Neuroscience 133 (2005) 437–451

ACTIVATING TRANSCRIPTION FACTOR 2 EXPRESSION IN THE ADULT HUMAN BRAIN: ASSOCIATION WITH BOTH NEURODEGENERATION AND NEUROGENESIS

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Abstract—Activating transcription factor 2 (ATF2) is a member of the activator protein-1 family of transcription factors, which includes c-Jun and c-Fos. ATF2 is highly expressed in the mammalian brain although little is known about its function in nerve cells. Knockout mouse studies show that this transcription factor plays a role in neuronal migration during development but over-expression of ATF2 in neuronal-like cell culture promotes nerve cell death. Using immunohistochemical techniques we demonstrate ATF2 expression in the normal human brain is neuronal, is found throughout the cerebral cortex and is particularly high in the granule cells of the hippocampus, in the brain stem, in the pigmented cells of the substantia nigra and locus coeruleus, and in the granule and molecular cell layers of the cerebellum. In contrast to normal cases, ATF2 expression is down-regulated in the hippocampus, substantia nigra pars compacta and caudate nucleus of the neurological diseases Alzheimer's, Parkinson's and Huntington's, respectively. Paradoxically, an increase in ATF2 expression was found in the subependymal layer of Huntington's disease cases, compared with normal brains; a region reported to contain increased numbers of proliferating progenitor cells in Huntington's disease. We propose ATF2 plays a role in neuronal viability in the normal brain, which is compromised in susceptible regions of neurological diseases leading to its down-regulation. In contrast, the increased expression of ATF2 in the subependymal layer of Huntington's disease suggests a role for ATF2 in some aspect of neurogenesis in the diseased brain.

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Abbreviations: AD, Alzheimer's disease; AP-1, activator protein-1; ATF2, activating transcription factor 2; CA, cornu ammonis; CN, caudate nucleus; DAB, 3,3′-diaminobenzidine tetrahydrochloride; EP, ependymal layer; ERK, extracellular-related kinase; GFAP, glial fibrillary acidic protein; HD, Huntington's disease; JNK, c-Jun N-terminal kinase; PBS, phosphate-buffered saline; PBS-T, phosphate-buffered saline containing 0.2% Triton X-100; PCNA, proliferating cell nuclear antigen; PD, Parkinson's disease; SEL, subependymal layer; SNc, substantia nigra pars compacta; SNr, substantia nigra pars reticulata; TAK, TGF-β-activated kinase-1; TUNEL, terminal deoxynucleotidyl transferase-mediated dATFP biotin nick end labeling.

Key words: transcription factor, neuronal viability, subependymal layer, neurogenesis.

Activating transcription factor 2 (ATF2) is a constitutively expressed bZIP transcription factor and is a member of the activator protein-1 (AP-1) family. The AP-1 protein binds to the AP-1 and/or CRE/ATF motifs within the promoter region of target genes causing subsequent up- or down-regulation of transcription. Target genes of ATF2 include the cell cycle proteins cyclin A (Beier et al., 2000; Shimizu et al., 1998) and cyclin D1 (Beier et al., 1999), as well as c-Jun (van Dam et al., 1995), tumor necrosis factor α (TNFα; Tsai et al., 1996), transforming growth factor-β2 (TGF-β2; Kim et al., 1992), E-selectin (Read et al., 1997) and tyrosine hydroxylase (Suzuki et al., 2002). ATF2 is also a common target of Smad and TGF-β-activated kinase-1 (TAK) pathways in TGF-β-signaling and plays a key role in the TAK/Smad-mediated differentiation of a clonal P19 cell line into cardiomyocytes and the maturation of chick chondrocytes (Ionescu et al., 2003; Monzen et al., 2001; Sano et al., 1999). ATF2 also has intrinsic histone acetyltransferase (HAT) activity and is one of the only sequence-specific DNA-binding activators reported to date (Kawasaki et al., 2000).

