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Depletion of the AP-1 repressor JDP2 induces cell death similar to apoptosis

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Abstract

JDP2 is a ubiquitously expressed nuclear protein that efficiently represses the activity of the transcription factor AP-1. Thus far, all studies of JDP2 function have relied on the ectopic expression of the protein. In this study, we use a different approach: depletion of JDP2 from cells. Specific depletion of JDP2 resulted in p53-independent cell death that resembles apoptosis and was evident at 72 h. The death mechanism was caspase dependent as the cells could be rescued by treatment with caspase inhibitor zVAD. Our studies suggest that JDP2 functions as a general survival protein, not only following UV-irradiation, as reported earlier, but also under normal culture conditions. Thus, our data support that JDP2 is a cellular survival protein whose presence is necessary for normal cellular function.

Keywords: JDP2; AP-1; Programmed cell death; Caspase; Antisense

1. Introduction

AP-1 is a multipotent transcription factor involved in various central cellular processes. Numerous studies have demonstrated that AP-1 is actively taking part in development and tumorigenesis (involving processes such as oncogenic transformation, angiogenesis, proliferation, and metastasis) as well as in neuronal degeneration and programmed cell death [1]. Many of these properties of AP-1 are associated with its c-Jun component. The ubiquitously expressed bZIP family member JDP2 is a very potent AP-1 repressor, which dimerizes efficiently with c-Jun and represses its transcriptional activity [2]. JDP2 expression has been detected in all tissues and cell lines tested thus far. JDP2 expression is rather stable and has not been found to respond to extracellular stimuli other than UV-irradiation and RANKL treatment, which both have been shown to both modestly up regulate JDP2 protein levels [3].

Heterodimerized JDP2 binds TREs in conjugation with c-Jun, JunB, JunD, or c/EBPγ; or CREs in conjugation with ATF2. Experiments utilizing combinations of ectopically expressed AP-1 family members have revealed, that JDP2 is a potent repressor of both TRE- and CRE- driven transcription [2]. Jin and coworkers have further demonstrated a mechanism that might be central for these functions, by showing that JDP2 represses the retinoic acid induced transcription of c-Jun in F9 cells through recruitment of the HDAC3 [4].

Abbreviations: Aa, amino acid; CM, Complete medium; CMV, Cytolomegavirus; CRE, cyclic AMP response element; DTT, dithiothreitol; E1A and E1B, Adenovirus 5 early region 1A and 1B; FCS, Fetal calf serum; GFP, Green fluorescent protein; HDAC3, Histone deacetylase complex 3; JDP, Jun dimerization partner; LDH, Lactate dehydrogenase; Mdm2, Murine double minute 2; MEF, Mouse embryonic fibroblasts; PBS, Phosphate buffered saline; RANKL, receptor activator of NF-κB ligand; TRE, TPA responsive element; ZVAD, Z-Val-Ala-Asp(Ome)-CH2F

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Overexpression of JDP2 protects immortalized mouse embryonic fibroblasts from UV irradiation induced cell death. This protection is apparently achieved via the capability of JDP2 to repress transcription of the *p53* promoter by binding to a variant of AP-1 site, termed the PF-1 site, in the *p53* promoter together with c-Jun [3]. Interestingly, two recent studies have shown that JDP2 also possesses novel unexpected cellular functions. The first study demonstrates that JDP2 can act as transcriptional co-activator via association with the progesterone receptor [5]. In the second study, JDP2 was shown to induce terminal differentiation of myoblasts with a mechanism that includes up regulation of p21 and down regulation of cyclin D1, demonstrating that JDP2 can interfere with normal cell cycle progression when overexpressed [6]. It seems that JDP2 function in vivo is fairly complex and can possibly involve other mechanisms than dimerization with bZIP proteins. Despite of increasing information of the possible cellular function of JDP2, details of the physiological role of JDP2 as well as the mechanisms by which JDP2 acts remain to be clarified.

Based on its potent AP-1 repressor function, it would be reasonable to assume that JDP2 might possess anti-apoptotic abilities and maybe even general tumor suppressor properties. The potential tumor suppressor role was very recently demonstrated in a work utilizing JDP2 over-expression [7]. We reasoned that inactivation/depletion of JDP2 from cells should release AP-1 repression and thus could promote proliferation and/or cell death depending on the cell type just as the increase in AP-1 activity does. Since previously published work had focused on overexpressed JDP2, we decided to examine the properties of JDP2 using the opposite approach: depletion of JDP2 from cells. Here, we have used an antisense approach and demonstrated that specific depletion of JDP2 from HEK-293 cells induces cell death that resembles apoptosis and involves caspases but not p53. Overall, our data suggest that JDP2 supports cellular survival function under normal culture conditions.

