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Localization of CKII β subunits in Lewy bodies of Parkinson's disease

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Abstract

We reported previously that phosphorylation by casein kinase II (CKII) regulates the interaction between alpha-synuclein and its binding partner synphilin-1, and that both CKII alpha and beta subunits co-localize with alpha-synuclein in cytoplasmic inclusions in transfected cells. In this study, we extended these observations to the brains of patients with Parkinson's disease (PD) and examined whether CKII subunits are present in Lewy bodies. Immunohistochemical studies on PD brains harboring Lewy bodies revealed a positive stain for CKII beta but not for CKII alpha. In addition, CKII beta subunits co-localized with alpha-synuclein in most Lewy bodies. These findings suggest that CKII beta subunits may play a role in the formation of intracytoplasmic inclusions in human alpha-synucleinopathies either through phosphorylation events or through a separate mechanism linked to the beta subunit itself.

Keywords: Lewy bodies; Casein kinase II; α-Synuclein; Phosphorylation; Parkinson's disease

1. Introduction

The accumulation of pathogenic proteins in inclusions is characteristic of several neurodegenerative disorders [1–4]. Although the molecular mechanisms that lead to the formation of these inclusions are not completely understood, elucidating their constituents can provide clues about the pathogenesis of the diseases and about the genesis of the inclusions. For example, α-synuclein, which is an abundant constituent of Lewy bodies [5,6], appears to have an important role in the pathogenesis of Parkinson's disease (PD) and other α-synucleinopathies. In addition, synphilin-1, which interacts with α-synuclein and induces the formation of cytoplasmic inclusion in cultured cells, is another component of Lewy bodies in the brains of patients with PD [7,8].

Casein kinase II (CKII) is a ubiquitous seryl/threonyl protein kinase which has a vital role in eukaryotic cells [9,10]. The holoenzyme is generally composed of two catalytic (α and/or α′) and two regulatory (β) subunits. α-Synuclein has several consensus sites for this kinase and is strongly phosphorylated by CKII, particularly at serine 129 [11]. CamKII, on the other hand, has only a weak phosphorylating activity on α-synuclein *in vitro* [11]. We reported previously that CKII mediated phosphorylation of

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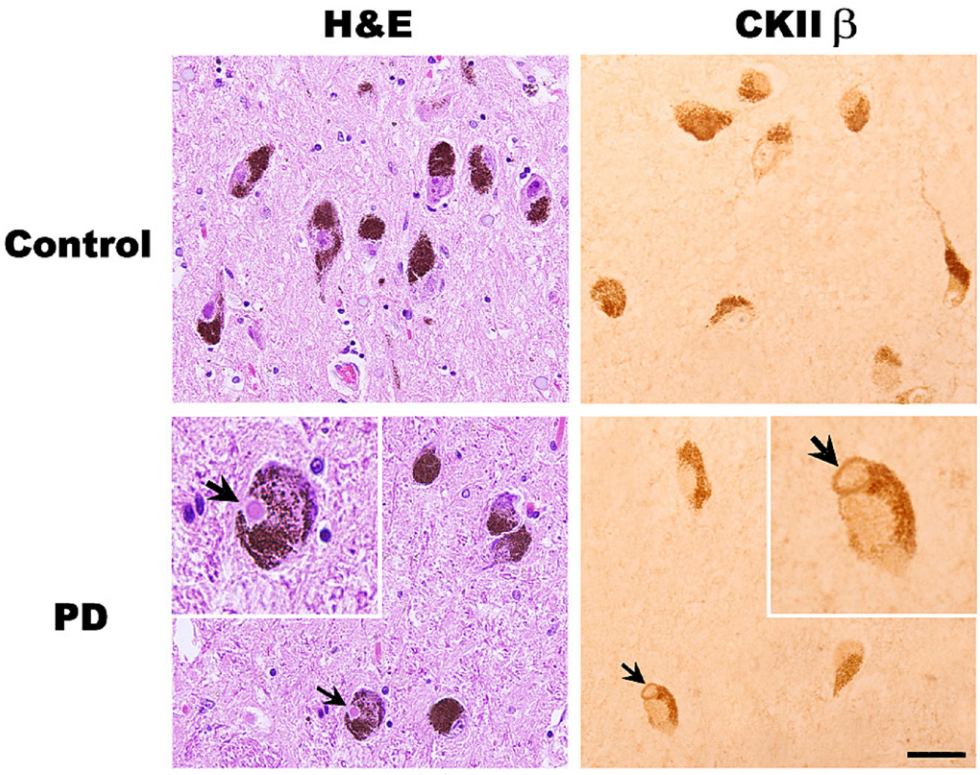

Fig.1. CKII β subunits are present in Lewy bodies of PD patients. Sections through the substantia nigra pars compacta (SN) were stained with hematoxylin and eosin (H&E) showing an eosinophilic Lewy body (arrow). The SN tissues of PD cases were stained immunohistochemically with anti-CKII β antibody. Three dopaminergic neurons with neuromelanin are seen in the field. The peripheral rim of an LB was predominantly stained with anti-CKII β antibody (*arrow*) in one neuron. The two other neurons in the field are Lewy body negative. The boxed neuron is shown at higher power magnification (*inset*). Omission of primary antibody gave no signal. Scale bar=50 μm.

synphilin-1 regulates α-synuclein/synphilin-1 interaction and thereby inclusion body formation [12]. We found that both CKII α and β subunits are present in cytoplasmic inclusions of cells transfected with these two protein partners. Therefore, CKII-induced phosphorylation may have an important role in the formation of inclusions in the context of α-synuclein and synphilin-1 interaction. However, the pathological relevance of this kinase to human α-synucleinopathies is unknown.

2. Subjects and methods

Postmortem human brain specimens were obtained from three clinically and pathologically confirmed cases of PD (2 females, 1 male; ages 76–83 years), and two control cases (both 73 year old males). These samples were obtained from the Research Resource Network through the National Center of Neurology and Psychiatry Musashi Hospital (Japan). This study was approved by the Scientific-Ethical Review Board of Ajou University Medical Center (AJIRB-CRO-06-056) and by the National Center of Neurology and Psychiatry (18-2-1).

Postmortem brain tissues from the midbrains of patients with PD and controls were fixed in formaldehyde and embedded in paraffin. Six-micrometer sections from substantia nigra (SN) were sectioned for immunohistochemistry. Tissue sections were deparaffinized in xylene followed by a descending concentration of ethanol solutions. Brain sections were permeabilized with 0.2% Triton X-100 in PBS for 30 min, and washed with PBS. Endogenous peroxidase was blocked by incubating sections in 3% hydrogen peroxide solution for 5 min, and then rinsed in PBS. The sections were then immunostained with mouse monoclonal antibodies to casein kinase II α and β-subunits (1:500, Calbiochem), which were characterized in our previous report [12]. Immunoreactivity was visualized with the avidin–biotin complex detection system (Vector Laboratories) according to the manufacturer's instructions, using 3,3-diaminobenzidine (DAB) as the chromogen. The sections were counterstained with Harris hematoxylin solution (ThermoShandon). To assess the co-localization of CKII subunits and α-synuclein, a double-labeling immunofluorescence study was performed on selected sections with a combination of monoclonal anti-CKII β subunit (1:500, Calbiochem) and polyclonal anti-α-synuclein (1:200, Sigma) antibodies. CKII β was visualized by anti-mouse IgG coupled with FITC (Chemicon), and α-synuclein was visualized with anti-rabbit IgG coupled with rhodamine (Chemicon).