DEVELOPMENT OF NEUROPROTECTIVE STRATEGIES WITH BDNF IN HUNTINGTON'S DISEASE

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Duration: 3 years
1. Summary of the project

Huntington’s disease (HD) is a neurodegenerative disorder triggered by the mutation of the huntingtin gene. This leads to the death of striatal projection neurons. To date, there is no treatment for this devastating hereditary disorder, because the pathophysiology of HD is not known. The present project studies the interaction of mutant huntingtin with other intracellular mechanisms that induce the specific vulnerability of striatal neurons. This proposal is focused on the involvement of neurotrophic factors, mainly BDNF, in the regulation of the toxic activity of mutant huntingtin. Therefore, there are three main objectives: (1) to improve BDNF signaling, (2) to increase endogenous BDNF, and (3) the administration of exogenous BDNF. In vitro (knock in striatal cell lines), and in vivo (R6/1, R6/1 BDNF+/-, and conditional mouse model of HD) models are used. The first step is to analyze how mutant huntingtin affects the BDNF signaling pathways, such as ERK1/2, Akt, PLCγ, calcineurin and PHLPP-1. Furthermore anti- and pro-apoptotic proteins of the bcl-2 family are also studied, mainly Bax, Bim and Bid. We also evaluate the effect of mutant huntingtin in intracellular trafficking of BDNF.

The last part is to develop strategies to release BDNF in the brain parenchyma, since BDNF does not cross the blood brain barrier. Thus, we develop a new model that releases BDNF under the promoter of GFAP. In this mouse BDNF is only over-expressed under pathologic conditions. These studies can give us new data for understanding the pathophysiology of Huntington’s disease, which may make it possible to design new neuroprotective treatments.

2. Results

In the present project, we have provided new data about the pathophysiological mechanisms involved in the neurodegenerative processes in Huntington’s disease (HD). These results make it possible to
identify new therapeutic targets for the treatment of this neurological disorder. The main results of this project are:

1. **BH3-only proteins Bid and BimEL are differentially involved in neuronal dysfunction in mouse models of Huntington’s disease.** To study the mechanisms of cell death activated by mutant huntingtin, we examined the regulation of Bcl-2 family proteins in three different mouse models of HD with exon 1 mutant huntingtin: the R6/1, the R6/1:BDNF+-/- and the conditional Tet/HD94 mice. Our results show that enhanced Bid protein levels represent an early mechanism linked to the continuous expression of mutant huntingtin that, together with enhanced BimEL, may be a reporter of the progress and severity of neuronal dysfunction (*J. Neurosci. Res.*, 85, 2756-2769, 2007). Furthermore, using Bax knockout mice, we identified that Bax is required to induce apoptosis in the excitotoxic model of HD (*Mol.Cell.Neurosci.*, 37, 663-672, 2008).

2. **Disruption of striatal glutamatergic transmission induced by mutant huntingtin involves remodelling of both postsynaptic density and NMDA receptor signaling.** We study the striatal susceptibility to NMDA receptor (NMDAR)-mediated injury of two Huntington’s disease (HD) transgenic mice: R6/1 and R6/1:BDNF+-/- mice. We found that R6/1:BDNF+-/- mice, which express reduced levels of BDNF, were more resistant than R6/1 mice to intra-striatal injection of quinolinate. This increased resistance is related to a differential reduction in expression of NMDAR scaffolding proteins, MAGUKs (PSD-95, PSD-93, SAP-102 and SAP-97) but not to altered levels or synaptic location of NMDAR. The specific regulation of MAGUKs and αCaMKII in striatal neurons may reflect a protective mechanism against expression of mutant huntingtin (*Neurobiol.Dis.*, 29, 409-421, 2008).

3. **Calcineurin regulates the excitotoxicity associated with Huntington’s disease.** We studied the role of calcineurin, a serine/threonine phosphatase activated by calcium/calmodulin, in the vulnerability to excitotoxicity of striatal neurons expressing mutant huntingtin. Stimulation of the NMDA receptor produced a greater increase in calcineurin activity. Moreover, the transfection of calcineurin in wild-type cells promoted a significant increase
in cell death compared to that recorded in GFP-transfected wild-type cells after NMDA treatment. The inhibition of calcineurin using the pharmacological inhibitor FK-506 produced a more robust reduction in cell death in mutant huntingtin knock-in cells (J. Neurochem., 105, 1596-1612, 2008). Using transgenic models, we observed that calcineurin A and B were differentially regulated during disease progression with a specific reduction of calcineurin A protein levels and calcineurin activity at the onset of the disease in R6/1:BDNF+/- mice. Analysis of the conditional mouse model Tet/HD94 showed that mutant huntingtin specifically controls calcineurin A protein levels. These results point to the important role played by calcineurin in striatal excitotoxic-mediated cell death in HD (Neurobiol. Dis. 36:461-9, 2009).

