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BRAIN RESEARCH

Research Report

p300 expression is induced by oxygen deficiency and protects neuron cells from damage

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ABSTRACT

Low oxygen level or oxygen deficiency (hypoxia) is a major factor causing neuronal damage in many diseases. Inducing cell adaptation to hypoxia is an effective method for neuroprotection that can be achieved by either inhibiting the death effectors or enhancing the survival factors. Transcription coactivator p300 is necessary for hypoxia-induced transcriptional activation and plays an important role in neuron survival. However, the alteration of p300 expression under hypoxia condition and its role in hypoxia-induced neuronal damage remain unclear. In this study, the distribution of p300 in rat brain and the alteration of its expression in rat hippocampus during hypobaric hypoxia exposure were detected. In addition, the role of p300 in neuronal-like PC12 cell damage induced by oxygen deficiency (3% oxygen) was evaluated. Our results showed that p300 protein was mainly expressed in the cells expressed β-tubulin *III* in the cerebral cortex, hippocampus, cerebellum cortex, medulla oblongata and hypothalamus. Less or no positive signal of p300 expression was observed in β-tubulin *III* negative cells. This indicated that p300 was predominantly expressed in neurons of rat brain. Furthermore, p300 expression was up-regulated in rat hippocampus during hypoxia exposure and in neuronal-like PC12 cells under 3% oxygen condition. Interestingly, neuronal-like PC12 cell damage induced by oxygen deficiency (3% oxygen) was increased by suppression of p300 expression with short hairpin RNA (shRNA). These data indicate that p300 is an important molecule for neuroprotection under hypoxia.

1. Introduction

Low oxygen level or oxygen deficiency (hypoxia) is a major factor causing neuronal damage in stroke, heart failure, and related diseases. Inducing cell adaptation to hypoxia is an effective method for neuroprotection that can be achieved by either inhibiting cell death effectors or enhancing survival factors. However, cell responses to hypoxia involved in cell survival or death are mediated by multiple transcriptional factors, such as p53, HIF-1 and NF-κB. This suggests that the

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Abbreviations: HIF-1, hypoxia-inducible factor 1; BCIP, 5-bromo-4-chloro-3-indoly-phosphate; NBT, nitroblue tetrazolium chloride; BSA, bovine serum albumin; DMSO, dimethyl sulfoxide; MTT, 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide; NGF, nerve growth factor; NPC12, neuronal-like PC12 cell; PBS, phosphate buffered saline; RT-PCR, reverse transcription-polymerase chain reaction; RNAi, RNA interference; shRNA, small hairpin RNA; HAT, histone acetyltransferase

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balance between positive and negative regulation of transcriptional factors is crucial in determining cell fate under hypoxic condition.

p300 is a transcriptional co-activator that mediates the interaction of DNA-binding factors with the basal transcriptional complex, and is important for cell responses to hypoxia (Eckner et al., 1994; Vo and Goodman, 2001). p300 possesses intrinsic histone acetyltransferase (HAT) (Vo and Goodman, 2001). Acetylation of histones is associated with a relaxed chromatin configuration, which is thought to facilitate transcription factor access to DNA. A previous report has shown that p300 can acetylate nonhistone nuclear proteins, including transcriptional factors, such as p53. Acetylation of p53 strongly increases its DNA binding activity in vitro, and provides us with a potential mechanism(s) for p300-mediated transcriptional control (Vo and Goodman, 2001).

In addition, p300 binds to HIF-1α, the hypoxia-inducible subunit required for HIF-1-induced transcriptional activation, and enhances the transcriptional activation of HIF-1 targeted genes (Arany et al., 1996). Other hypoxia-induced transcriptional regulators, including p53 and NF-κB, also need to bind with p300 for their transcriptional activation (Vo and Goodman, 2001). Previously, it has been shown that p53 inhibits HIF-1 by competitively binding to p300, which enhances neuronal cell death caused by DNA damage reagents or ischemia (Blagosklonny et al., 1998; Schmid et al., 2004a,b; Vleugel et al., 2006). Exogenous expression of p300 can attenuate such inhibition and prevents neuronal death. Up-regulation of p300 expression prevents neuronal degeneration, while decrease in p300 protein level promotes neuronal death (Culmsee et al., 2003; Ravi et al., 1998; Schmid et al., 2004b). These observations suggest that p300 is an effective molecular in regulating the balance between positive and negative activation of cell death pathways and plays a neuroprotective role. However, alterations in p300 expression under hypoxic condition and its role in hypoxia-induced neuronal damage remain unclear.

