Ablation of huntingtin in adult neurons is nonleterious but its depletion in young mice causes acute pancreatitis

Guohai Wang, University of Chinese Academy of Sciences
Xudong Liu, University of Chinese Academy of Sciences
Shihua Li, Emory University
Marta A. Gaertig, Emory University
Xiao-Jiang Li, Emory University

Journal Title: PNAS
Volume: Volume 113, Number 12
Publisher: United States National Academy of Sciences | 2016-03-22, Pages 3359-3364
Type of Work: Article | Final Publisher PDF
Publisher DOI: 10.1073/pnas.1524575113
Permanent URL: https://pid.emory.edu/ark:/25593/vm9tb

Final published version: http://dx.doi.org/10.1073/pnas.1524575113

Copyright information:
© 2016 National Academy of Sciences.

Accessed October 18, 2023 11:38 PM EDT
Ablation of huntingtin in adult neurons is nondeleterious but its depletion in young mice causes acute pancreatitis

Guohao Wang\textsuperscript{a,b,c}, Xudong Liu\textsuperscript{a,b,c}, Marta A. Gaertig\textsuperscript{b}, Shihua Li\textsuperscript{b,1}, and Xiao-Jiang Li\textsuperscript{a,b,1}

\textsuperscript{a}State Key Laboratory of Molecular Developmental Biology, Institute of Genetics and Developmental Biology, Chinese Academy of Sciences, Beijing 100101, China; \textsuperscript{b}Department of Human Genetics, Emory University School of Medicine, Atlanta, GA 30322; and \textsuperscript{c}University of Chinese Academy of Sciences, Beijing 100049, China

Edited by Solomon H. Snyder, Johns Hopkins University School of Medicine, Baltimore, MD, and approved January 29, 2016 (received for review December 14, 2015)

Huntington’s disease (HD) protein, huntingtin (HTT), is essential for early development. Because suppressing the expression of mutant HTT is an important approach to treat the disease, we must first understand the normal function of Htt in adults versus younger animals. Using inducible Htt knockout mice, we found that Htt depletion does not lead to adult neurodegeneration or animal death at >4 mo of age, which was also verified by selectively depleting Htt in neurons. On the other hand, young Htt KO mice die at 2 mo of age of acute pancreatitis due to the degeneration of pancreatic acinar cells. Importantly, Htt interacts with the trypsin inhibitor, serine protease inhibitor Kazal-type 3 (Spink3), to inhibit activation of digestive enzymes in acinar cells in young mice, and transgenic HTT can rescue the early death of Htt KO mice. These findings point out age- and cell-type-dependent vital functions of Htt and the safety of knocking down neuronal Htt expression in adult brains as a treatment.

Huntingtin | aging | degeneration | acinus | pancreas

Significance

Because of the toxicity gain of expanded polyglutamine (polyQ) repeats, many studies have used RNAi and other approaches to inactivate the mutant HTT gene in Huntington’s disease. However, Htt is essential for early embryonic development, and normal function of Htt in adult animals remains unknown. Using conditional Htt knockout mice, we found that loss of Htt causes the lethal phenotype only in postnatal mice but not in adult mice, and this early death is due to acute pancreatitis. Htt interacts with serine protease inhibitor Kazal-type 3 (Spink3) to inhibit trypsin activity in pancreatic cells and thus prevents acinar cell degeneration and pancreatitis. This age- and cell-type-dependent function indicates the safety in knocking down neuronal HTT in adult brains for treating Huntington’s disease.
Depletion of Neuronal Htt in Adult Mice Does Not Cause Death. The above studies suggested that depletion of Htt in the brain might not be responsible for the death of young mice. To confirm this, we crossed floxed Htt mice with transgenic mice expressing Nestin-CreER, such that the resulting crossed mice (neuronal KO) had selectively depleted Htt expression in neuronal cells after tamoxifen injection. Cre expression driven by the neuronal Nestin promoter. Western blot analysis confirmed that tamoxifen injection caused Htt depletion in various brain regions in neuronal KO mice at different ages (Fig. 2A). As expected, very few (<18%) 2-mo-old neuronal KO mice died, and all neuronal KO mice >4 mo lived normally after Htt was depleted by tamoxifen injection (Fig. 2B). These living mice showed the same body weight, rotorod performance, and gripping ability as their controls (Fig. 2C). Because depletion of Htt is known to affect the neurogenesis or survival of developing neurons (16, 17), we examined 2-mo-old ubiquitous KO mice, but found no difference in the brain size and volume between controls and Htt KO mice (Fig. 3A and B). For neuronal KO mice, depletion of Htt by tamoxifen for 90 d did not show any difference in the brain volume compared with control mice (Fig. 3B). Histological examination showed no morphologic defects in neuronal KO mice (Fig. S2A), which is supported by Western blotting results that detected no significant changes in NeuN, β-tubulin III, GFAP, P62, LC3II, and caspase-3, except mouse Htt protein (Fig. 3 C and D). Immunofluorescent staining also did not show any changes in LC3II, β-tubulin III, and cleaved caspase-3 staining in ubiquitous and neuronal KO mouse brains (Fig. 3E and Fig. S2B). In addition, we crossed floxed Htt mice to transgenic mice that express CreER in the forebrain under the control of the neuronal promoter CaMKII. The crossed mice, upon tamoxifen injection, showed normal growth and survival without any detectable brain atrophy or degeneration (Fig. S3). Taken together, our results demonstrate that ubiquitous KO mice show an age-dependent death that is unlikely due to the depletion of Htt in neuronal cells or neuronal degeneration.

