12

ZNF395 (HDBP2 /PBF) is a Target Gene of Hif-1α

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1. Introduction

The extention of the polyglutamin (polyQ) repeats within the N-terminus of the Huntingtin (Htt) protein causes Huntington´s disease (D) associated with aging and the accumulation of mutant (mt) Htt in the diseased neurons. The level of the mtHtt proteins and the length of the polyQ repeat determine the severity and progression of the disease. The intracellular aggregation of mtHtt to form insoluble inclusion bodies may be crucial to the development of the disease. One of the most important mechanisms by which the mutant Htt leads to cell cytotoxicity comprises transcriptional dysregulation (Cha, 2007). A variety of mechanisms have been attributed to result in large changes in the expression of coding and non-coding RNAs. In addition to the cytoplasm, the proteolytic fragments comprising the N-terminus of mtHtt aggregate in the nucleus where they sequester several co-factors and transcription factors such as CBP, p300, mSIN3a and Sp1. This might limit their access to DNA and decrease their normal transcriptional activity (Buckley et al., 2010).

Genes repressed by mtHtt include those controlling adaption to low mitochondrial energy charge. This may cause mitochondrial dysfunction resulting in aberrant energy metabolism which is one of the primary defects in Huntington´s D (Cui et al., 2006). A way to compensate for mitochondrial energy deficit is to shift the cell´s energy production from oxidative phosphorylation towards aerobic glycolysis, as observed upon adaption to hypoxia. The transcriptional upregulation of glycolytic enzymes in the presence of low O_2 tension occurs primarily through the hypoxia inducible transcription factor-1α (Hif-1α) that functions as a global regulator of O_2 homeostasis and adaption to low energy (reviewed in (Denko, 2008; Majmundar et al., 2010)). Under normoxia Hif-1α is an unstable protein. In the presence of O_2, prolylhydroxylases (PHD) act as oxygen sensors and hydroxylate a prolin in Hif-1α, which is a signal to initiate the proteasome mediated degradation of Hif-1α. Upon hypoxia, PHD are inactive, resulting in the stabilization of Hif-1α, which can then dimerize with its interaction partner Hif-1β and bind to its specific recognition sequence, the hypoxia response element (HRE), present in the control regions of its targets genes. Hif-1α activates more than 100 genes associated with the adaption to hypoxic stress, including genes involved in angiogenesis, cell survival and aerobic glycolysis. Hif-1α stimulates glycolytic energy production by transactivating genes involved in extracellular glucose import (such as GLUT1) and coding for enzymes responsible for the breakdown of intracellular glucose. Small molecule inhibitors of PHD have been shown to protect neurons from ischemic or

oxidative injury. PHD inhibitors, resulting in activation of Hif-1α, are able to prevent neuronal death induced by mitochondrial toxins and have therapeutic implications in the treatment for Huntington´s D and Alzheimer D (Niatsetskaya et al., 2010). Inhibition of PHD may also prevent mitochondrial toxicity in glioma cells. Thus, PHD inhibitors are regarded as promising candidates for preventing cell death in Huntington´s D as well as other neurodegenerative Ds associated with metabolic stress (Harten et al., 2010). On the other side, Hif-1α was among the genes whose expression was significantly upregulated in brain from post-mortem Huntington´s D patients as well as in blood samples from symptomatic patients in contrast to non symptomatic patients and healthy individuals, suggesting a role of these factors in disease development. Elevated level of these factors including Hif-1α was also correlated with disease progression and response to treatment (Borovecki et al., 2005; Lovrecic et al., 2009) indicating that Hif-1α activation may not only be beneficial for the disease outcome. In order to consider PHD inhibitors as neurological therapeutics, it is necessary to characterize their effect in the cells at the molecular level. We describe here that Hif-1α activates the expression of Huntington´s D binding protein 2 (HDPB2), a protein binding to a DNA segment within the Htt promoter that mediates neuronal cell specific activation of Htt expression (Tanaka et al., 2004) and discuss its potential implication for Huntington´s D.

