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Altered transcriptional regulators in response to serum in immortalized lymphocytes from Alzheimer's disease patients

Natividad de las Cuevas, Úrsula Muñoz, Ofelia G. Hermida, Ángeles Martín-Requero*

Department of Pathophysiology and Human Molecular Genetics, Centro de Investigaciones Biológicas (CSIC), Ramiro de Maeztu 9, 28040-Madrid, Spain

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Abstract

Cell cycle disturbances may precede neuronal death in Alzheimer's disease (AD). We described alterations, in lymphocytes from AD patients, on the activity of two transcription factors, E2F and NF-κB, involved in cell proliferation and survival regulation, demonstrating that cell cycle dysfunction also occurs in peripheral cells. The analysis of E2F–DNA binding activity revealed lower signal intensity of protein–DNA complexes in AD cells, which correlated with increased phosphorylation of retinoblastoma (pRb) related proteins and enhanced proliferation. The calmodulin (CaM) antagonist calmidazolium (CMZ) abrogated the increased activity of AD cells by partially dephosphorylating pRb and p130. The NF-κB–DNA binding activity increased as cell progress through the cell cycle. The reduced NF-κB activation observed in AD cells appears not to be related to the increased phosphorylation of the pRb family proteins nor with the enhanced proliferative activity of AD cells, but seems to protect them from death induced by the loss of trophic support. Ca^{2+}/CaM antagonists rescue NF-κB–DNA binding activity and sensitize AD cells to serum withdrawal. These observations suggest that disruption of Ca^{2+}/CaM signaling pathway could be linked mechanistically to its pro cell survival actions, promoting enhanced proliferation or decreased cell death depending on the presence of growth-stimulatory signals.

Keywords: Alzheimer's disease; Lymphocytes; Calmodulin; E2F; NF-κB

1. Introduction

Recent studies have shown the presence of cell cycle proteins in neurons within affected brain areas during AD [8,10,11,37,42]. In addition, truly DNA replication has been detected, by the use of fluorescent in situ hybridation techniques, in hippocampal neurons in autopsy material from AD patients [55]. These observations suggest the attempt of certain neurons to re-enter the cell cycle. However, since postmitotic neurons lack the machinery to fulfill the cell division, this emergence out of quiescence may have deleterious consequences and, eventually, could induce neuronal death.

The source of signals that drives the neurons of AD patients to begin the cell division process is not yet known, but numerous neurotrophic and potential mitogenic compounds have been found to be elevated early in the course of the disease [12,23,52]. Moreover, cell surface amyloid precursor protein (APP) seems to posses a neurite-promoting activity [13] and the product of its degradation, β-amyloid peptide, the main component of amyloid plaques, has been shown to induce a proliferative response in neuronal cultures [10].

Recent research has provided evidence that AD has systemic expression at cellular and molecular levels [27]. For this reason, and considering the difficulties of using postmortem material to study dynamic events, peripheral cells have been used as experimental models to study cellular aspects of AD etiopathogeny at any level of the disease pro-

* Corresponding author. Tel.: +34 91 837 3112; fax: +34 91 536 0432.
E-mail address: amrequero@cib.csic.es (Á. Martín-Requero).

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gression [38,51]. In general, non-neuronal cell types like fibroblasts or lymphocytes obtained from AD patients show a distinct responsiveness to extracellular activators [20,28]. Of particular relevance for this work is the finding of cell cycle disturbances in peripheral cells from AD patients [41,48] suggesting that dysfunction of cell cycle is a more general phenomenon affecting cells other than neurons.

Previous work from our laboratory had shown altered Ca^{2+} and pH homeostasis associated with enhanced proliferation of lymphoblasts from AD patients [29,51]. The increased cell growth of AD lymphoblastoid cell lines seems to be consequence of activation of G1 cyclin-dependent kinases (CDKs) and consequently phosphorylation and inactivation of the pRb family proteins [14]. To further define the role of these cell cycle proteins in neurodegeneration associated with AD, we tried to determine whether there is a distinct regulation of the activity of E2F, a family of transcription factors involved in cell cycle progression [17,43]. In addition, the activity of the transcription factor NF-κB has been investigated in lymphoblasts from control and AD subjects. The rationale behind finds support in the recent finding of a novel role of pRb family proteins as potential activators of NF-κB and inducers of G1 cyclins [49]. Besides its widely known role in inflammation and immune responses, NF-κB is also involved in the control of cell division and apoptosis [16,36]. Postmortem brain tissues from AD patients have revealed altered NF-κB activity in cells in the vicinity of amyloid plaques [30]. Moreover, it has been shown that the conditioned medium from β-amyloid-stimulated glial cells triggers neuronal cell division [54], suggesting that the inflammatory process may be one of the mitotic pressures in AD.