2. Materials and methods

2.1. Plasmids

Following plasmids were used in this study: pEBS7 [8], pCMV-E2F-1 [9], pCMV-CD20 [10], pH2B-GFP [11], and p53wt and p53(H175) [12]. The Mdm2-luc reporter was a gift from Kanaga Sabapathy and the p53(N315) belongs to E. Shaulian. The plasmid pEGFPC3 was purchased from Clontech, pNull renilla luciferase (pNull-rluc) from Promega and pcDNA3.1 HisA from Invitrogen.

Database search for human ESTs with a sequence highly similar to the published sequences of mouse and rat JDP2 [2] yielded one EST assumed to encode a protein with 94% and 96% of the sequence identical to the entire sequence of mouse and rat JDP2, respectively (Analysed in Clustal W multiple alignment, [13]). A plasmid encoding this EST was obtained from the I.M.A.G.E. Consortium (CloneID: 2316213) [14] and the insert encoding the putative human JDP2 sequence was used as a template for subsequent PCR amplifications. A set of four different primers leading to four differently sized and partially overlapping PCR-products were planned. The primers hJDP2/X1 (5′-tgc**tctaga**atgatgcctgggcagatc-3′) (hybridising sequence is underlined, restriction endonuclease recognition sites in bold) and hJDP2/H3 (5′-ccc**aagctt**gttccgtcatcgggctgc-3′) were used for the insert in pJDP2AS1, the primers hJDP2/X1 and hJDP2/H1 (5′-ccc**aagctt**gtcacttcttctcgagct-3′) for the insert in pJDP2AS2, the primers hJDP2/X4 (5′-tgc**tctaga**cggccctgactgtggagg-3′) and hJDP2/H3 for the insert in pJDP2AS3 and the primers hJDP2/X4 and hJDP2/H1 for the insert in pJDP2AS4 leading to products identical to the areas 1–273 bp (corresponding aa 1–91), 1–492 bp (corresponding aa 1–163), 82–273 bp (corresponding aa 28–91) and 82–492 bp (corresponding aa 28–163) of the human JDP2 CDS. All PCR reactions were carried out utilizing the Advantage 2 PCR System (Clontech Laboratories) according to the manufacturers protocol with the following settings: 1′ at 95 °C, 30×(15″ at 94 °C, 30″ at 55 °C and 60″ at 72 °C), 10′ at 72 °C. All PCR products were designed to have an *Xba*I site upstream the amplified region and a *Hind*III site downstream and were clone to respective sites in the pEBS7 vector. The human JDP2 cDNA clone was also used as a template for PCR amplifications of the full length cDNA of the sense JDP2 using the primers hJDP2-*Kpn*I (5′-cggggtac-caatgatgcctgggcagatcc-3′) and hJDP2- *Eco*RI (5′-ccggaattcgtcacttcttctcgagctgc-3′). The amplification was carried utilizing the Advantage 2 PCR System. The PCR fragment was subcloned into pcDNA3.1 HisA vector (Invitrogen) with *Kpn*I and *Eco*RI. All the DNA constructs were verified by sequencing.

2.2. Cell culture and transfection methods

Cells were propagated in a humidified atmosphere containing 10% CO_2. HEK-293 CM consisted of DMEM medium (Invitrogen), 10% heat inactivated FCS (Biological Industries), 100 μg/ml streptomycin, and 100 U/ml penicillin; while MCF-7 CM consisted of RPMI-1640 medium (Invitrogen), 6% heat inactivated FCS (Biological Industries), 100 μg/ml streptomycin, and 100 U/ml penicillin. When needed, cells were selected with 150 U/ml of Hygromycin B (Calbiochem). HEK-293 cells were transfected using the calcium-phosphate precipitation method [15]. After 8 to 16 h, the medium was replaced with new. Transfection efficiencies were estimated using co-transfected pEGFP-C3 (Clontech) and was generally estimated to be 70%. Transfection of MCF-7 cells was done using the Fugene 6 transfection agent (Roche) as described in the protocol supplied from the manufacturer. Both HEK-293 and the MEC-7 cells were from ATCC.