfected with the mHTT gene. They evaluated the extent of cell death through a condensation assay, which identifies cells undergoing apoptosis, or programmed death (Oberhammer et al., 1994). The results showed that the overexpression of the fusion protein reduces apoptosis (Figure 1). This finding implies that the restoration of RanGAP1 concentrations allows for the breakdown of Ran and the re-establishment of nuclear export in striatal neurons. Moreover, the researchers found that NUPs are modified through the addition of beta-N-acetylglucosamine (O-GlcNAc), a component of glucose. This process, known as O-GlcNAcylation, prevents the NUP from being tagged for degradation (Zhu et al., 2016), so it maintains the NPC's ability to selectively import materials. A protein called O-GlcNAcase is known to remove O-GlcNAc from the NPC. Thiamet-G, however, is a potent inhibitor of O-GlcNAcase, meaning that it prevents O-GlcNAcase from lowering O-GlcNAC concentrations. The effects of Thiamet-G concerning NPC function were assessed through RanGAP1 immunofluorescence staining, and the results indicate that the application of Thiamet-G relays Ran protein concentrations from the cytoplasm back to the nucleus, meaning that RanGAP1 regains the ability to break down Ran to provide fuel for nuclear export via the NPC (Figure 1).

It is imperative to understand the NPC's mechanisms because functional changes impact longevity. Lord et al. (2015) studied a species of yeast called S. cerevisiae, and they discovered that the presence and absence of FG NUPs alter life span. They found that the deletion of NUP116, for instance, significantly decreases the yeast species' life span, and the removal of NUP100 significantly increases longevity (Lord et al., 2015). These results show that NUP dysfunction does not always lead to neurodegeneration. This contradiction to the results in Grima et al. (2017)s emphasizes the need to conduct further research on NUPs and their roles in the NPC. Ultimately, further investigation of the NPC and its relation to the progression of Huntington's Disease may narrow down a potential target for developing treatment.

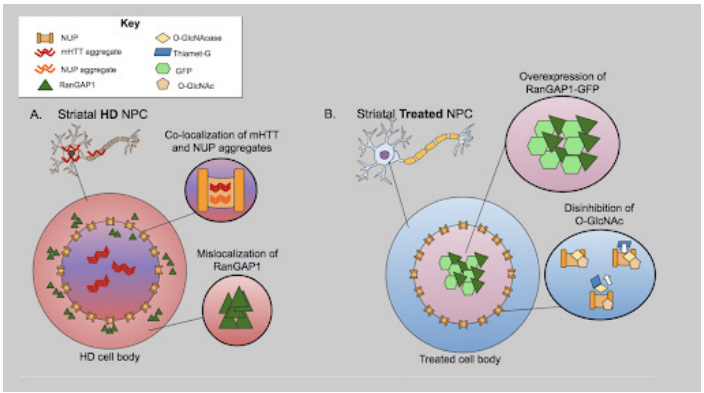

Figure 1. A comparison between diseased and treated NPC within the cell body of SPNs based on Grima et al. (2017). A.) The co-localization of mHTT and NUP aggregates along with the mislocalization of RanGAP1 disrupts nucleocytoplasmic transport via the NPC. B.) The overexpression of the RanGAP1-GFP fusion protein and the removal of inhibition on O-GlcNAc through the binding of Thiamet-G both restore nuclear export in SPNs.

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