Htt Depletion Causes Acute Pancreatitis That Results from Acinar Cell Degeneration in Young Mice. We next focused on the peripheral tissue morphology of dead ubiquitous KO mice and found abnormal and inflamed abdominal cavities (Fig. 4A). There was significant inflammation in the abdominal organs, including intestine,
vessels, and pancreas, as evidenced by hemorrhagic, black-brown necrosis of the pancreatic parenchyma and peripancreatic fat (Fig. 4A and Fig. S4A). The pancreas was swollen and did not show the typical tan, lobulated architecture. All these features indicate acute pancreatitis. We then examined 2-mo-old ubiquitous KO mice after injecting tamoxifen to induce Htt depletion for different days and found the typical acute pancreatitis changes: the acinar cells were initially enlarged at day 3, and then the mice started to have ascites, ileus, and splanchnic hyperemia before their death at day 7 (Fig. 4B). Western blot results confirmed that Htt in pancreas is depleted in ubiquitous KO mice (Fig. 4C). However, histological examination revealed that living ubiquitous KO mice at 4 and 8 mo of age, which had been injected with tamoxifen for 5 d, show no abnormal pancreatic histology (Fig. S4B). Examination of ubiquitous KO mice that were injected with tamoxifen at 4 or 8 mo of age and then used to isolate their tissues 2 mo later did not reveal any abnormal histology in their pancreases.

Elastase 3B or protease E (CELA3B), which is secreted from acinar cells, nuclear chromatin clumping, and margination, indicating apoptosis, mitochondria enlargement, and edema, as well as lysosomes containing degenerated cytoplasmic elements or organelles (Fig. 4F). Quantification of zymogene granules and apoptosis or necrosis in pancreatic acinar cells also confirmed that Htt depletion increased the accumulation of zymogene granules and acinar cell death (Fig. S4D).

Htt Interacts with Spink3 to Inhibit Trypsin Activity. The phenotypes and morphological studies of 2-mo-old ubiquitous KO mice clearly indicate acute pancreatitis caused by Htt depletion. The acute pancreatic inflammation can be induced by activation of digested enzymes in pancreatic zymogens in acinar cells (19–21). This activation normally involves a hydrolysis reaction of inactivated forms by trypsin. Because we saw the degeneration of acinar cells, we focused on the molecules known to inhibit trypsin activity in acinar cells. Western blot results show that Spink3, a mouse homolog of human SPINK1 that inhibits trypsin in acinar cells (22), is selectively decreased in 2-mo-old mouse pancreatic tissues compared with 4- and 8-mo-old mouse pancreatic tissues (Fig. 5A). Because mutations in the human Spink3 homolog SPINK1 cause pancreatitis (23–26) and the selective decrease of Spink3 in young mouse pancreas is associated with acute pancreatitis in 2-mo-old ubiquitous KO mice, we further investigated whether Htt associates with Spink3 to inhibit trypsin. Using immunoprecipitation of pancreatic tissues from wild-type mice at 2, 4, and 8 mo of age, we found an age-dependent association of Htt with Spink3, which declines from 2 to 8 mo (Fig. 5B). This result suggests that the association of Htt with Spink3 in pancreatic tissues in young mice may be more important for Spink3’s function.

To verify the functional relevance of this binding, we transfected a series of Htt and Spink3 constructs into primary cultured acinar cells from ubiquitous KO mice. The cells were treated with tamoxifen (KO) to deplete endogenous Htt and then transfected with full-length Htt (HttT), N-terminal Htt (1–208 aa, nHtt), or truncated Htt (tHtt) lacking N-terminal 169 amino acids. Measuring trypsin activities of these transfected cells revealed that HttT and HttT, but not nHtt, could restore the inhibitory function of Htt on trypsin activity when endogenous Htt has been depleted (Fig. 5D). We also performed transfection of HEK293 cells with a series of Htt and Spink3 constructs. Without overexpression of Spink3 and Htt, HEK293 cell lysates show detectable trypsin activity when incubated with porcine trypsin and the substrate Boc-Gln-Ala-Arg-7-amino-4-methylcoumarin (AMC). This activity was inhibited by expressing Spink3 and could be further reduced when additional tHtt was also expressed (Fig. S5A). However, nHtt was unable to promote this inhibition, supporting the idea that a truncated Htt lacking the N-terminal region is able to bind Spink3 to inhibit trypsin activity.

