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PAT1 induces cell death signal and SET mislocalization into the cytoplasm by increasing APP/APLP2 at the cell surface

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Abstract

The cleavage of amyloid precursor protein (APP) by caspases unmasks a domain extending from membrane to caspase cleavage site. This domain induces apoptosis *in vitro* and *in vivo* when overexpressed in neurons through the help of an internalization vector. In this model, we previously showed that SET rapidly binds to the internalized domain and is involved in downstream deleterious effects. Under these conditions SET mislocalizes from the nucleus to the cytoplasm, as in Alzheimer's disease (AD). In this report using the same model, we show that PAT1 attaches to the internalized domain earlier than SET and that this binding causes an increase in the levels of APP and APLP2 at the cell surface. Down regulation experiments of PAT1 and of APP and APLP2 show that the increase of the levels of APP and APLP2 at the cell surface triggers the cell death signal and SET mislocalization into the cytoplasm. In the context of AD these data suggest that mislocalization of SET into the cytoplasm may occur downstream of first cell death signal events involving PAT1 protein.

Keywords: Amyloid precursor protein; Neuron; Cell death; PAT1; SET; Subcellular localization; Alzheimer

1. Introduction

Amyloid precursor protein (APP) is a key molecule in Alzheimer's disease (AD), as it generates the amyloid peptide involved in AD pathology (Selkoe, 1999). However the function of APP remains unclear. The characterization of APP interactions with specific proteins would provide insight into APP-function and the dysregulation of APP in AD. The cytoplasmic domain of APP carries information for APP trafficking and signaling. The cytoplasmic juxtamembrane domain of APP has been shown to be important both for sorting to the basolateral side of MDCK cells and for endocytosis (Lai et al., 1995; Haass et al., 1995). Few interacting proteins have been identified for this juxtamembrane region, possibly due to its conformation. The entire cytoplasmic domain contains two type I reverse turns, the first of which is located at 'TPEE' residues, four amino-acids downstream from the caspase cleavage site at residue D664 (Kroenke et al., 1997). This type I turn in the APP cytoplasmic domain may mask interactions with putative targets. Indeed, one such putative target, PAT1, has been reported to interact primarily with the first 11 amino-acids of the cytoplamic juxtamembrane domain (Zheng et al., 1998). The strength of the PAT1–APP interaction decreases if the APP cytoplasmic sequence is extended to residue 29 or to the entire cytoplasmic domain. These findings suggest that the APP cytoplamic juxtamembrane domain could be exposed by the caspase-mediated cleavage of APP at residue D664 (Zheng et al., 1998). We termed this domain Jcasp since the caspase site is found at the C-terminal end of this region. Caspase cleavage should facilitate the binding of the unmasked Jcasp domain to PAT1.

The caspase-mediated cleavage of APP has been observed in the brains of control adults but its levels are significantly elevated in the hippocampus of AD patients, and detected in the neuronal cytoplasm and nuclei at early stages of the

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disease (Ayala-Grosso et al., 2002; Zhao et al., 2003; Banwait et al., 2008). Studies in a mouse model of AD carrying a mutation at the caspase cleavage site have demonstrated that this cleavage plays an essential role in the generation of AD-like deficits, as this mutation abolishes synaptic loss, astrogliosis, dentate gyrus atrophy, behaviour anomalies (Galvan et al., 2006). APP cleavage by caspase does not prevent the formation of amyloid peptides. This suggests that peptides are formed from the region extending from the secretase sites to the caspase site, ending with the Jcasp domain. Indeed, several APP peptides with a caspase cleavage site at the C-terminal end, consistent with the presence of a Jcasp domain, have been described in the brains of AD patients and in apoptotic neurons (Lu et al., 2000; LeBlanc et al., 1999). We have previously showed that accumulation of Jcasp peptide in neurons via an internalization vector, induces apoptosis *in vitro* and *in vivo*, and that mutation of tyrosine in this sequence abolishes the effect (Bertrand et al., 2001). Similar results were obtained for the intraneuronal accumulation of peptides extending from the gamma secretase site to the caspase site (Gcasp) (Madeira et al., 2005).