2. ZNF395 is identical to HDBP2, binding to a neuronal specific regulatory element of the Htt promoter

HDBP1 and HDBP2 are two closely related proteins that were identified as transcription factors binding to a 7bp GC rich sequence which resides in triplicate at intervals of 13bp within and proximal to the -20bp direct repeat sequences of the Htt promoter (for overview see Fig. 1A). Two years earlier, we have identified the cellular factor HDBP2 by its ability to bind to regulatory regions in papillomaviruses (PV) and subsequently called the protein papillomavirus binding factor (PBF) (Boeckle et al., 2002). The official gene name is ZNF395, which we will use here. HDBP1 is identical to GLUT4-Enhancer factor (GEF) which activates the gene expression of GLUT4, a glucose transporter. HDBP1/GEF and ZNF395 are closely related to the mouse glucocorticoid induced gene 1 (GIG1, human ZNF704). It has been suggested that these three proteins that are conserved from drosophila to vertebrates build up a new family of transcription factors. They share three conserved regions CR1, CR2 and CR3, a domain rich in serines and prolines and have the potential to form a zinc-finger structure (see Fig. 1C for overview). The C-terminal CR3 is responsible for DNA-binding (Sichtig et al., 2007a; Tanaka et al., 2004). This region is highly similar to the 30-amino acid auxiliary DNA interaction motif present in "E" variants of TCF transcription factors (Atcha et al., 2007). Although the recognition motif of ZNF395 has not yet been determined, it recognizes GC rich sequences which is supported by the finding that ZNF395 was "very strongly" excluded from DNA after CpG methylation in a genome-wide screen (Bartke et al., 2010). Moreover, it binds to GCCGGCG in the Huntington´s D gene promoter (Tanaka et al., 2004) and a CCGG in HPV8 (Boeckle et al., 2002) while GEF binds ACCGG within GLUT4 (Knight et al., 2003; Oshel et al., 2000). While in Drosophila GEF was found to be required for normal wing-positioning (Yazdani et al., 2008), a physiological role of these factors in vertebrates is unknown. ZNF395 was characterized as a nucleo-cytoplasmic shuttling protein (Tanaka et al., 2004). We could show that its subcellular localization seems to be regulated by growth factors, since recombinant ZNF395 entered the nucleus upon withdrawal of growth factors from the cell

culture medium. The binding to 14-3-3β contributes to the control of the subcellular localization of ZNF395. Moreover, over-expression of ZNF395 resulted in inhibition of cell growth (Sichtig et al., 2007b). ZNF395-mediated growth inhibition of osteosarcoma cell lines was shown to rely on apoptosis (Tsukahara et al., 2008).

2.1 ZNF395 is a repressor of PV gene expression

In the case of PV, mutations abolishing the DNA binding of ZNF395 reduced the promoter activity, from which we concluded that ZNF395 is a transcriptional activator (Boeckle et al., 2002). Surprisingly, the over-expression of ZNF395 resulted in repression of transcription from the PV promoters. This repression was dependent on the recruitment of the mSIN3A/HDAC1/2 complex via a direct interaction of ZNF395 with Sin3A associated protein of 30kDa (SAP30), a component of this complex. Moreover, transcriptional repression required the intact CR3, indicating that ZNF395 has to bind to DNA (Sichtig et al., 2007a).

