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CYSTATIN C IMMUNOREACTIVITY AND NEURONAL DEGENERATION IN AMYOTROPHIC LATERAL SCLEROSIS

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Abstract Cystatin C (CC), a cysteine protease inhibitor involved in protein degradation, is a marker of Bunina bodies in lower motor neurons in amyotrophic lateral sclerosis (ALS). TDP-43-immunoreactive inclusions are also histological hallmark of ALS. However, immunohistochemical localization of CC in ALS motor neurons with or without inclusions is uncertain. Recently, we demonstrated that the majority of anterior horn cells showed moderate to intense immunoreactivity for CC in controls and that CC immunoreactivity was severely decreased in anterior horn cells in ALS. The proportion of CC-immunolabeled anterior horn cells was reduced regardless of whether those neurons contained Bunina bodies or not. In contrast, the proportion of CC-immunolabeled anterior horn cells was significantly reduced owing to the presence of TDP-43 inclusions. These findings suggest that the formation of TDP-43 inclusions, but not of Bunina bodies, reduces the content of CC in spinal motor neurons and that perturbations in endogenous levels of CC in neurons may participate in neurodegenerative process in ALS.

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Key words: Amyotrophic lateral sclerosis; Immunohistochemistry; Bunina body; Cystatin C; TDP-43.

1. Introduction

Cystatin C (CC) is a cysteine protease inhibitor involved in lysosomal and endosomal protein degradation[1,2] as well as in cell-matrix interaction[3,4]. Previous immunohistochemical studies have demonstrated that antibodies against CC labeled Bunina bodies (BBs) observed in amyotrophic lateral sclerosis (ALS), an adult-onset neurodegenerative disorder affecting both upper and lower motor neurons[2,5]. BBs are small eosinophilic intracytoplasmic inclusions, 1-3 μm in diameter, in residual lower motor neurons[6]. Electron microscopic studies showed that the inclusions consist of a homogeneous, electron-dense granular matrix surrounded by vesicular and tubular structures[7]. Ubiquitinated inclusions, including skein-like and round inclusions, are also histological hallmark of ALS[8]. TDP-43 is now known as a major disease protein of ubiquitinated inclusions in ALS and frontotemporal lobar degeneration with ubiquitin-positive inclusions with or without motor neuron disease[9,10].

Recently, several investigators reported that the levels of TDP-43 protein in the cerebrospinal fluid from patients with ALS and frontotemporal lobar degeneration were higher than those in controls[11,12]. In contrast, the concentration of CC in the cerebrospinal fluid from patients with ALS is significantly lower than that in normal controls, suggesting an increased sequestration of CC into BBs[13,14]. However, immunohistochemical localization of CC in motor neurons with or without inclusions in ALS is uncertain. In the present study, we examined the expression of CC in the spinal cord from patients with ALS and control subjects by immunohistochemistry and immunoelectron microscopy. In this paper, we report that CC immunoreactivity is severely decreased in the anterior horn cells in ALS.

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2. CC immunoreactivity in the central nervous system

2.1. Decrease in CC immunoreactivity in anterior horn cells in ALS

Previous immunohistochemical studies have shown that in the brain, CC immunoreactivity is widely distributed in the neurons, astrocytes and choroid plexus epithelium[15]. Motor neurons in the brainstem and spinal cord show diffuse granular cytoplasmic immunoreactivity in normal human specimen[16]. CC has been reported to be up-regulated in response to various injuries to the brain. In Alzheimer's disease, CC immunostaining was increased in pyramidal neurons of the temporal cortex[17]. In patients with temporal lobe epilepsy, CC immunoreactivity was increased in astrocytes in the molecular layer of the dentate gyrus[18,19]. CC expression was up-regulated in neurons and reactive astrocytes in the murine hippocampus following transient forebrain ischemia[20,21] and perforant pathway transection[22]. The level of CC mRNA and protein was increased in neurons, astrocytes and microglia in the striatum following dopamine depletion[23]. Thus, there is a multitude of evidence that CC plays a key role in the protection against tissue injuries via inhibition of cysteine proteases[24]. We demonstrated that CC immunoreactivity is severely decreased in anterior horn cells in ALS (Fig. 1)[25]. These findings suggest that there may be some differences in the roles of CC between ALS and other neurological disorders described above. Considering that CC is one of the cysteine protease inhibitors, decreased level of CC may imply increased proteolysis via up-regulation of cysteine proteases in motor neurons in ALS. This is supported by the finding that the immunoreactivity of cathepsin B, one of the cysteine proteases that are inhibited by CC, was increased in the cytoplasm of anterior horn cells in ALS[26].