Here, we demonstrate in knock-in HD striatal cells that mutant huntingtin enhances dopamine-mediated striatal cell death via dopamine D₁ receptors. Moreover, we show that NMDA receptors specifically potentiate the vulnerability of mutant huntingtin striatal cells to dopamine toxicity as pretreatment with NMDA increased D₁R-induced cell death in mutant but not wild-type cells. As potential underlying mechanism of increased striatal vulnerability, we identified aberrant cyclin-dependent kinase 5 (Cdk5) activation. These findings provide new insights into the molecular mechanisms underlying the selective vulnerability of striatal cells in HD and identify p25/Cdk5 as an important mediator of dopamine and glutamate neurotoxicity associated to HD (J. Neurosci., 28, 10090-101001, 2008).

5.- Brain derived neurotrophic factor modulates the severity of cognitive alterations induced by mutant huntingtin: Involvement of phospholipaseCγ activity and glutamate receptor expression. We observed that BDNF modulates cognitive function in different learning tasks, even before the onset of motor symptoms. R6/1:BDNF+/- mice showed earlier and more accentuated cognitive impairment than R6/1 mice. We observed a decrease in phospholipaseCγ activity, but not ERK, in R61, BDNF+/- and R6/1:BDNF+/- hippocampus at the age when LTP was altered. These
results show that BDNF modulates the learning and memory alterations induced by mutant huntingtin. This interaction leads to intracellular changes, such as specific changes in glutamate receptors and in BDNF-trkB signaling through phospholipaseCy (Neuroscience, 158, 1234-1250, 2009).

6.- Mutant huntingtin impairs post-golgi trafficking to lysosomes by delocalizing Optineurin/Rab8 complex from the Golgi apparatus. Colocalization studies and Western blot analysis of isolated Golgi membranes showed a reduction of huntingtin in the Golgi apparatus of cells expressing mutant huntingtin. These findings correlated with a decrease in the levels of optineurin and Rab8 in the Golgi apparatus. Our results indicate that mutant huntingtin perturbs post-Golgi trafficking to lysosomal compartments by delocalizing the optineurin/Rab8 complex, which, in turn, affects the lysosomal function (Mol. Biol.Cell, 20, 1478-1492, 2009)

7.- PH domain leucine-rich repeat protein phosphatase 1 helps to maintain the activation of the PI3K/Akt pro-survival pathway in Huntington’s disease striatum. Our results show decreased PHLPP1 protein levels in knock-in models (HdhQ111/Q111 mouse striatum and STHdhQ111/Q111 cells), in the striatum of N-terminal exon-1 mutant huntingtin transgenic mouse models (R6/1; R6/1:BDNF +/-, R6/2 and Tet/HD94) and in the putamen of HD patients. The analysis of the conditional mouse model Tet/HD94 disclosed that after mutant huntingtin shutdown PHLPP1 levels returned to wild-type levels whereas phospho-Akt levels were partially reduced. In conclusion, our results show that mutant huntingtin downregulates PHLPP1 expression. In the striatum, these reduced levels of PHLPP1 can contribute to maintain high levels of activated Akt that may delay cell death and allow the recovery of neuronal viability after mutant huntingtin silencing (Cell Death Diff., 17, 324–335, 2010).

8.- BDNF regulation under GFAP promoter provides engineered astrocytes as a new approach for long term protection in Huntington disease. Here we generated transgenic mice overexpressing BDNF under the promoter of the glial fibrillary acidic protein (GFAP) (pGFAP-BDNF mice). These mice are more resistant to quinolinate. pGFAP-BDNF derived astrocytes showed
higher levels of BDNF and larger neuroprotective effects than the wild-type ones when quinolinate was injected 30 days after grafting. These findings demonstrate that astrocytes engineered to release BDNF can constitute a therapeutic approach for Huntington’s disease (Gene Ther., 17, 1294-308, 2010).