2.2 Recombinant ZNF395 is a repressor of the Htt promoter

Similar to the situation observed with PV promoters, mutations within the 7bp motif that abolished binding of GEF/HDBP1 and ZNF395/HDBP2, reduced the Htt promoter activity in a neuronal cell line, while there was no effect in HeLa cells (Tanaka et al., 2004), indicating that these two factors are involved in neuronal specific gene expression of Htt. However, neither the direct involvement of these two factors in the control of Htt expression nor their specific activity has been analyzed. In order to investigate the role of ZNF395 on the expression of Htt we performed transient transfections with two different reporter constructs. The first construct contained the Htt promoter and 1032bp of its upstream regulatory region (-1032-Htt-Luc) while the second had 324bp of the upstream region of the Htt promoter (-324-Htt-Luc). Both constructs that were kindly provided by Coles et al. (Coles et al., 1998) contained the 21 base pair repeat flanked by three copies of the 7bp GC rich sequence, the putative DNA segment bound by HDBP1/GEF and HDBP2/ZNF395 (Tanaka et al., 2004; see Fig. 1A). We used an immortalized keratinocyte cell line, since we have initially isolated the ORF for ZNF395 from these cells. As shown in Fig. 1, transfecting increasing amounts of an expression vector for ZNF395 resulted in a dose dependent repression of the promoter up to 90%. The level of repression was similar with both constructs. Thus, consistent with the situation in PV, heterologous ZNF395 acts as repressor of Htt. In order to exclude that a cell specific factor is required for ZNF395 to activate we used U87 MG cells, a human glioblastoma cell line. Again, over-expression of ZNF395 induced 80% repression of the promoter with the -324-Htt-Luc reporter. In order to further address the mechanism of repression we tested a set of ZNF395 mutants. Most of the repression was relieved when over-expressing ZNF395mtCR3, devoid of DNA-binding, indicating that ZNF395 has to bind to DNA to act as transcriptional repressor. ZNF395Δ280-312 also revealed a reduced repression although it was still able to decrease luc activity by 60%. Thus, recruitment of the mSIN3A/HDAC1 complex via interaction of amino acids 280-312 with SAP30 may be involved, although regions outside contribute to the repression. The co-transfection of an expression vector for HDBP1/GEF did not stimulate the activity of the HD promoter as well. In contrast to ZNF395, GEF only slightly repressed the HD promoter in this assay (Fig. 1C), even when increasing amounts of expression vectors have been transfected (data not shown), indicating that both proteins might affect Huntington´s D gene expression differentially.

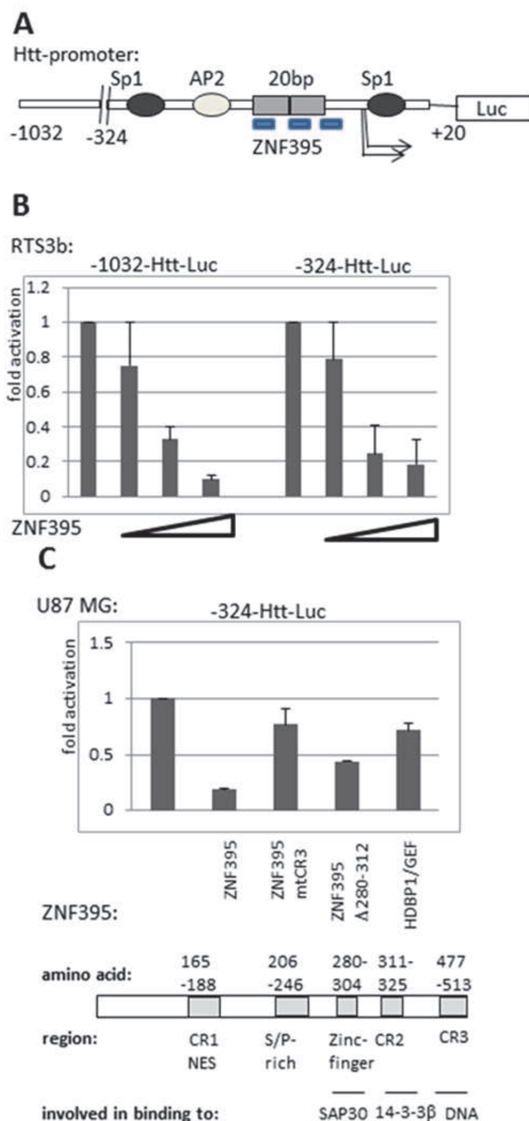

Fig. 1. ZNF395 represses the Huntington´s D gene promoter. RTS3b cells (B) and U87 MG cells (C) were transiently transfected with luciferase reporter constructs containing the Htt promoter including its upstream region up to -324 or -1032, respectively, together with an expression vector for ZNF395 (5, 10ng and 20ng in B and 10ng in C) or for HDBP1/GEF (described in Knight et al., 2003) as indicated. The cells were transfected by the FuGene reagent (Roche diagnostics) and 48h later luciferase activity was determined. The results represent the means of two (in C) and three (in B) independent experiments and the standard deviations are shown. The structure of the Htt promoter is given in (A) and the structure of ZNF395 with its domains that are described in the text in (C).

