Therapeutic potential of insulin-like growth factor 2 in Huntington's disease: controlling proteostasis to alleviate the load of misfolded protein

Paulina Troncoso-Escudero, Claudio Hetz*, Rene L. Vidal*

Huntington's disease (HD) is an inherited autosomal dominant neurodegenerative disorder characterized by the development of adult-onset motor dysfunction, psychiatric disturbances and intellectual decline. HD is associated with an expansion of CAG repeat sequence in the huntingtin gene (*Htt*). Exon 1 of *Htt* normally contains between 6 to 35 CAG repeats, whereas in patients affected with HD it contains more than 40 trinucleotides. The mutant Htt protein (mHtt) exhibits gain-of-toxic properties that cause neuronal dysfunction and death (Saudou and Humbert, 2016). Protein misfolding and aggregation is a common molecular feature of HD, suggesting that impairment in the buffer capacity of the proteostasis network contributes to the pathogenesis of the disease. Multiple studies in cell culture and animal models, in addition to the analysis of postmortem human tissue, have indicated that one of the main nodes of the proteostasis network affected in HD involves the function of the endoplasmic reticulum (Vidal et al., 2011). XBP1 is a master regulator of the unfolded protein response (UPR), the main adaptive pathway to cope with endoplasmic reticulum stress (Hetz et al., 2020). We previously reported that the genetic disruption of the transcription factor XBP1 delays disease progression and reduces protein aggregation in models of HD (Vidal et al., 2012), in addition to amyotrophic lateral sclerosis (ALS; Hetz et al., 2009), Parkinson´s disease (Valdes et al., 2014) and Alzheimer´s disease (Duran-Aniotz et al., 2017).

To identify disease-modifier genes involved in the neuroprotective effects of XBP1 deficiency, we performed gene expression profiling from brain tissue of these animals. This unbiased screening uncovered insulin-like growth factor 2 (IGF2) as the major upregulated gene (García-Huerta et al., 2020). IGF2 is a member of the insulin-like peptide family, which includes IGF1 and insulin, having important roles in brain physiology and neurodegeneration. We studied the significance of IGF2 to HD and performed cell culture studies demonstrating that IGF2 expression reduces the load of intracellular aggregates of mHtt and a polyglutamine peptide (polyQ, a peptide made of 79 glutamines), which was associated with a decrease in the half-life of mHtt. Importantly, IGF2 treatment protected medium spiny neurons derived from HD patients, in addition to spinocerebellar ataxia 3 patients, the second most common polyQ disease.

Our previous studies in XBP1 deficient mice suggested that the upregulation of autophagy explained part of the protection observed in HD and ALS models (Hetz et al., 2009; Vidal et al., 2012). However, the reduced protein aggregation observed in cells stimulated with IGF2 was independent of the activity of autophagy and the proteasome pathways, the two main routes for mHtt clearance (García-Huerta et al., 2020). Instead, we observed that IGF2 signaling enhanced the secretion of soluble polyglutamine peptide (polyQ) tract species through exosomes and microvesicles. To determine the signaling pathways downstream of the IGF2 receptor that control mHtt secretion, we determined the proteome changes upon stimulation with IGF2 by quantitative proteomics. This study revealed changes in actin dynamics, including several actin-binding proteins, Rho GTPases, among other factors. In fact, IGF2 treatment increased actin dynamics and Rac1 activity, a modulator of actin polarization, which mediated the release of polyQ into the extracellular space (**Figure 1A**).

To determine the significance of IGF2 to the progression of HD, we developed a gene therapy strategy to deliver IGF2 into the brain. Administration of IGF2 to the striatum resulted in a marked decrease in the levels of mHtt aggregation in three different animal models of HD. Finally, due to the dramatic effects of IGF2 on intracellular mHtt levels, we moved forward to explore possible changes in IGF2 levels in the brain and blood samples derived from HD patients. We observed a marked reduction of IGF2 protein levels in caudate-putamen samples when compared with healthy donors. Moreover, we evaluated the presence of IGF2 in peripheral blood mononuclear cells from HD patients. Although control samples presented a clear expression of IGF2, HD-derived blood cells showed an almost 70% decrease in IGF2 protein levels. Since IGF2 is a soluble secreted factor, we measure the amount of IGF2 in plasma from HD patients using ELISA, revealing a small but significant decrease of circulating IGF2 in plasma samples derived from HD patients (**Figure 1B**). Overall, our study reinforces the idea that the administration of IGF2 into the brain of patients might have important neuroprotective effects in protein misfolding disorders. In addition, our results demonstrate that a deregulation in IGF2 expression may enhance the pathological consequences of mHtt.