Transgenic Htt Rescues the Early Death of Ubiquitous KO Mice. We have generated transgenic mice that express tHtt lacking N-terminal 169 amino acids under the control of the human Htt promoter (Fig. 6A and B). Because we have found that tHtt is able to bind Spink3 (Fig. 5C), we wanted to see if this transgenic Htt could rescue the acute pancreatitis and early death of ubiquitous KO mice. Thus, we crossed HttT transgenic mice with ubiquitous KO mice. PCR genotype indicates that the transgenic Htt existed in the crossed mice (Fig. 6C). The crossed mice were then injected with tamoxifen at the age of 6 wk to deplete the endogenous full-length Htt in mice. Mice carrying the transgenic Htt, however, continued to live versus the ubiquitous KO mice that did not express transgenic Htt (Fig. 6D). After depleting endogenous Htt for 5 mo, examination of the body weight of mice also revealed...
that these KO mice expressing tHTT (tHTT/KO) are indistinguishable from control mice (Fig. 6E). We then isolated the pancreatic tissues from tHTT/KO mice to perform Western blotting, which showed the expression of tHTT that is smaller than the endogenous full-length Htt in wild-type mouse pancreatic tissues (Fig. 6F). Moreover, the proteins for apoptosis (cleaved caspase3), necrosis (RIP3), autophagy (P62, LC3I/II), and CELA3B enzyme showed no alteration compared with those in the control mouse pancreatic tissues (Fig. 6G). Thus, this in vivo rescue effect also supports the idea that Htt is important for preventing pancreatic inflammation in young mice. Based on the results we obtained, we propose a model for the acute pancreatitis caused by the loss of Htt in young mice (Fig. S5B). In the pancreatic tissues of young mice, Htt binds Spink3 to help inhibit trypsin activity. Without Htt, this inhibition is abolished, leading to trypsin activation in acinar cells and resulting in acinar cell degeneration and pancreas inflammation, as well as early death. This function is not only cell-type specific but also age dependent, as aging increases the expression of Spink3, whose inhibitory effect on trypsin may also be dependent on other age-related factors.

Discussion

Htt is known to be essential for early embryonic development, and conditional knockout of Htt during the postnatal stage can also cause neurodegeneration (16). Interestingly, mutant Htt with expanded polyQ can rescue this embryonic lethality (27, 28). Deletion of polyQ or the proline-rich domain does not affect mouse development (29, 30). Also, humans homozygous for mutant expanded polyQ Htt age to adulthood with no obvious enhancement in pathology (31, 32), indicating that the expansion or lack of polyQ does not affect early development, but polyQ expansion causes late-onset neurodegeneration and neurological symptoms. Based on these findings, great efforts have gone into suppressing the expression of mutant Htt in the treatment of HD (13–15). Nevertheless, because of the essential function of Htt, a major concern has always been the possible side effects of depleting Htt. Our studies suggest that such concerns may be allayed if depletion of Htt occurs only in the brain or in older adults.

The previous studies using CaMKII-driven Cre transgenic mice show the degeneration of some neuronal cells and the degree of the degeneration appears to depend on age; the later depletion of Htt yielded a lesser extent of neuronal degeneration (16). Thus, Htt function in the brain appears to be age dependent, although its role in developing neuronal cells has been clearly shown by a number of studies (17, 33–35). Our studies show that in adulthood, Htt knockout does not affect neuronal survival or cause any significant movement or behavioral phenotypes. This finding is also supported by mice with selectively depleted Htt expression in the brain via Nestin-CreER or CaMKII-CreER. It should be pointed out that a few Nestin-Cre/KO mice die early, which is likely due to the expression of Cre in pancreatic acinar cells driven by the Nestin promoter (36). Thus, our studies suggest that depletion of Htt in adult brain would not cause severe phenotypes as seen in early development, providing a rationale to design a more...
efficient strategy to completely eliminate the expression of mutant HTT in adults.