2.3 ZNF395 is over-expressed in cancers and is a target gene of Hif-1α

Data obtained from transcriptional profiling implied that ZNF395 is a target gene of Hif-1α. For instance, ZNF395 was among the genes activated by hypoxia, by over-expression of Hif-1α, in the absence of von Hippel Lindau (VHL) proteins or by treating the cells with a chemical inducer of Hif-1α, DMOG (dimethyloxalyl-glycine) (Jiang et al., 2003; Lal et al., 2001). Consistent with these reports, ZNF395 was among hypoxia inducible genes that represent a hypoxic signature in neuroblastoma cell lines and neuroblastomas as well as in glioblastomas (Fardin et al., 2010; Murat et al., 2009). These reports imply that ZNF395 over-expression may have a functional role in cancer progression and in Hif-1α regulated pathways.

2.3.1 Hif-1α activates the expression of ZNF395

In order to investigate a role of Hif-1α in the regulation of expression of ZNF395, we treated RTS3b cells with DMOG for 24h prior harvesting. The Western Blot shown in Fig. 2 demonstrates that the level of Hif-1α protein increased, which is in line with the stabilization of Hif-1α due to the inhibition of PHD. Only from extracts of cells that have been treated with DMOG, we were able to precipitate ZNF395 by a specific antibody, indicating that Hif-1α mediated activation of ZNF395 expression is also reflected by increased protein level. In order to address the role of Hif-1α in regulation of ZNF395 expression in more detail, we cloned a cellular DNA fragment harboring the putative promoter of ZNF395 by PCR. A fragment spanning 1190 bases upstream of the initiation site (-1190) to 51 bases downstream (+51) of the mRNA for ZNF395 was amplified from total genomic DNA of RTS3b cells and cloned into a luciferase reporter gene vector. An analysis of putative transcription factor binding sites predicted a high affinity HRE at pos. -815 (Fig. 2B). The co-transfection of an expression vector for Hif-1α increased the promoter activity 1.5 fold. The deletion of the segment from -830 to –565, thus removing the HRE at pos. -815, eliminated this small activation indicating that the effect is specific and the HRE is required, although the activation is much smaller than observed in microarrays, where up to 7 fold inductions of ZNF395 specific mRNA level were described (Jiang et al., 2003; Lal et al., 2001). A second putative HRE located 2000bp further upstream and not included in the DNA segment in our reporter construct might contribute to the Hif-1α mediated regulation of the ZNF395 expression. Moreover, our preliminary results indicate that Hif-1α cooperates with other transcription factors binding to the promoter region of ZNF395 as well (own unpublished results).

3. Conclusions

Activation of the Hif-1α-pathway by inhibitors of PHDs was shown to prevent neuronal cell death in a Huntington´s D cell culture model, thus PHD inhibitors might be considered as therapeutics. Our data shown here imply that PHD inhibitors will also induce ZNF395 via Hif-1α. ZNF395 will then bind to the Htt promoter and contribute to the control of the expression of mtHtt. Our findings that ZNF395 represses the Htt-promoter implicate that ZNF395 contributes to an amelioration of the disease and/or a slowing down of disease progression achieved by PHD inhibitors. However, until now an involvement of ZNF395 in the regulation of Htt is not convincingly shown at all and has to be analyzed carefully.

