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BRAIN RESEARCH

Research Report

Dendritic and axonal localization of cytotoxic T-lymphocyte antigen-2 alpha protein in mouse brain

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ABSTRACT

Cytotoxic T-lymphocyte antigen-2 alpha (CTLA-2α) is a novel cysteine proteinase inhibitor protein originally discovered and expressed in mouse activated T-cells and mast cells. Expressed recombinant CTLA-2α is shown to exhibit selective inhibition of cathepsin L-like cysteine proteinases. We have recently reported the expression pattern of CTLA-2α mRNA in mouse brain by *in situ* hybridization, demonstrating that it is mainly enriched within neuronal populations. In this study we present the distribution profile of the protein by immunohistochemical analysis. Results showed that CTLA-2α protein is preferentially localized in dendritic and axonal compartments. In telencephalon, strong labeling was detected in dendrites in the cerebral cortices, stratum radiatum and stratum lacunosum moleculare and within axonal fibers of stratum lucidum where mossy fibers emanating from all parts of the granule cell layer of dentate gyrus terminate at pyramidal neurons and interneurons. In diencephalon, moderate staining was found in all thalamic nuclei but was strong in medial habenular nucleus and the hypothalamic nuclei including suprachiasmatic nucleus, optic chiasm, arcuate nucleus and median eminence. In mesencephalon, strong immunoreactivity was detected in superior colliculus, inferior colliculus and paramedian raphe nucleus. In the rhombencephalon, the pontine nucleus and transverse fibers of the pons revealed strong staining but were moderate in vestibular nuclei. Strong immunoreactivity was also observed in the internal white matter, granule cell layer and Purkinje cell layer within cerebellum. On Western blot analysis, a band of 14 kDa for CTLA-2α from protein extracts of the cerebrum, cerebellum, pons and medulla was detected. The distribution pattern and functional considerations of CTLA-2α in the brain are discussed.

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Abbreviations: CTLA-2α, Cytotoxic T-lymphocyte antigen-2 alpha; CNS, central nervous system; SCN, suprachiasmatic nucleus; Arc, arcuate nucleus; opt, optic nucleus; mf, mossy fibers; Rad, stratum radiatum; Py, pyramidal neurons; py, pyramidal tract; LMol, stratum lacunosum moleculare; Mol, molecular layer of dentate gyrus; DG, dentate gyrus; GrDG, granule cell layer of dentate gyrus; SLu, stratum lucidum; CA1, 2, 3, Cornu Ammonis fields 1, 2, 3; MHb, medial habenular nucleus; MAP-2, microtubule associated protein-2; NeuN, neuron-specific nuclear protein

[1] These authors have equal contribution to the manuscript.

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1. Introduction

Cysteine proteinases are widely distributed in a variety of biological tissues and fluids, where they are involved in the process of intra- and extra-cellular protein degradation and turnover (Reddy et al., 1995) and hence in disease-related tissue remodeling (Barrett and Kirschke, 1981; Bond and Butler, 1987; Denizot et al., 1989; Deussing et al., 2002; Cowan et al., 2005). Cysteine proteinases are synthesized as inactive proenzymes with N-terminal propeptide regions. Activation processes of the enzymes include removal of the propeptide regions. Several functions have been ascribed to the propeptides including proper folding of a newly synthesized enzyme (Barrett and Kirschke, 1981; Bond and Butler, 1987) and stabilization of the enzyme against denaturation at neutral to alkaline pH conditions (Abrahamson, 1994; Katutuma and Kominami, 1995). It has also been shown that the propeptides purified from a given proteinase, can inhibit the activity of that proteinase *in vitro* (Delaria et al., 1994). Several attempts have been made to develop specific inhibitors of individual cysteine proteinases for various purposes such as selective inhibition of cysteine proteinases of insect digestive system for pest control (Denizot et al., 1989; Yamamoto et al., 2002).

Cytotoxic T-lymphocyte antigen-2 alpha (CTLA-2α) was originally discovered and expressed in mouse activated T-cells and mast cells (Denizot et al., 1989). Structurally, CTLA-2α is reported to be homologous to the proregion of cysteine proteinases (Denizot et al., 1989; Yamamoto et al., 2002; Kurata et al., 2003; Deshapriya et al., 2007). The propeptide of cysteine proteinases proregion is part of the enzyme but CTLA-2α is a protein expressed independently and is designated as a member of I29 protease inhibitor family (MEROPS peptidase database number I29.002 (http://merops.sanger.ac.uk/). CTLA-2α is shown to exhibit selective inhibition of mouse cathepsin L-like cysteine proteinases (Kurata et al., 2003). Other propeptide-like cysteine proteinase inhibitor proteins homologous to CTLA-2α have been discovered in other organisms including the *Bombyx* cysteine proteinase inhibitor (BCPI) identified in *Bombyx mori* (Yamamoto et al., 1999a,b; Kurata et al., 2001) and the crammer peptide (CG10460 gene product) found in *Drosophila melanogaster* (Comas et al., 2004; Deshapriya et al., 2007).

