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Amyloid peptide attenuates the proteasome activity in neuronal cells

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Abstract

Previous studies have suggested a possible relationship between the ubiquitin–proteasome pathway and some pathological manifestations of Alzheimer's disease (AD). This study investigated the possibility that the Aβ peptides interact with the ubiquitin–proteasome pathway inside neuronal cells. The ubiquitin–proteasome activity decreased with age in the brains of Tg2576 mice while the $A\beta_{1-42}$ levels increased. In cultured neuronal cells, an extracellular treatment of Aβ markedly decreased the proteasome activity and extracellular treated Aβ peptides were found in the cytoplasmic compartment. These results suggest that the extracellular Aβ peptides enter the cell and inhibit the proteasome activity, which might play a role in the pathogenesis of AD.

Keywords: Tg2576; Proteasome; Chymotrypsin-like activity; Amyloid β-peptide

1. Introduction

Proteasome is a multicatalytic complex that has been considered to be the proteolytic machinery responsible for the final clearance of the ubiquitinated intracellular proteins (Checler et al., 2000; Coux et al., 1996). The 26S proteasome, which constitutes the catalytic machinery of ubiquitin–ATP-dependent protein degradation, is distributed in the cytoplasmic and nuclear compartments. This pathway plays a role in the constitutive turnover of the intracellular proteins, the rapid degradation of the abnormal proteins and the critical regulatory proteins including the cell cycle proteins and transcriptional factors, modulation of the cell surface receptors and ion channels, and the generation of peptides presented by the major histocompatibility complex-I (MHC-I) molecules (Ciechanover et al., 2000; Coux et al., 1996; Groettrup et al., 1996; Murry, 1995; Palombella et al., 1994; Rock et al., 1994). Therefore, the ubiquitin–proteasome system plays an important role in maintaining cellular homeostasis by regulating the proper protein level.

Under pathological conditions, a malfunctioning proteasome can lead to the accumulation of ubiquitinated proteins in the cells (Schwartz and Ciechanover, 1999). In the case of Alzheimer's disease (AD), Lewy body disease, and inclusion body myositis (IBM), many studies have reported high levels of ubiquitin and the ubiquitin conjugates in abnormal intracellular inclusions (Askanas et al., 1993; Lowe and Mayer, 1990; Mori et al., 1987). This suggests that a malfunction in the ubiquitin–proteasome pathway might be related to the pathophysiology of Alzheimer's disease. In support of this hypothesis, previous studies have suggested a possible relationship between the proteasome function and the AD-related proteins. Lopez Salon et al. (2003) reported that a blockade of the 26S proteasome function by lactacystin caused a marked decrease in $A\beta_{1-42}$ degradation,

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and Keck et al. (2003) reported that PHF-tau directly inhibits the proteasome activity in AD brains. Moreover, Shringarpure et al. (2000) showed that severely oxidized Aβ could not be easily degraded by the proteasome, and inhibited the proteasome instead. These reports support the possibility that an abnormal proteasome function contributes to the pathogenesis of AD, and that Aβ and PHF-tau, which are the pathological hallmark proteins of AD, attenuate the proteasome activity. Intracellular, as well as extracellular Aβ, have been detected in several laboratories (Haass and Selkoe, 1993; Shoji et al., 1992; Wertkin et al., 1993). There are reports of Aβ internalization from the cell culture media into the normal human fibroblast via the lysosomal–endosomal pathway (Knauer et al., 1992). Cellular Aβ immunoreactivity is specifically associated with intraneuronal neurofibrillary tangle-bearing bodies (Grundke-Iqbal et al., 1989; Murphy et al., 1994; Spillantini et al., 1990). These findings suggest that Aβ exists inside the cells under pathological conditions in the affected neurons, where they are available for an interaction with the cellular pathways (Gregori et al., 1995, 1997). Furthermore, a recent finding showing that Aβ binds two distinct yeast proteasome subunits highlighted the ability of Aβ to interact with the proteasome (Sahassrabidhe and Hughes, 1995).