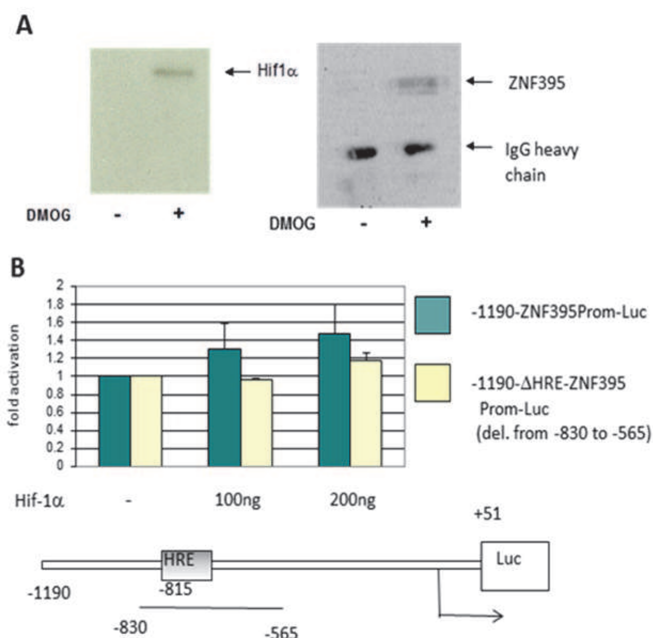

Fig. 2. Hif-1α activates ZNF395 expression. (A) RTS3b cells were treated with 1mM DMOG for 24h. Cell extracts were used in Western Blot developed with an antibody against Hif-1α (from Epitomics) (on the left) or for an immunoprecipitation with an antibody against ZNF395 followed by Western Blot, developed with the anti-ZNF395 antibody described in (Boeckle et al., 2002). The positions of Hif-1α and ZNF395 are indicated. (B) RTS3b cells were transiently transfected as described in figure 1, with a reporter construct containing a 1190 bp fragment upstream of the initiation site of the ZNF395 gene in front of the luciferase and an expression vector for Hif-1α. In the construct -1190-ΔHRE-ZNF395Prom-Luc the segment from -830 to -565 has been deleted. The structure of the ZNF395 promoter with the position of the putative HRE is shown beneath the graph. The graph represents the means of three independent experiments and the standard deviations are shown.

Tanaka et al. concluded that ZNF395 acts as activator since the mutations that reduced promoter activity also resulted in loss of binding of ZNF395 in vitro (Tanaka et al., 2004). However, it cannot not be excluded that the mutations affected the binding of another factor recognizing a similar sequence and mediating activation. A chromatin immunoprecipitation assay might be performed to reveal the presence of endogenous ZNF395 on the Htt promoter in stratial cells. A neuronal cell specific knock out of ZNF395 in Htt mouse models that have been described will provide evidence for the implication and the specific role of ZNF395 in the control of Htt expression. The effect of ZNF395 on the Htt promoter is strikingly similar to that observed for the PV promoters. Consistently, eliminating the binding of ZNF395 in vitro reduced the PV-promoter activity, but over-expression of the recombinant protein efficiently repressed the PV promoter, which required the DNA-binding domain of ZNF395 and the segment binding to SAP30 (Sichtig et al., 2007a). This may reflect that ZNF395 acts as activator or as repressor of transcription. The specific effect

of ZNF395, including its stability and subcellular localization might be controlled by post-translational modifications such as phosphorylation and ubiquitination. In line with this, we found that Akt-kinase-mediated phosphorylation of ZNF395 at S447/449/451 creates an interaction motif for 14-3-3β, which contributes to the control of the subcellular localization of ZNF395 and its cell growth inhibitory function (Sichtig et al., 2007b). Elucidating these modifications, the associated pathways and their consequences for the activity of ZNF395 is a prerequisite to understand a role in Huntington´s D.

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5. References

Atcha, F. A., Syed, A., Wu, B., Hoverter, N. P., Yokoyama, N. N., Ting, J. H., Munguia, J. E., Mangalam, H. J., Marsh, J. L. & Waterman, M. L. (2007). A unique DNA binding domain converts T-cell factors into strong Wnt effectors. *Mol Cell Biol* 27, 8352-8363.