Little is known regarding the cellular localization and physiological function of CTLA-2α protein in the brain. However, in our previous study (Luziga et al., 2007); we reported the expression pattern of CTLA-2α mRNA in the mouse brain by *in situ* hybridization, demonstrating that the mRNA is preferentially enriched within various neuronal populations. We developed interest to know the cellular localization of the protein because such information is crucial for the accurate interpretation of neuroscientific data regarding the function of CTLA-2α in central nervous system (CNS).

In an approach to study the localization of CTLA-2α protein in the mouse brain, we prepared in rabbit a specific antibody raised against the entire amino acid sequence of CTLA-2α. The antibody was then used for immunohistochemical analysis to visualize CTLA-2α protein in the mouse brain. The specificity of the antibody was characterized by Western blot.

2. Results

2.1. *Detection of CTLA-2α protein in mouse brain by Western blot analysis and control tests for the specificity of CTLA-2α signal*

The affinity purified polyclonal antibody raised against CTLA-2α specifically detected a band with a molecular mass of 14 kDa in the cerebrum, cerebellum, pons and medulla equal to that found in the positive control extract of decidua (maternal placenta) at gestation day 10.5 (Fig. 1A). In contrast to specific labeling for CTLA-2α protein observed with CTLA-2α antibody, no immunoblot signal was found in the lane loaded with the recombinant CTLA-2α [His-Tag], brain samples and decidua when CTLA-2α antiserum absorbed with the recombinant CTLA-2α antigen was used in place of the primary antibody (Fig. 1B). The specificity of anti-CTLA-2α antibody was also tested by immunohistochemistry in comparison with the absorbed CTLA-2α antiserum. Positive staining was observed in sections incubated with the anti-CTLA-2α antibody (Fig. 2A) but was not observed in sections incubated with the absorbed antiserum (Fig. 2B). Same results were also obtained by immunofluorescence control tests (Fig. 2C, D). The CTLA-2α antibody was subsequently used to examine the cellular distribution pattern of CTLA-2α protein in sagittal and coronal sections of the mouse brain with streptavidin-peroxidase and immunofluorescence methods.

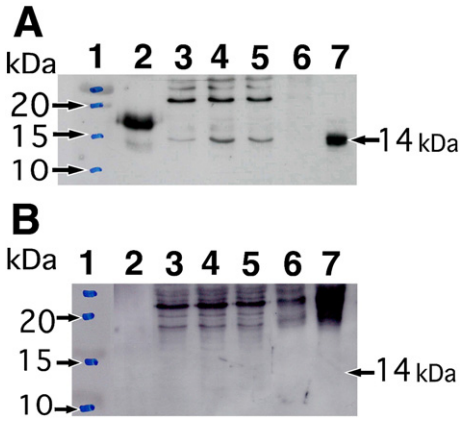

Fig. 1 – Immunoblot analysis for the detection of CTLA-2α protein in mouse brain and control tests for the specificity of CTLA-2α signal. Lane 1, molecular marker protein; lane 2, recombinant CTLA-2α [(His-Tag) 0.01 μg total protein]; lane 3, cerebrum (10 μg); lane 4, cerebellum (10 μg); lane 5, medulla and pons (10 μg); lane 6, liver [(10 μg) negative control]; lane 7, D 10.5 decidua/maternal placenta [(2 μg) positive control]. (A) Western blot probed with a polyclonal rabbit anti-CTLA-2α primary antibody showing a higher band of recombinant CTLA-2α (His-Tag) in lane 2 and lower bands corresponding to CTLA-2α (14 kDa) in the brain samples (lanes 3, 4, and 5) which match well with that in decidua (lane 7) used as positive control but is not observed in the liver (lane 6) used as negative control. (B) Western blot probed with a polyclonal rabbit anti-CTLA-2α primary antibody absorbed with the recombinant CTLA-2α antigen. No signal is detected in all tissues samples including lane 2 of the recombinant CTLA-2α (His-Tag).