This study further examined the relationship between the proteasome function and Aβ. The relationship between proteasome activity and Aβ was first examined in an animal model of AD. The results showed that the Aβ level increased with age, whereas proteasome function decreased. The hypothesis that Aβ peptides directly attenuate the proteasome function inside cells was then tested. This study examined the effect of an Aβ treatment on the proteasome activity with the purpose of determining if extracellular Aβ enters the cultured neuronal cells. The results showed that an extracellular treatment of Aβ indeed inhibited the proteasome activity and the treated Aβ proteins could be found inside the cells. The results suggest that over-produced Aβ proteins enter the neuronal cells and attenuate the proteasome function, thereby causing the accumulation of the ubiquitin conjugates and the acceleration of neurodegeneration.

2. Materials and methods

2.1. Cell culture and transient transfection

B103 rat neuroblastoma cells, which do not express the endogenous APP and Aβ peptides, were used in this study (Schubert et al., 1974). The cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM; GibcoBRL, Grand Island, NY) supplemented with 10% fetal bovine serum (FBS; HyClone, Logan, UT) in 8% CO_2 at 37 °C. This study used a reporter construct, pEGFP-C1-CL-1, containing a short degron, CL1 (a generous gift from Dr. Ron Kopito, Stanford University, Palo Alto, CA) (Bence et al., 2001) to investigate proteasome activity in the cells. The B103 cells were transfected with 1–3 μg of appropriate pEGFP-C1-CL1 and pEGFP-C1 DNA (Clonetech, Palo Alto, CA) using the LipofectAMINE plus Transfection Reagent (Life Technologies, Gaithersburg, MD).

2.2. Chemical and peptides

Synthetic amyloid beta 25–35 ($A\beta_{25–35}$) and $A\beta_{1–42}$ (U.S. Peptide, Inc., Fullerton, CA), were dissolved to 10 mM in dimethylsulfoxide (DMSO; Amresco, Solon, OH) and diluted in PBS to final concentration of 1 mM of the Aβ peptides.

2.3. Cell viability assay

The cell viability was assessed using a modified 3-(4,5-dismethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay (Kim et al., 2001).

2.4. Subcellular fractionation and Western blotting for Aβ uptake

The cells were cultured in the presence or absence of 1 μM $A\beta_{1–42}$ for 12 h. The cells were then washed five times in ice-cold Ca^{2+}–Mg^{2+} free PBS (CMF-PBS), and harvested by centrifugation at 700 × *g*. The cell extracts were resuspended in buffer A (20 mM Tris–HCl [pH 7.5], 20 mM EDTA, 2 mM EGTA and 250 mM sucrose) containing 100 μM Na_3VO_4, 1 μg/ml aprotinin, 1 μg/ml leupeptin, 1 μg/ml pepstatin, 100 μg/ml phenylmethylsulfonyl fluoride (PMSF) and 100 μg/ml dithiothreitol (DTT) for 30 min and then sonicated. The cytosolic fraction was collected by ultracentrifugation at 104,000 × *g* for 90 min. The supernatants (cytosolic fraction) were subjected to Western blot analysis using the 4G8 anti-Aβ antibody (Signet Laboratories, Dedham, MA).

2.5. Immunocytochemical studies and confocal laser scanning microscopy for Aβ uptake

The cells were fixed with 4% paraformaldehyde (PFA) in CMF-PBS for 2 h at room temperature in order to examine the Aβ uptake. The Aβ peptides on the cell surface were detected by adding the primary antibody (6E10, 1:200 dilution, Signet Laboratories) to the cells without cell permeabilization overnight at 4 °C. The primary antibody was detected using fluorescein isothiocyanate (FITC). After adding MeOH–acetone (1:1), which was used to permeate the cell membrane, the Aβ peptides in the cell were detected by adding the primary antibody (6E10) to the cells permeated overnight at 4 °C. The primary antibody was detected using tetramethyl rhodamine isothiocyanate (TRITC)-conjugated secondary antibodies. In order to identify the localization of Aβ, an optical slice section