Bartke, T., Vermeulen, M., Xhemalce, B., Robson, S. C., Mann, M. & Kouzarides, T. (2010). Nucleosome-interacting proteins regulated by DNA and histone methylation. *Cell* 143, 470-484.

Boeckle, S., Pfister, H. & Steger, G. (2002). A new cellular factor recognizes E2 binding sites which mediate transcriptional repression by E2. *Virology* 293, 103-117.

Borovecki, F., Lovrecic, L., Zhou, J., Jeong, H., Then, F., Rosas, H. D., Hersch, S. M., Hogarth, P., Bouzou, B., Jensen, R. V. & Krainc, D. (2005). Genome-wide expression profiling of human blood reveals biomarkers for Huntington's disease. *Proc Natl Acad Sci U S A* 102, 11023-11028.

Buckley, N. J., Johnson, R., Zuccato, C., Bithell, A. & Cattaneo, E. (2010). The role of REST in transcriptional and epigenetic dysregulation in Huntington's disease. *Neurobiol Dis* 39, 28-39.

Cha, J. H. (2007). Transcriptional signatures in Huntington's disease. *Prog Neurobiol* 83, 228-248.

Coles, R., Caswell, R. & Rubinsztein, D. C. (1998). Functional analysis of the Huntington's disease (HD) gene promoter. *Hum Mol Genet* 7, 791-800.

Cui, L., Jeong, H., Borovecki, F., Parkhurst, C. N., Tanese, N. & Krainc, D. (2006). Transcriptional repression of PGC-1alpha by mutant huntingtin leads to mitochondrial dysfunction and neurodegeneration. *Cell* 127, 59-69.

Denko, N. C. (2008). Hypoxia, HIF1 and glucose metabolism in the solid tumour. *Nat Rev Cancer* 8, 705-713.

Dunah, A. W., Jeong, H., Griffin, A., Kim, Y. M., Standaert, D. G., Hersch, S. M., Mouradian, M. M., Young, A. B., Tanese, N. & Krainc, D. (2002). Sp1 and TAFII130 transcriptional activity disrupted in early Huntington's disease. *Science* 296, 2238-2243.

Fardin, P., Barla, A., Mosci, S., Rosasco, L., Verri, A., Versteeg, R., Caron, H. N., Molenaar, J. J., Ora, I., Eva, A., Puppo, M. & Varesio, L. (2010). A biology-driven approach identifies the hypoxia gene signature as a predictor of the outcome of neuroblastoma patients. *Mol Cancer* 9, 185.

Harten, S. K., Ashcroft, M. & Maxwell, P. H. (2010). Prolyl hydroxylase domain inhibitors: a route to HIF activation and neuroprotection. *Antioxid Redox Signal* 12, 459-480.

Jiang, Y., Zhang, W., Kondo, K., Klco, J. M., St Martin, T. B., Dufault, M. R., Madden, S. L., Kaelin, W. G., Jr. & Nacht, M. (2003). Gene expression profiling in a renal cell carcinoma cell line: dissecting VHL and hypoxia-dependent pathways. *Mol Cancer Res* 1, 453-462.

Knight, J. B., Eyster, C. A., Griesel, B. A. & Olson, A. L. (2003). Regulation of the human GLUT4 gene promoter: interaction between a transcriptional activator and myocyte enhaner factor 2A. *Proc Natl Acad Sci U S A* 100, 14725-14730.

Lal, A., Peters, H., St Croix, B., Haroon, Z. A., Dewhirst, M. W., Strausberg, R. L., Kaanders, J. H., van der Kogel, A. J. & Riggins, G. J. (2001). Transcriptional response to hypoxia in human tumors. *J Natl Cancer Inst* 93, 1337-1343.

Li, S. H., Cheng, A. L., Zhou, H., Lam, S., Rao, M., Li, H. & Li, X. J. (2002). Interaction of Huntington disease protein with transcriptional activator Sp1. *Mol Cell Biol* 22, 1277-1287.

