Short communication

Immunocytochemical study on the distribution of p53 in the hippocampus and cerebellum of the aged rat

Yoon Hee Chung, Chung-Min Shin, Myeung Ju Kim, Byung-Kwon Lee, Kyeong Han Park, Choong Ik Cha

Department of Anatomy, Seoul National University College of Medicine, 28 Yongon-Dong, Chongno-Gu, Seoul 110-799, South Korea
Neuroscience Research Institute, Medical Research Center, Seoul National University, 28 Yongon-Dong, Chongno-Gu, Seoul 110-799, South Korea
Biomedical Research Center, KNIH, Seoul, South Korea

Accepted 29 August 2000

Abstract

A role for p53-mediated modulation of neuronal viability has been suggested by the finding that p53 expression is increased in damaged neurons in models of ischemia and epilepsy. P53 gene upregulation precedes apoptosis in many cell types, and a potential role for this molecule in apoptosis of neurons has already been demonstrated in Alzheimer’s disease. Recent studies suggest that p53-associated apoptosis may be a common mechanism of cell loss in several important neurodegenerative diseases. In the present study, we examined changes in p53-immunoreactive (IR) neurons in the brains of aged rats for the first time employing immunocytochemical and in situ hybridization methods. P53-IR neurons were found in the CA1 region of hippocampus, septal region and cerebellum in the aged rats, but there was no p53-IR cell in the brains of adult rats. In the hippocampus of the aged rat, p53-IR cells predominated in the stratum oriens and pyramidal layers, while the molecular layer contained relatively few p53-IR cells. The most prominent population of immunoreactive labeling in cerebellar cortex was localised within the cell bodies of Purkinje cells and dendrites in molecular layers. Upregulation of p53 in the Purkinje cells observed in this study suggests that significant loss of Purkinje cells with aging may be regulated with several apoptosis-controlling factors including p53 and oxidative stress mechanism. Further investigations are required to establish whether direct functional relations exist between p53 and the apoptotic neuronal death in normal aging or Alzheimer brains.

The tumor suppressor gene p53 encodes a nuclear phosphoprotein involved in the control of cell growth. Normally, tissue levels of p53 protein and transcriptional activity are very low [14], but can be induced by various DNA-damaging agents, such as UV light, X-radiation and chemotherapy [7,16,17]. Several lines of evidence have led to the idea that terminally differentiated neurons undergo apoptosis when prompted to re-enter an aberrant cell cycle. In this regard, both apoptosis and mitosis share certain morphological features. In addition, genes that play a role in modulating cell proliferation, such as c-myc, c-fos, c-jun, cyclin D1, and p53, have been implicated in apoptosis [2,6,8,13,23,24].

Enormous interest in p53 has developed in recent years because of a high percentage of human malignancies that are associated with p53 mutations. A number of studies have shown that overexpression of wild-type p53 protein arrests the cell cycle at the G1-S interphase. P53-mediated cell cycle arrest following genomic damage is an important mechanism of cell growth control that probably facilitates DNA repair prior to replication. Under certain conditions, nuclear accumulation of wild-type p53 protein results in apoptosis. Accordingly, transfection of p53 constructs into cells that normally lack endogenous p53 expression causes apoptosis [21]. Further, p53 induction in vivo results in...
tumor regression in nude mice that is morphologically consistent with apoptosis [22]. These examples indicate that increased p53 expression may not only serve as a cellular marker of apoptosis, but also play an active role in the cell death process. Recent studies suggested that p53-associated apoptosis may be a common mechanism of cell loss in several important neurodegenerative diseases [4].

Based on the increased expression of p53 seen with DNA damage, p53 has been proposed to be a component of cell cycle checkpoints that inhibit the progression of cells through the cell cycle until damaged DNA is repaired. Inhibition of p53 function has been reported to extend the proliferative lifespan of human fibroblast, suggesting a significant role in cellular senescence and a possible link between senescence and DNA damage. Recently, de la Monte et al. [4] reported p53-immunoreactivity in the human aged brain.

In the present study, we examined changes in p53-IR neurons in the brains of aged rats for the first time employing immunocytochemical and in situ hybridization methods.