Our findings support important emerging evidence that highlights the importance of IGF2 in normal brain function and its relevance to neurodegenerative diseases. IGF2 is highly expressed in the hippocampus, and several groups have demonstrated the importance of IGF2 expression in shaping synaptic plasticity, impacting learning and memory in rodents. Interestingly, administration of recombinant IGF2 also improved memory in wild type animals (Chen et al., 2011). Besides memory formation, IGF2 is also important for the extinction of memories in the hippocampus, especially fear memories associated with anxiety and mood disorders (Pardo et al., 2019). These functions could be mediated in part by the fact that IGF2 acts as a regulator of synapse formation and spine maturation in hippocampal neurons, involving the activation of the IGF2R. IGF2 is decreased in the hippocampus of aged animals, and overexpression of IGF2 in the hippocampus reverses memory and dendritic spine density impairments (Pascual-Lucas et al., 2014). Furthermore, IGF2 expression is also decreased in the hippocampus of Alzheimer's disease patients and in a mouse model of the disease, and gene therapy to deliver IGF2 into the hippocampus of transgenic aged mice reverses memory deficit and restores spine density. Interestingly, IGF2 overexpression reduces Aβ plaques in the hippocampus of transgenic mice (Pascual-Lucas et al., 2014) (**Figure 1C**). Taken together, these evidences highlight IGF2 as an interesting candidate for the treatment of cognitive impairments.

The physiological function of IGF2 in the hippocampus has been attributed to the regulation of neurogenesis, where IGF2 has important roles in the maintenance of brain neuronal stem cells (NSCs), which are present in the subventricular zone of the lateral ventricles and in the subgranular zone of the dentate gyrus in the hippocampus. IGF2 promotes NSCs self-renewal and stemness in the subventricular zone, which is mediated by the insulin receptor A, a high affinity receptor for IGF2 (Ziegler et al., 2014). Importantly, IGF2 is highly expressed in NSCs of the dentate gyrus, where it promotes adult neurogenesis both *in vitro* and *in vivo* (Pardo et al., 2019). Together with the proteostatic effects of IGF2, its function in neurogenesis could be further exploited for therapy because it may target the two pillars of neurodegenerative diseases: abnormal protein aggregation and synaptic dysfunction. Therefore, IGF2 represents an interesting candidate for the restoration of neurogenesis and cell function under pathological conditions.

As mentioned, the delivery of IGF2 into the brain has been associated with neuroprotective effects. IGF2 is highly expressed in resistant motoneurons in ALS, and treatment of human spinal motoneurons from ALS patients with IGF2 protected motoneurons from degeneration. Furthermore, adeno-associated virus-mediated delivery of IGF2 into muscles of ALS transgenic mice preserved motoneuron function and induced axonal regeneration, extending the lifespan of ALS transgenic mice. Also, in a mouse model of autism, the systemic injection of IGF2 reversed abnormal social, cognitive and repetitive behaviors in mice, whereas the intranasal administration of IGF2 ameliorated learning and memory impairments in a mouse model of Fragile X syndrome (Pardo et al., 2019). Since IGF2 is a soluble factor that can spread through the brain, all these results highlight the therapeutic potential of gene therapy to administrate IGF2 in a sustained manner to multiple pathological conditions affecting the brain, ranging from neurodegenerative diseases to neurodevelopmental and psychiatric disorders (**Figure 1C**).

In conclusion, although IGF2 is less explored than IGF1 and insulin, increasing evidence suggests important functions of IGF2 in brain physiology and as a protective factor counteracting pathological events observed in diverse brain diseases. Our recent study