ATF2 is ubiquitously expressed in the brain and throughout the mammalian body (Kara et al., 1990; Maekawa et al., 1989; Takeda et al., 1991). Knockout mouse studies have demonstrated that the presence of ATF2 is required for correct neurological development and neuronal migration (Reimold et al., 1996); furthermore, ATF2 is required for resistance to apoptosis and survival of at least three different non-neuronal cancer cell lines (Hayakawa et al., 2003; Ronai et al., 1998; Zoumpourlis et al., 2000). In human tissue, ATF2 immuno-reactivity is reported to be found predominantly in white matter with very little neuronal staining (Yamada et al., 1997). In the rat brain, however, ATF2 is present in the nucleus of all neuronal cell populations, but not in glial cells, and its expression is decreased in axotomised neurons following nerve cell lesions (Ferrer et al., 1996; Herdegen et al., 1997; Martin-Villalba et al., 1998; Robinson, 1996). ATF2 down-regulation is also associated with a corresponding increase in c-Jun expression in cells surviving axotomy (Buschmann et al., 1998). In addition, ATF2 is phosphorylated in dying pyramidal cornu ammonis (CA1) neurons, but not in surviving dentate granule cells in the rat hippocampus following hypoxic–ischemic insult (Walton et al., 1998). Experiments in PC12 cells have also suggested

0306-4522/05$30.00+0.00
doi:10.1016/j.neuroscience.2005.02.029

ATF2 is more involved in promoting cell death (Leppa et al., 2001; Walton et al., 1998). In contrast, ATF2 immuno-labeling is reported in surviving retinal ganglion cells but not in axotomised neurons following partial optic nerve injury (Kreutz et al., 1999). These data suggest the context of ATF2 expression is important in determining its role in nerve cells.

The neurodegenerative diseases Alzheimer's, (AD) Parkinson's (PD) and Huntington's (HD) are characterized by neuronal loss in specific regions although the cause of cell loss in these disorders remains unknown. Neuronal loss in AD originates in the medial temporal lobe then spreads into the hippocampus, temporal cortex and into the frontal and parietal regions and subcortices (Braak and Braak, 1991). Within the hippocampus, the pattern of cell loss is well documented with the majority of cell loss occurring in the CA1 region and subiculum, while the granule cells of the dentate gyrus and CA3-4 regions remain relatively unaffected (Bobinski et al., 1997; Van Hoesen and Hyman, 1990). PD is characterized primarily by a loss of the dopaminergic neurons whose cell bodies lie within the substantia nigra pars compacta (SNc) and project predominantly to the striatum, however, neuronal loss also occurs in the dopaminergic neurons which originate in the ventral tegmental area of the midbrain and project to the cortex and limbic areas. (Hornykiewicz, 2001) In HD, the striatum is the first region to be affected, with loss of GABAergic cells, although by the end stages of the disease neuronal loss is widespread (Glass et al., 2000; Vonsattel et al., 1985). Expression of ATF2-related transcription factor, c-Jun is increased in the AD hippocampus and in PD animal models following medial forebrain transection (Buschmann et al., 1998; Herdegen et al., 1997; Winter et al., 2000).

In the present study, the regional distribution of ATF2 is determined in the normal human brain using light microscopy and confocal laser scanning. The expression of ATF2 is then investigated in the affected regions associated with AD, PD and HD, and compared with normal cases.

EXPERIMENTAL PROCEDURES

Brain tissue

Human brain tissue was obtained from the Neurological Foundation of New Zealand Human Brain Bank (Department of Anatomy with Radiology, The University of Auckland, Auckland, New Zealand). All protocols used in this study were approved by The University of Auckland Human Subject Ethics Committee. Neuropathological analysis of all cases used was undertaken by Dr B. Synek (Neuropathologist at Auckland City Hospital, Auckland, New Zealand) using standard criteria. AD was diagnosed according to the CERAD neuropathology protocol (Mirra et al., 1991), PD was diagnosed according to Hughes et al., 1992, and HD was diagnosed according to genetic studies and neuropathology using standard criteria (Vonsattel et al., 1985); and to exclude other brain pathology.