To uncover the mechanisms underlying cell death by Jcasp peptide, we have focused our efforts to identify the interacting proteins that bind the internalized Jcasp peptide soon after entry into the cell. We previously identified SET, also called template activating factor 1β or phosphatase 2A inhibitor 2, as a new target protein that interacts with Jcasp domain and induces apoptosis. Down regulation of SET abolishes the deleterious effect of Jcasp (Madeira et al., 2005). SET performs several functions including that of transcription factor and activator of tau phosphorylation as it both activates cdk5 and inhibits phosphatase 2A. Early in Jcasp-induced cell death, a partial mislocalization of SET from the nucleus to the cytoplasm was detected (Madeira et al., 2005). Also, genotoxic stress in neurons has recently been reported to decrease the SET levels in the nucleus and increase them in the cytoplasm (Qu et al., 2007). This change in distribution leads to cell death suggesting that SET is toxic when present in the cytoplasm (Qu et al., 2007). The toxicity of SET in the cytoplasm has also been associated with tau hyperphosphorylation in PC12 cells stably expressing tau (Chohan et al., 2006). We also observed that overexpression of SET in the cytoplasm of primary neurons induces cell death (Madeira et al., 2005). Interestingly, the mislocalization of SET from the nucleus to the cytoplasm was also observed in the hippocampus and the temporal cortex of AD patients (Tanimukai et al., 2005). The signal responsible for triggering this mislocalization remains unknown.

We investigated the signals that trigger SET mislocalization by studying the early events in the Jcasp-induced cell death model. We hypothesized that PAT1, a target of the Jcasp domain, may be involved in Jcasp-induced cell death and SET mislocalization. Here we show that PAT1 binds to the Jcasp domain before SET interacts with it, and that this binding is directly involved in accumulating APP, and its homolog amyloid precursor like protein 2 (APLP2), at the cell surface. This induces a cell death signal and the mislocalization of SET to the cytoplasm.

2. Materials and methods

2.1. Antibodies and chemicals

Anti-APP (22C11) antibody and anti-caspase cleaved APP were purchased from Millipore; anti-APLP2 (DII-2) and anti-APLP1 (CT11) antibodies were from Calbiochem; anti-tubulin antibody was from Sigma and anti-SET antibodies from Santa-Cruz. Rabbit antibody to APP C-terminal was previously used (Langui et al., 2004). Horse radish peroxydase labelled anti-mouse and anti-rabbit secondary antibodies for immunoblotting were from Amersham. Cy3 labelled anti-mouse and anti-rabbit antibodies were from Jackson Immunoresearch Laboratories. The supernatant of the hybridoma cell line mAb26 was used as the antiserum against PAT1 (Zheng et al., 1998). Trypsin, DNAse and trypsin inhibitors were from Sigma.

2.2. Primary cortical neurons

Primary cortical neurons were performed from E16 mouse embryos as previously described (Lafont et al., 1992). Briefly, sliced dissociated cells were plated on polyornithine-coated plastic dishes for biochemistry and glass coverslips for immunocytochemistry at a density of 15×10^4 cells/cm^2.

For cell death experiments, the dissected embryos removed from meninges in PBS glucose were sliced and incubated for 20 min at 37 °C with 0.25 mg/ml trypsin in buffer 1 (1.21 M NaCl, 48.3 mM KCl, 12.2 mM KH_2PO_4, 254.7 mM $NaHCO_3$ and 142.6 mM glucose) supplemented with 3 mg/ml bovine serum albumin and 0.3 mg/ml $MgSO_4$. Then trypsin digestion was stopped by adding a trypsin inhibitor solution (0.25 mg/ml) supplemented with DNAse (0.04 mg/ml) in the same buffer. The suspension was washed and centrifuged and the pellet was resuspended in the medium. This procedure resulted in lower levels of cell death in the control than dissociation in the absence of trypsin treatment.

Both procedures gave the same data concerning the changes in APP and APLP2 at the surface and the mislocalization of SET to the cytoplasm after Jcasp peptide internalization.

2.3. HEK cell lines

HEK cell lines stably overexpressing wild type APP 751, or the mutant protein truncated at the epsilon site, and the mock cell line transfected with the vector only were a gift from Dr Frédéric Checler (Lefranc-Jullien et al., 2006). For Jcasp peptide internalization the cells were transferred to DMEM without serum 3 h before internalization.