Twelve adult (4–6 month old) and 15 aged (20–29 month old) Sprague–Dawley rats were examined in this study. The authors conformed to the Seoul National University Ethical Committee Guidelines for Laboratory Animals. The animals were perfused transcardially with cold phosphate buffered saline (PBS, 0.02 M, pH 7.4), and then with ice-cold 4% paraformaldehyde for 10 min at a flow rate of 50–60 ml/min. Brains were removed immediately and sliced into blocks 4–6 mm thick. These were immersed in a cold fixative for 6–12 h and then washed in a series of cold sucrose solutions of increasing concentrations. Frozen sections were cut at 40 μm in the coronal plane, and were incubated using the free-floating method for 48–72 h at 4°C in primary antiserum containing Triton X-100 (0.3%), bovine serum albumin (0.5 mg/ml) and normal goat serum (3 drops/10 ml). Monoclonal mouse anti-human p53 protein antibodies (DAKO™, Code No. M 7001, Lot 056 at a dilution of 1:100) were used as primary antibodies and visualized according to the avidin–biotin complex (ABC) method, using an ABC kit (Vectorstain™). Sections were then developed for peroxidase reactivity signals in the pyramidal layer of the CA1 region and cerebellar hemisphere and the vermis of the aged rat.

Animals used by in situ hybridization were killed by decapitation and the brains were quickly removed, frozen and sliced at 12 μm on a cryostat. These sections were fixed, acetylated, dehydrated and treated with chloroform. Hybridization was performed with digoxigenin-11-UTP (BM) labeled antisense p53 cRNA probe, and visualized with NBT/BCIP (Promega).

In the adult rats (control group), no p53-IR cell was found in any region of the central nervous system (Table 1).

In contrast, p53-IR neurons were found in the hippocampus, septal region and cerebellum of the aged rat (Table 1). P53-IR cells were found in all laminae of the CA1 region of hippocampus except for the molecular layer of Ammon’s horn, where they were sparse. P53-IR cells predominated in the stratum oriens and pyramidal layers, while the molecular layer contained relatively few p53-IR cells (Fig. 1). Both the stratum oriens and pyramidal layer harbored many p53-IR cells with broad differences in somal morphology and dendritic branching patterns. In Ammon’s horn, they ranged from small and fusiform to medium or large multipolar neurons. Some neurons in the pyramidal layer were bipolar, with short or long spinous dendrites that were oriented perpendicular to the pial surface. The morphology of p53-IR cells ranged from small, round bipolar cells with sparsely branching dendrites to relatively large cells with widely branching dendrites trees.

In the septal region, p53-IR cells were scattered throughout the rostral septal area, mostly in the lateral septal nucleus and adjacent to the lateral ventricle. P53-IR neurons were medium in size and multipolar or fusiform in shape. P53-IR cells were also found in the cerebellum. The most prominent population of immunoreactive labeling in cerebellar cortex was localized within the cell bodies of Purkinje cells and dendrites in molecular layers (Fig. 2).

In agreement with immunocytochemistry, p53 mRNA signals were also localized in the CA1 region of hippocampus, cerebellar hemisphere and the vermis of the aged rat. P53 mRNA in situ hybridization showed intensely positive signals in the pyramidal layer of the CA1 region and Purkinje cell layer of cerebellum. However, the stratum

<table>
<thead>
<tr>
<th>Area</th>
<th>Subdivision</th>
<th>Control</th>
<th>Aged</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hippocampus</td>
<td>CA1</td>
<td>0</td>
<td>42±11</td>
</tr>
<tr>
<td></td>
<td>CA2</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>CA3</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Cerebellum</td>
<td>vermis</td>
<td>0</td>
<td>98±18</td>
</tr>
<tr>
<td></td>
<td>intermediate zone</td>
<td>0</td>
<td>115±23</td>
</tr>
<tr>
<td></td>
<td>hemisphere</td>
<td>0</td>
<td>48±15</td>
</tr>
<tr>
<td>Septal region</td>
<td></td>
<td>0</td>
<td>24±8</td>
</tr>
</tbody>
</table>

*Data are presented as mean±s.d.*
oriens of the CA1 region showed relatively lower p53 mRNA level than immunoreactivity (Fig. 3).