For analysis of ATF2 expression in control brains, tissue was obtained from 15 subjects (average age 66 years; range 46–81 years; average postmortem interval 12 h) with no history of neurological or psychiatric disorders. For analysis of ATF2 expression in AD subjects, tissue was obtained from nine subjects (average age 75 years, range 60–83 years; average postmortem interval 9 h). For PD cases, tissue was obtained from seven subjects (average age 75 years; range 70–84 years; average postmortem interval 16 h). For analysis of HD cases, tissue was obtained from seven subjects (average age 60 years; range 39–78 years; average postmortem interval 11 h). Details of each case are listed in Table 1. Table 2 lists the brain regions, including spinal cord, studied from the various cases.

The two hemispheres of the forebrain from each case were separated; blocks of tissue from one hemisphere were cut then frozen quickly in crushed dry ice and stored at −80 °C; Tissue from this hemisphere was used for Western blot analysis. For immunohistochemical studies, the other hemisphere was fixed by perfusion through the basilar and internal carotid arteries with phosphate-buffered saline (PBS) containing 1% sodium nitrite, followed by 15% formalin in 0.1 M phosphate buffer, pH 7.4. Blocks were then dissected out and kept in the same fixative for 24 h. These blocks were then cryoprotected in 20% sucrose in 0.1 M phosphate buffer containing 0.1% sodium azide for 2–3 days, and then in 30% sucrose in 0.1 M phosphate buffer containing 0.1% sodium azide for a further 2–3 days. Fifty micron sections were cut from blocks on a freezing microtome and stored in PBS with 0.1% sodium azide until required for use.

Immunohistochemical procedures

Microtome-cut sections of adult human brain tissue were processed for immunohistochemical staining as previously described (Baer et al., 2003; Waldvogel et al., 2003).

Primary antibodies. ATF2 was detected with a rabbit polyclonal antibody raised against the carboxy terminus of human ATF2 (sc-187; Santa Cruz Biotechnology, Inc., CA, USA). Proliferating cell nuclear antigen (PCNA) was detected with a mouse monoclonal antibody (sc-56; Santa Cruz Biotechnology, Inc.); glial fibrillary acidic protein (GFAP) was detected with a mouse monoclonal antibody (G3893; Sigma, St. Louis, MO, USA); NeuN was detected with a mouse monoclonal antibody (MAB377; Chemicon International, Inc., CA, USA) and microtubule-associated protein-2 (MAP-2) was detected with mouse monoclonal antibody (MAB3418) obtained from Chemicon International, Inc.

All antibodies were diluted in immunobuffer consisting of 1% goat serum in PBS containing 0.2% Triton X-100 and 0.4% Thimerosol (Sigma).

Single immunoperoxidase labeling. Sections were processed free-floating in tissue culture wells using standard immunohistochemical procedures. All washes were performed with PBS containing 0.2% Triton X-100 (PBS-T) three times for 15 min. For PCNA immuno-labeling procedures an additional initial antigen retrieval step was required. Tissue sections were incubated in 0.1 M sodium citrate solution (pH 4.5) overnight then heated in a 650 W microwave oven on high for 30 s and left to cool before washing with PBS-T. All sections were then incubated in 50% methanol and 1% H_2O_2 for 20 min before washing in PBS-T and incubation with primary antibodies for 48–72 h at 4 °C with gentle shaking. The rabbit antibody to ATF2 was used at 1:1000 and the mouse antibody to PCNA was used at 1:500. The primary antibodies were then washed off with PBS-T and the sections then incubated with the appropriate species-specific biotinylated secondary antibodies (1:500; goat anti-mouse or goat anti-rabbit; Sigma) for 24 h at room temperature with gentle shaking. Following this, sections were again washed and then incubated with ExtraAvidin™ (1:1000; Sigma) for 4 h at room temperature. Finally, sections were washed again before being immersed in 0.05% 3,3′-diaminobenzidine tetrahydrochloride (DAB; Sigma) and 0.01% H_2O_2 in 0.1 M phosphate buffer, pH 7.4, for 10–20 min until a brown reaction was visualized. Sections were washed once in PBS before mounting onto chrome-alum-coated