9. **Impaired TrkB-mediated Erk1/2 activation in Huntington’s disease knock-in striatal cells involves reduced p52/p46 shc expression.** We demonstrate reduced TrkB mediated Ras/MAPK/ERK1/2 signaling but unchanged PI3K/Akt and PLC-gamma activation in knock-in HD striatal cells. These results suggest that in addition to reduced BDNF, diminished Ras/MAPK/ERK1/2 activation is involved on neurotrophic deficits associated to HD pathology. Therefore pharmacological approaches aimed to directly modulate the MAPK/ERK1/2 pathway may represent a valuable therapeutic strategy in HD (J. Biol. Chem., 285, 21537-2148, 2010).

10. **Altered cholesterol homeostasis contributes to enhanced excitotoxicity in Huntington’s Disease.** Our results indicate that mutant huntingtin alters cholesterol distribution that contributes to NMDA-mediated excitotoxicity. Administration of drugs that recover this effect, such as simvastatin could be beneficial for the treatment of Huntington’s disease (J. Neurochem., 115(1):153-67, 2010).

3. **Relevance and possible clinical applicability of the final results**

This project was focused on identifying new intracellular targets involved in the pathogenesis of Huntington disease. This neurodegenerative disorder induces severe motor and cognitive disturbances. To date there is no treatment for this disease. Therefore, it is very important to understand the pathophysiology involved in the neurodegenerative processes activated by mutant huntingtin to develop new neuroprotective treatments. In this project, we have identified new intracellular pathways that are affected by mutant huntingtin. These results may make it possible to develop new drugs which modulate these intracellular pathways to halt the pathologic
effects induced by mutant huntingtin. Another relevant issue of this project is that we also studied the cognitive alterations. These new data are very important in identifying complementary treatments for the cognitive disturbances observed in Huntington disease.

4. Publications


**BH-only proteins Bid and BimEL are differentially involved in neuronal dysfunction in mouse models of Huntington’s disease.**

JOURNAL OF NEUROSCIENCE RESEARCH, 85(12), 2756-2769, 2007 (IF 2.986)


**Disruption of striatal glutamatergic transmission induced by mutant huntingtin involves remodelling of both postsynaptic density and NMDA receptor signaling.**

NEUROBIOLOGY OF DISEASE, 29, 409–421, 2008 (IF 4.518)


**Bax deficiency promotes an up-regulation of BimEL and Bak during striatal and cortical postnatal development, and after excitotoxic injury.**

MOLECULAR AND CELLULAR NEUROSCIENCE, 37, 663-672, 2008 (IF 3.569)


**Calcineurin is involved in the early activation of NMDA-mediated cell death in mutant huntingtin knock-in striatal cells.**

JOURNAL OF NEUROCHEMISTRY, 105, 1596-612, 2008 (IF 3.999)

*Dopaminergic and glutamatergic signaling crosstalk in Huntington’s disease neurodegeneration: the role of p25/cyclin-dependent kinase 5.*

JOURNAL OF NEUROSCIENCE, 28(40):10090–10101, 2008 (IF 7.178)


*Brain Derived Neurotrophic Factor modulates the severity of cognitive alterations induced by mutant huntingtin: Involvement of phospholipaseCγ activity and glutamate receptor expression.*

NEUROSCIENCE, 158:1234-1250, 2009 (IF 3.292)


*Mutant Huntingtin Impairs Post-Golgi Trafficking to Lysosomes by Delocalizing Optineurin/Rab8 Complex from the Golgi Apparatus.*

MOLECULAR BIOLOGY OF THE CELL. 20:1478-1492, 2009 (IF 5.979)


*Reduced calcineurin protein levels and activity in exon-1 mouse models of Huntington’s disease: role in excitotoxicity.*

NEUROBIOLOGY OF DISEASE, 36(3):461-9, 2009 (IF 4.518)


*PH domain leucine-rich repeat protein phosphatase 1 contributes to maintain the activation of the PI3K/Akt pro-survival pathway in Huntington’s disease striatum.*


Ginés S., P. Paoletti and J. Alberch.

*Impaired TrkB-mediated ERK1/2 activation in Huntington’s disease knock-in striatal cells involves reduced p52/p46 Shc expression.*


*BDNF regulation under GFAP promoter provides engineered astrocytes as a new approach for long-term protection in Huntington’s disease.*  

GENE THERAPY, 7(10):1294-1308, 2010 (IF 4.745)


*Altered cholesterol homeostasis contributes to enhanced excitotoxicity in Huntington’s disease.*  