Our findings also show that the interaction of Htt with Spink3 is important for preventing acute pancreatitis at young ages. Spink3 is the mouse homolog of serine protease inhibitor Kazal-type 1 (SPINK1) in humans, which is expressed at high levels in the pancreas (37, 38). SPINK1 is involved in pancreatitis in humans (24, 39, 40), as patients heterozygous for a SPINK1 mutation have a 20- to 40-fold increased risk of developing pancreatitis (23, 41, 42), and this risk may be as high as 500-fold in individuals with homozygous mutations (26). However, germ-line knockout of the mouse Spink3 does not lead to acute pancreatitis but autophagic death of pancreatic acinar cells, although the homozygous KO mice die postnatally. This difference could be due to the species- and age-dependent role of Spink3 in mouse, as Spink3 also plays important roles in the proliferation and/or differentiation of various cell types during mouse development (43). Indeed, a progressive degradation of the exocrine pancreas is seen at 16.5 days postcoitum in Spink3 KO mice, which was attributed to autophagic cell death (44). However, in adult mice, the expression level of Spink3 appears to be important for acute pancreatitis, because transgenic expression of pancreatic secretory trypsin inhibitor-1 rescues Spink3-deficient mice and restores a normal pancreatic phenotype (45).

Another issue is why the loss of Htt-mediated death is age dependent. We saw that Spink3 is expressed at a lower level in pancreatic tissues in young mice. We also show that Htt binds more Spink3 at younger ages. These results suggest that the association of Htt with Spink3 is particularly important for Spink3 to inhibit trypsin activities when Spink3 is at a low level in pancreatic acinar cells at young ages. In older mice, the higher levels of Spink3 could be due to its interaction with other proteins, which may substitute the function of Htt.

Our studies suggest that Htt possesses a cell type-specific function that is also age dependent, adding more complexity to this large protein. Although this is somewhat surprising, it is reasonable given that Htt associates with so many proteins (46, 47). It is also known that mutant Htt with expanded polyQ repeats could cause a loss of function. For example, polyQ expansion can inhibit the normal function of Htt by altering its interactions with other proteins. Such consequence could be different from the complete loss of normal Htt, as the complete loss of HTT may be compensated by other molecules, whereas a partial loss of normal function of htt due to polyQ expansion may also affect the function of other

---

Fig. 6. Rescued effect of transgenic HTT on Htt KO-induced pancreatitis. (A) THTT construct used for generation of the THTT transgenic mice. (B) Western blotting showing that THTT is expressed in the mouse brain cortex. Wild-type mice cortex was used as control. (C) PCR genotyping verified the presence of transgenic THTT in heterozygous (THTT/Ctl) and homozygous (THTT/KO) ubiquitous KO mice that also carry CAG-CreER. Ctl, control. (D) Survival rate of THTT/KO mouse and ubiquitous KO mice showing that THTT can prevent early death. (E) Body weight was tested at the fifth month after tamoxifen injection in the THTT/KO and control (THTT/Ctl) mice (paired two-tailed t test; n.s. represents no significant difference, n = 8, THTT/Ctl, male = 5, female = 3; control, male = 4, female = 4). (F) Western blotting showing that THTT is expressed in the ubiquitous KO mouse pancreas; heterozygous KO mouse pancreas was used as control. Note THTT is smaller than full-length Htt (Htt). Three mouse pancreases of each genotype were examined. (G) Western blotting of the CELA3B, caspase-3, P62, RIP3, and LC3II protein in the 2-mo-old thTT/KO and control mouse pancreas.

Wang et al.
proteins. The compensatory mechanisms for the loss of Htt may be cell-type and age dependent and can confound the interpretation of the function of Htt and the absence of overt phenotypes in adult mice that lack Htt. Despite further studies being required to understand the cell type-specific function of Htt, our results provide evidence for the first time to our knowledge that depletion of Htt causes cell type- and age-dependent phenotypes and also indicate the safety for a more efficient way of depleting neuronal Htt in adult brain to achieve better treatments for HD.

Materials and Methods

Htt floxP-flanked mice were provided by Scott Zeitlin, University of Virginia, Charlottesville, VA, and were maintained at the Emory University Animal Facility in accordance with the institutional animal care and use committee guidelines. All animal procedures were approved by the institutional animal care and use committee of Emory University. Additional information is provided in SI Materials and Methods.

ACKNOWLEDGMENTS. We thank Dr. Scott Zeitlin for providing floxed Htt KO mice, Heju Zhang at the Transgenic Mouse/Gene Targeting Core Facility at Emory University for generating transgenic mice, Dr. Hong Yi of the Robert P. Arkipian Integrated Electron Microscopy Core at Emory University for help with electron microscopy, and Cheryl Strauss for critical reading of this manuscript. This work was supported by National Natural Science Foundation of China Grant 91332206, National Institutes of Health Grants N5041669 and AG019206 (to X.-J.L.) and N5095279 (to S.L.), and the State Key Laboratory of Molecular Developmental Biology, China.