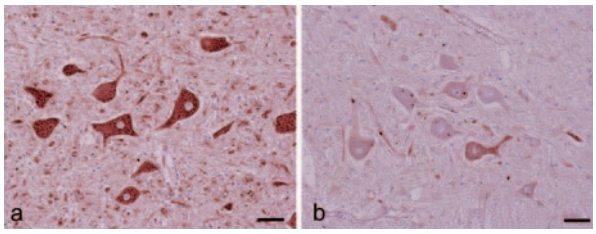

Figure 1 Cystatin C immunoreactivity in the spinal cord in control (a) and amyotrophic lateral sclerosis (ALS) (b). (a) Anterior horn cells showing strong diffuse granular cytoplasmic staining. (b) Anterior horn cells in ALS showing no or only weak immunoreactivity. Bars = 50 μm.

2.2. Relationship between CC immunoreactivity, BBs and TDP-43 positive inclusions

Van Welsem et al. (2002) reported the relationship between the severity of neuronal loss and the proportion of skein-like inclusion-containing neurons in the spinal cord, although no relation was found with BBs[16]. We reported that the presence of BBs had no influence on the decrease of CC immunoreactivity, whereas the presence of TDP-43-positive inclusions significantly decreased the proportion of anterior horn cells with CC immunoreactivity[25]. These findings suggest that the formation of TDP-43-positive inclusions, but not of Bunina bodies, reduces the content of CC in spinal motor neurons, or reduction of CC in neurons may accelerate the formation of TDP-43-positive inclusions, but not Bunina bodies.

Previously, electron microscopic studies showed that BBs consist of a homogeneous, electron-dense granular matrix surrounded by vesicular and tubular structures[7]. Our immunoelectron microscopic study clearly demonstrated that CC is localized to the vesicular membranous structures, but not to the electron-dense material, of BBs (Fig. 2a, b)[25]. It is noteworthy that CC immunoreactivity of BBs is decreased with the maturation of BBs (Fig. 2c, d)[25]. Moreover, the presence of BBs had no influence on the decrease of CC immunoreactivity of anterior horn cells in ALS[25]. It is likely that the decrease of CC immunoreactivity of anterior

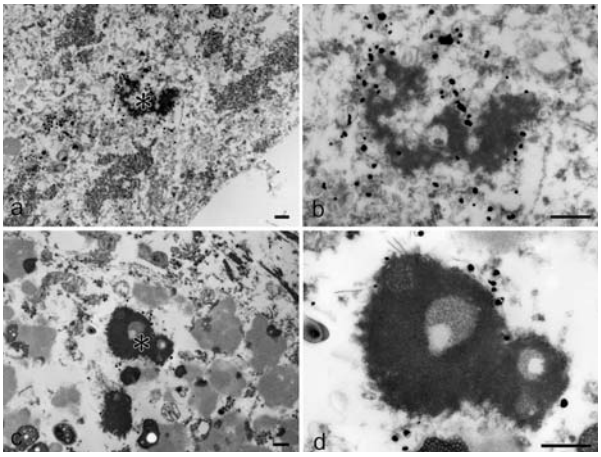

Figure 2 Immunoelectron microscopy using cystatin C antibody in the spinal anterior horn cells of patients with amyotrophic lateral sclerosis (a-d). (a) Early-stage Bunina bodies. (b) Higher magnification view of t the area indicated by asterisk in (a) showing gold labeling on the vesicular structures within and around BBs. (c) Advanced-stage BBs with scant vesicular structures located in the lipofuscin granules. (d) Higher magnification view of the area indicated by asterisk in (c) showing only a few gold particles in the periphery of BBs. Bars = 0.5 μm.

horn cells is not simply due to an increased sequestration of CC into BBs. We demonstrated that the decreased CC immunoreactivity in the spinal motor neurons is closely associated with the presence of TDP-43-positive inclusions in patients with ALS. Perturbations in endogenous levels of CC in neuronal may participate in neurodegenerative process in ALS.

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