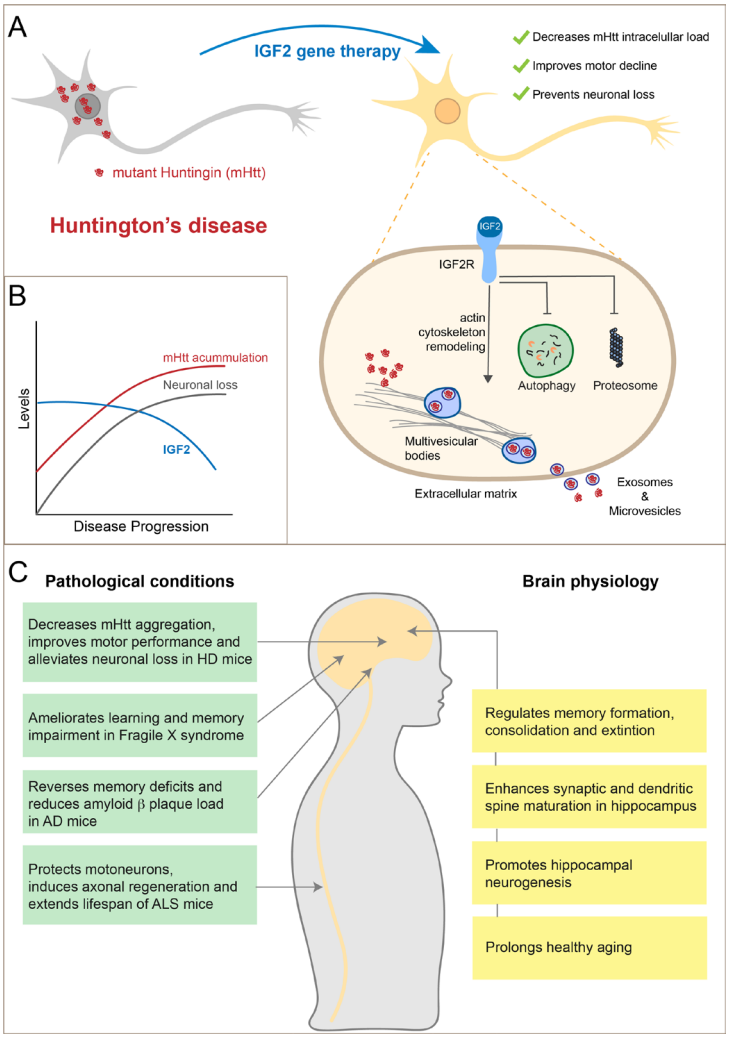

Figure 1 | **The new discovered function of IGF2 expression in HD models and its roles in brain physiology and in neuropathological conditions.**
(A) In cellular and preclinical models of HD, IGF2 decreases the intracellular load of mutant huntingtin via signaling through IGF2 receptor. IGF2 does not activate autophagy or the proteasome-mediated degradation, but instead causes actin cytoskeleton remodeling inducing mHtt secretion to the extracellular compartment. *IGF2* gene therapy reduces mHtt levels, prevents neuronal loss, and improves motor behavior in preclinical models. (B) In humans, IGF2 levels are reduced in the brain and blood from HD patients, which might be associated with increase accumulation of mutant huntingtin, and an increase in neuronal degeneration. (C) Under physiological conditions, IGF2 has important functions in the hippocampus, mediating memory formation, consolidation and extinction, participates in adult hippocampal neurogenesis, and in synapses formation and spine maturation. We speculate that IGF2 treatment would have beneficial effects in aging, possibly prolonging life span of treated subjects. Under pathological conditions with IGF2 treatment, the overexpression of IGF2 has shown neuroprotective effects in Alzheimer's disease, amyotrophic lateral sclerosis, Fragile X syndrome, and HD. AD: Alzheimer's disease; ALS: amyotrophic lateral sclerosis; HD: Huntington's disease; IGF2: insulin-like growth factor 2.

demonstrates a novel pathway regulating proteostasis in the brain governed by IGF2 to alleviate the load of abnormal protein aggregates. Available evidence regarding IGF2 function in the central nervous system highlights the importance of studying the biological significance of IGF2 to other brain illnesses. Given that IGF2 crosses the blood-brain barriers and is a soluble secreted factor, it emerges as an interesting candidate for potential translational applications.

This work was supported by Agencia Nacional de Investigación y Desarrollo (ANID) and Fondo de Financiamiento de Centros de Investigación en Áreas Prioritarias (FONDAP), No. 15150012 (to CH and RLV); Millennium Institute, No. P09-015-F (to CH and RLV); Fondo de fomento al desarrollo científico (FONDEF), No. ID16I10223, D11E1007 (to CH); Fondo Nacional de Desarrollo Científico y Tecnológico (FONDECYT), No. 1180186 (to CH) and 1191003 (to RLV); U.S. Air Force Office of Scientific Research 20RT0419; Michael J Fox For Parkinson's Research Target Validation ID 12473.01 (to CH) and Consejo Nacional de Ciencia y tecnología (CONICYT), No. 21160843 (to PTE).

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Date of submission: August 23, 2020
Date of decision: September 30, 2020
Date of acceptance: November 21, 2020
Date of web publication: January 7, 2021

https://doi.org/10.4103/1673-5374.303020
How to cite this article: *Troncoso-Escudero P, Hetz C, Vidal RL (2021) Therapeutic potential of insulin-like growth factor 2 in Huntington's disease: controlling proteostasis to alleviate the load of misfolded protein. Neural Regen Res 16(8):1564-1565.*

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C-Editors: Zhao M, Wang L; T-Editor: Jia Y