Lovrecic, L., Kastrin, A., Kobal, J., Pirtosek, Z., Krainc, D. & Peterlin, B. (2009). Gene expression changes in blood as a putative biomarker for Huntington's disease. *Mov Disord* 24, 2277-2281.

Majmundar, A. J., Wong, W. J. & Simon, M. C. (2010). Hypoxia-inducible factors and the response to hypoxic stress. *Mol Cell* 40, 294-309.

Murat, A., Migliavacca, E., Hussain, S. F., Heimberger, A. B., Desbaillets, I., Hamou, M. F., Ruegg, C., Stupp, R., Delorenzi, M. & Hegi, M. E. (2009). Modulation of angiogenic and inflammatory response in glioblastoma by hypoxia. *PLoS One* 4, e5947.

Niatsetskaya, Z., Basso, M., Speer, R. E., McConoughey, S. J., Coppola, G., Ma, T. C. & Ratan, R. R. (2010). HIF prolyl hydroxylase inhibitors prevent neuronal death induced by mitochondrial toxins: therapeutic implications for Huntington's disease and Alzheimer's disease. *Antioxid Redox Signal* 12, 435-443.

Oshel, K. M., Knight, J. B., Cao, K. T., Thai, M. V. & Olson, A. L. (2000). Identification of a 30-base pair regulatory element and novel DNA binding protein that regulates the human GLUT4 promoter in transgenic mice. *J Biol Chem* 275, 23666-23673.

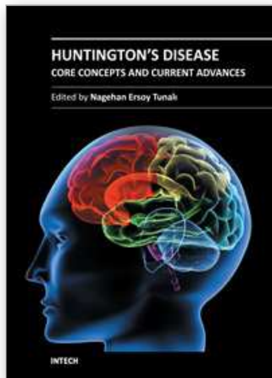
Sichtig, N., Körfer, N. & Steger, G. (2007a). Papillomavirus binding factor represses transcription via recruitment of the Sin3/HDAC co-repressor complex. *Archives of Biochemistry and Biophysics* 467, 67-75.

Sichtig, N., Silling, S. & Steger, G. (2007b). Papillomavirus binding factor (PBF) mediated inhibition of cell growth is regulated by 14-3-3 ß. *Archives of Biochemistry and Biophysics* 464, 90-99.

Tanaka, K., Shouguchi-Miyata, J., Miyamoto, N. & Ikeda, J.-E. (2004). Novel nuclear shuttle proteins, HDBP1 and HDBP2, bind to neuronal cell-specific cis-regulatory element in the promoter for the human Huntington disease gene. *J Biol Chem* 279, 7275-7286.

Tsukahara, T., Kimura, S., Ichimiya, S., Torigoe, T., Kawaguchi, S., Wada, T., Yamashita, T. & Sato, N. (2009). Scythe/BAT3 regulates apoptotic cell death induced by papillomavirus binding factor in human osteosarcoma. *Cancer Sci.* 100, 47-53.

Yazdani, U., Huang, Z. & Terman, J. R. (2008). The glucose transporter (GLUT4) enhancer factor is required for normal wing positioning in Drosophila. *Genetics* 178, 919-929.



Huntington's Disease - Core Concepts and Current Advances
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Huntington's Disease is one of the well-studied neurodegenerative conditions, a quite devastating and currently incurable one. It is a brain disorder that causes certain types of neurons to become damaged, causing various parts of the brain to deteriorate and lose their function. This results in uncontrolled movements, loss of intellectual capabilities and behavioural disturbances. Since the identification of the causative mutation, there have been many significant developments in understanding the cellular and molecular perturbations. This book, "Huntington's Disease - Core Concepts and Current Advances", was prepared to serve as a source of up-to-date information on a wide range of issues involved in Huntington's Disease. It will help the clinicians, health care providers, researchers, graduate students and life science readers to increase their understanding of the clinical correlates, genetic aspects, neuropathological findings, cellular and molecular events and potential therapeutic interventions involved in HD. The book not only serves reviewed fundamental information on the disease but also presents original research in several disciplines, which collectively provide comprehensive description of the key issues in the area.

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