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Research Report

Intracellular translocation of histone deacetylase 5 regulates neuronal cell apoptosis

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ABSTRACT

Histone deacetylase 5 (HDAC5) undergoes signal-dependent shuttling between the nucleus and cytoplasm, which is regulated in part by calcium/calmodulin-dependent kinase (CaMK)-mediated phosphorylation. Here, we report that HDAC5 regulates the survival of cortical neurons in pathological conditions. HDAC5 was evenly localized to the nucleus and cytoplasm in cultured cortical neurons. However, in response to 50 μM NMDA conditions that induced neuronal cell apoptosis, nuclear-distributed HDAC5 was rapidly phosphorylated and translocated into cytoplasm of the cultured cortical neurons. Treatment with a CaMKII inhibitor KN93 suppressed HDAC5 phosphorylation and nuclear translocation induced by NMDA, whereas constitutively active CaMKIIα (T286D) stimulated HDAC5 nuclear export. Importantly, we showed that ectopic expression of nuclear-localized HDAC5 in cortical neurons suppressed NMDA-induced apoptosis. Finally, inactivation of HDAC5 by treatment with the class II-specific HDAC inhibitor trichostatin A (TSA) promoted NMDA-induced neuronal cell apoptosis. Altogether, these data identify HDAC5 and its intracellular translocation as key effectors of multiple pathways that regulate neuronal cell apoptosis.

1. Introduction

Epigenetic changes induced by chemical exposure are believed to be involved in the pathogenesis of chronic neurological disorders including Alzheimer's, Parkinson's and Huntington's diseases (Arias et al., 1998; Schiefer et al., 2002; Song et al., 2011). However, little is known about the exact epigenetic mechanisms underlying neurotoxic chemical exposure in nervous system and the relevance of these epigenetic changes to the pathogenesis of neurodegenerative diseases. Histone modification, including methylation, phosphorylation, acetylation, and ubiquitination, has been linked to many human diseases in recent years (Somech et al., 2004). Among the various histone modifications, the acetylation of specific lysine residues in the N-terminal tails of histones has been increasingly recognized as a crucial mechanism in several important phenomena in the brain, including neuronal differentiation (Hsieh and Gage, 2004), neurodegeneration (Song et al., 2011), circadian rhythm (Etchegaray et al., 2003) and memory formation (Levenson et al., 2004). This elaborate epigenetic process is carried out by many classes of chromatin-modifying enzymes. A solid body of evidence suggests that HDACs regulate neuron viability (Kazantsev and Thompson, 2008; Majdzadeh et al., 2008; Morrison et al., 2006; Sleiman et al., 2009).

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Histone deacetylases (HDACs) are enzymes that modulate the acetylation level of histone and non-histone proteins to regulate chromatin structure and gene expression. It is well known that class IIa HDACs play important roles in brain development and neuron survival (Bolger and Yao, 2005; Chen and Cepko, 2009; Ma and D'Mello, 2011). Class IIa HDACs (HDAC4, 5, 7, 9) are specifically expressed in a variety of tissues, with highest expression in the brain and skeletal muscle. Some of the class II HDACs can shuttle between the nuclear and the cytoplasm depending on their phosphorylation status. The examination of the sequences surrounding the conserved phosphorylation residues in class IIa HDACs revealed that they are closely related to the consensus phosphorylation sites of calcium/calmodulin-dependent kinase (CaMK) (McKinsey et al., 2000a; McKinsey et al., 2000b). Phosphorylation recruits the phosphor-binding protein 14-3-3, and the resulting complex is exported efficiently from the nucleus (de Ruijter et al., 2003). Importantly, nuclear export of HDAC5 can be observed in apoptotic cardiac myocytes (Zhang et al., 2011). Moreover, upon prolonged genotoxic stress, HDAC5 is phosphorylated and undergoes nuclear export, which triggers extensive apoptosis of cancer cells (Sen et al., 2013). However, the role of HDAC5 in neurodegenerative diseases is not completely understood.

A wide range of acute and chronic brain injury diseases, such as stroke/ischemia, epilepsy and certain neurodegenerative disorders have been linked to NMDA receptor-mediated neuronal cell death (Gielen et al., 2009; Liu et al., 2010; Xia et al., 1996). The NMDA receptor complex constitutes an ion channel which is preferentially permeable to Ca^{2+} (Li et al., 2008). Excessive activation of NMDA receptors may cause intracellular calcium overload, leading to an enzymatic cascade of events resulting ultimately in cell death known as excitotoxicity (Takei and Endo, 1994). CaMKII is a general integrator of Ca^{2+} signaling that has been linked to regulation of apoptosis (Chen et al., 2013; Liu and Templeton, 2007). Furthermore, HDAC5 is a key target of activated CaMKII (Renthal et al., 2007), suggesting it has function there. Thus, the role of HDAC5 in NMDA-induced neuronal cell apoptosis was evaluated in the present study.