In view of this, we detected the distribution of p300 protein in the rat brain and the hypoxia-induced alterations of p300 expression in the rat hippocampus. A differentiated neuron-like PC12 cell model (neuronal-like PC12 cells) was used to investigate the potential role of p300 in hypoxia-induced neuronal cell damage by using small hairpin RNA (shRNA).

2. Results

2.1. *Distribution of p300 in the rat brain*

We used dual-labeling fluorescence immunohistochemistry to detect the distributions of p300 and β-tubulin *III* (a marker of mature neuron) in the rat brain. DAPI was used to label nuclei. p300 protein was detected in the cerebral cortex, hippocampus, cerebellar cortex, medulla oblongata and hypothalamus. Furthermore, p300 was mainly distributed in those cells that also expressed β-tubulin *III*. Less or no positive signal was observed in β-tubulin *III* negative cells. This data indicated that p300 is mainly expressed in neurons of rat brain, and suggests that it is of particular importance for normal neuronal function (Fig. 1).

2.2. *Expression of p300 in rat hippocampus and neuronal-like PC12 cells after hypoxia exposure*

In order to explore the role of p300 in hypoxia-induced neuronal damage, we examined the alteration of p300 expression in rat hippocampus following exposure to hypoxic condition (simulated high altitude of 5000 m). The expression of β-actin was used as internal control. The p300 mRNA levels increased 1 day after hypoxic exposure and reached a peak after 5 days. After which, the hypoxia-induced increase in p300 mRNA level was declined, but remained higher than that of control value (Fig. 2A). Concomitantly, p300 protein levels increased at day 1 and peaked at day 5. By day 15, the hypoxia-induced increase in p300 protein levels had declined, but was still higher than that of control level (Fig. 2B). No difference in the β-actin expression was observed (Figs. 2A and B).

The PC12 cell line is hypoxia-sensitive, which is a useful model for studying oxygen-sensitive molecular and cellular mechanisms (Seta et al., 2002). Sympathetic-like neurons differentiated from PC12 cells by stimulation with nerve growth factor (NGF) have been used widely to investigate hypoxia-induced neuronal damage (Conrad et al., 2001; Spicer and Millhorn, 2003). We used this neuronal-like PC12 cell (NPC12 cells) as an in vitro model to investigate the alteration of p300 expression under hypoxic conditions. Undifferentiated PC12 cells were proliferated to form clone-like cell clusters (Fig. 3A) and then exposure to NGF for 7 days, which resulted in 92% of the cells displaying a differentiated neuronal morphology (Fig. 3B). The NGF and serum media in the 7 day cultures was replaced and the cells grown under hypoxic condition (3% O_2). p300 expression levels were detected after different culture durations. The results showed that the p300 mRNA levels increased 3 h after hypoxic treatment and remained high levels at 6 h, 12 h and 24 h (Figs. 3C and D). Concomitantly, p300 protein levels increased 12 h after hypoxic treatment and remained elevated at 24 h (Figs. 3E and F). No difference in the β-actin expression was detected (Figs. 3C–F).

2.3. *Role of p300 in the hypoxia-induced neuronal-like PC12 cells damage*

We down-regulated p300 expression using shRNA in order to explore the relationship of p300 up-regulation with hypoxia-induced neuronal damage. Semi-quantitative RT-PCR and western blot were used to analyze the knockdown efficiency. Twenty-four hours after the onset of p300-shRNA transfection, p300 mRNA levels in the cells were reduced significantly compared with the cells treated with control shRNA as negative control and transfection agent only as blank control (Figs. 4A and B). p300 protein levels in the cells transfected with p300-shRNA were 48.52%, 49.92% and 51.40% of the blank control values at 24 h, 48 h and 72 h post transfection, respectively (Figs. 4A and B). Cells in negative control and blank control groups showed no changes in p300 protein and mRNA levels ($p>0.05$; Figs. 4A and C). These results suggest that p300 shRNA is effective and specific in reducing p300 expression. In order to ensure that cell activity post shRNA transfections were similar before hypoxic treatment, we detected cell activity at 30 h and 48 h by MTT assay after the