In this study, we demonstrated that the immunoreactivity of p53 was upregulated. Immunocytochemistry showed intensely stained p53-IR neurons in the hippocampus, septal region and cerebellum in the aged rat, but no p53-IR cells were observed in the control group (Table 1). Hippocampal pyramidal cells and cerebellar Purkinje cells are among the most vulnerable in the nervous system to hypoxia and ischemia. Likewise, hippocampal pyramidal cells and cerebellar Purkinje cells are among the largest neurons in the nervous system; their dendritic fields are among the most highly arborized; their projection distances are lengthy; and their metabolic requirements are among the highest [20]. So there is possibility that decreased blood flow in the aged rat brain is one of the causative factors to the up-regulation of p53 with aging. Recently, de la Monte et al. [4] reported p53-immunoreactivity in the human aged brain.

Recent studies have provided supportive evidence for a relationship between p53 gene activation and neuronal death in the CNS. Following middle cerebral artery occlusion, increased p53 mRNA and protein were detected.
in regions of severe neuronal damage [15]. Similar results have been obtained following photochemical brain injury [18]. Thus, p53 induction may be a response to a variety of insults that result in permanent neuronal loss. Using the transgenic mice expressing a p53 null allele, Crumrine et al. [3] has shown that loss of p53 affords relative protection against focal ischemic neuronal damage. In addition, cerebellar granule cells in p53 null mice are resistant to radiation-induced apoptosis [26]. Furthermore, the absence or suppression of p53 expression has been shown to protect cultured neurons from excitotoxicity-mediated [27] and spontaneous cell death [5]. Conversely, overexpression of p53, mediated by adenoviral gene delivery, induced neuronal cell death with features characteristic of apoptosis [10]. These results indicate that p53 induction is a marker of neuronal apoptosis and suggest that the p53 protein is actively involved in the death pathway.

One hypothesis of aging is that it is a consequence of the accumulation of oxidative damage to cells, an inevitable consequence of leakage of reactive oxygen species during normal metabolism. Several reports indicate that p53 regulates sensitivity to oxidative damage in CNS neurons [26,27] and cerebellar Purkinje cells are highly sensitive to oxidative stress [1,19]. So up-regulation of p53 in the Purkinje cells observed in this study suggests that significant loss of Purkinje cells with aging may be regulated with several apoptosis-controlling factors including p53 and oxidative stress mechanism. Recently, Inamura et al. [9] reported increased number of p53-positive neurons in the Purkinje cell layer using organotypic slice culture exposed to bleomycin and indicated that p53 is involved in DNA strand break-induced apoptosis of fully postmitotic central nervous system neurons.

Apoptotic-like cell death has been described in association with various human neurodegenerative disorders such as amyotrophic lateral sclerosis, Parkinson's disease, Alzheimer's disease and Huntington's disease [4,25]. Recently, two laboratories reported that p53 is involved in the pathogenesis of Alzheimer's disease. La Ferla et al. [12] have derived transgenic mice with intracellular expression of Aβ specifically in neurons with the hope of defining the successive steps, which may reflect Alzheimer's disease pathogenesis. Interestingly, they found that Aβ can activate the p53 dependent apoptotic pathway and that extracellular deposition of Aβ occurs secondary to neuronal cell death. Another indirect evidence supporting the involvement of the p53 in the pathogenesis of Alzheimer's disease is the report of Kitamura et al. [11]. They measured the level of p53 in the temporal cortices in eight control and eight Alzheimer's disease cases, and they found that the level of p53 was increased approximately two-fold in the Alzheimer's disease brain compared to control brains.

Further investigations are required to establish whether direct functional relations exist between p53 and the apoptotic neuronal death in normal aging or Alzheimer brains.

Acknowledgements

This study was supported by Seoul National University Hospital Research Fund (800-20000239). This study was supported in part by year 2000 BK21 project for Medicine, Dentistry and Pharmacy.

References