2. Results

2.1. *Nuclear export of HDAC5 is involved in NMDA-induced apoptosis*

To assess whether HDAC5 is involved in NMDA-induced neuronal cell apoptosis, we first detected distribution of HDAC5 in cortical neurons, which were treated or not for 3 h with 50 μM NMDA. As shown in Fig. 1A, HDAC5 was evenly localized to the nucleus and cytoplasm in normal cortical neurons, whereas HDAC4 exhibited little nuclear accumulation. Furthermore, after exposure of cultured cortical neurons to NMDA, endogenous HDAC5 shifted out of the nucleus. To confirm these results, the cytoplasmic (Cyto) and nuclear (Nucl) cell fractions were separated from the cortical neurons, HDAC4 and HDAC5 levels were detected by western blot (Fig. 1B). Nuclear export of HDAC5 but not HDAC4 was induced by NMDA treatment (Fig. 1C and D).

The phosphorylation of class IIa HDACs is a crucial event that determines whether they are localized in the nucleus or cytoplasm (McKinsey et al., 2000a). We then analyzed the phosphorylation of HDAC5 at S259 and S498 in cortical neurons by western blot (Fig. 1E). Treatment with 50 μM NMDA induced a time-dependent increase in activation of caspase-3 (Fig. 1F), which was associated with increased phosphorylation of HDAC5 at S259 and S498 (Fig. 1G). These data suggest that nuclear export of HDAC5 is involved in NMDA-induced neuronal cell apoptosis.

2.2. *NMDA-induced HDAC5 phosphorylation and nuclear export is dependent of CaMKII activation*

It is known that calcium signaling-mediated Ca^{2+}/calmodulin-dependent kinase II (CaMKII) activity is the most crucial of the pathway involved in regulation of apoptosis (Chen et al., 2013; Liu and Templeton, 2007). To identify the mechanism underlying NMDA-induced phosphorylation of HDAC5, we conducted kinase inhibition assays to determine whether CaMKII signaling is involved. Phosphorylation of CaMKIIα and HDAC5 were detected by western blot (Fig. 2A). Treatment with 50 μM NMDA for 3 h increased phosphorylation of CaMKIIα at T286, whereas CaMKII inhibitor NK-93 inhibited this effect of NMDA (Fig. 2B). Furthermore, KN-93 decreased NMDA-induced phosphorylation of HDAC5 at S259 and S498 (Fig. 2C). To confirm these results, cortical neurons were co-transfected with expression vectors for HDAC5-EGFP and CaMKIIα(WT)-DsRed or constitutively active CaMKIIα(T286D)-DsRed using calcium phosphate. Pretreatment with KN-93 suppressed NMDA-induced HDAC5 nuclear export, whereas constitutively active CaMKIIα(T286D) blocked the inhibitory effect of KN-93 on the nuclear export of HDAC5 in cortical neurons (Fig. 2D). Further results from western blot confirmed the above results (Fig. 2E). These findings suggest that NMDA-induced HDAC5 phosphorylation and nuclear export is dependent of the activation of CaMKIIα.

2.3. *Nuclear localization of HDAC5 inhibits NMDA-induced apoptosis*

To further confirm the involvement of the HDAC5 during NMDA-induced neuronal cell apoptosis, a non-phosphorylatable mutant HDAC5(S259A/S498A)-EGFP was constructed. Cortical neurons were co-transfected with expression vectors for CaMKIIα-DsRed and HDAC5(WT)-EGFP or HDAC5(S259A/S498A)-EGFP using calcium phosphate. As shown in Fig. 3A, nuclear export of wild-type HDAC5 was observed in apoptotic neuron induced by NMDA. In contrast, the distribution of phosphorylation-deficient mutant of HDAC5 (HDAC5S259A/S498A) was not affected by NMDA, and this nuclear-localized HDAC5 inhibited NMDA-induced neuronal cell apoptosis. Further results from western blot confirmed the above results (Fig. 3B). These findings clearly support the idea that nuclear export of HDAC5 is required for NMDA-induced apoptosis in cortical neurons.

2.4. *Inhibition of histone deacetylases increases NMDA-induced apoptosis in cortical neurons*

If nuclear accumulation of HDAC5 is important for neuron survival, then inhibition of HDAC5 activity in the nucleus should be more facilitative to NMDA-induced neuronal cell apoptosis.