E2F is a generic name given to a group of heterodimeric transcription factors consisting of one member of E2F family of proteins (E2F1–6) together with one member of DP family (DP1–3). Phosphorylation of pRb-related proteins by CDKs, results in release and activation of E2F, which then activates genes required for S phase transition [53].

NF-κB is a homo or heterodimer of proteins belonging to the NF-κB/Rel family. The dimer p50/p65 is the most prominent and is considered to be the prototype of the NF-κB factors [22]. NF-κB exists in the cytoplasm as an inactive dimer bound to inhibitory proteins IκB [4]. Upon cellular stimulation, NF-κB dimer is released from the corresponding IκB, and then translocated to the nucleus where it activates its target genes by binding to specific DNA response elements in their regulatory regions.

In this work, we have assessed the influence of the serum-mediated changes in the levels and phosphorylation status of cell cycle regulatory proteins on the transcriptional activities of E2F and NF-κB. We report here that whereas the activity of E2F correlates with increased phosphorylation of pRb-related proteins and enhanced cell proliferation, NF-κB–DNA binding activity does not. Instead, the transcriptional activity of NF-κB appears to be related to cell vulnerability to serum deprivation.

2. Methods

2.1. Materials

Radioactive compounds were purchased from Amersham (Uppsala, Sweden). Poly(vinylidene) fluoride (PVDF) membranes for Western blots were purchased from Bio-Rad (Richmond, CA). All the antibodies used in this study were from Santa Cruz Biotechnologies (Santa Cruz, CA) and include antibodies to pRb (sc-50), p130 (sc-317), cyclin D1 (sc-8396), p50 (sc-7178) and p65 (sc-7151). The enhanced chemiluminiscence (ECL) system was from Amersham (Uppsala, Sweden). Tissue culture media and reagents were obtained from GIBCO-BRL. (Gatithersburg, MD). MTT [3-(4,5-dimethylthiazol-2-yl]-2,5 diphenyltetrazolium bromide) was from Sigma (Saint Louis, MO). 15-deoxy-$\Delta^{12,14}$-prostaglandin J_2 (15d PGJ_2) was from Calbiochem–Novabiochem (Darmstadt, Germany). Amyloid-β-protein (25–35) was from Bachem AG (Switzerland). All other reagents were of molecular biology grade.

2.2. Cell lines

A total of 20 patients diagnosed by the department of Neurology of the University Hospital Doce de Octubre (Madrid, Spain) of probable Alzheimer according to NINCDS–ADRDA (National Institute of Neurological and Communicative Diseases and Stroke–Alzheimer's Disease and Related Disorders Association) criteria were used for this study. The average age of onset of the disease was 74 ± 2 years. A group of 20 non-demented age-matched individuals were used as controls. In all cases, peripheral blood samples were taken after written informed consent of the patients or their relatives.

Establishment of lymphoblastic cell lines was performed in our laboratory as previously described [27] by infecting peripheral blood lymphocytes with the Epstein Barr virus [31]. Cells were grown in suspension in T flasks in an upright position, in approximately 10 ml of RPMI-1640 (Gibco-BRL) medium that contained 2 mM L-glutamine, 100 μg/ml streptomycin and, unless otherwise stated, 10% (v/v) fetal bovine serum (FBS) and maintained in a humidified 5% CO_2 incubator at 37 °C. Fluid was routinely changed every two days by removing the medium above the settled cells and replacing it with an equal volume of fresh medium.

2.3. Analysis of DNA fragmentation

Fragmentation of lymphoblasts DNA was assessed as previously described [35]. Cytoplasmic DNA was extracted after elimination of intact nuclei. Purified DNA was then electrophoresed in a 2% agarose gel and visualized by UV fluorescence after staining with ethidium bromide.