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Preventing apoptotic cell death by a novel small heat shock protein

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Abstract

NCBI database analysis indicated that the human C1orf41 protein (small heat shock-like protein—Hsp16.2) has sequence similarity with small heat shock proteins (sHsps). Since sHsps have chaperone function, and so prevent aggregation of denatured proteins, we determined whether Hsp16.2 could prevent the heat-induced aggregation of denatured proteins. Under our experimental conditions, recombinant Hsp16.2 prevented aggregation of aldolase and glyceraldehyde-3-phosphate dehydrogenase, and protected *Escherichia coli* cells from heat stress indicating its chaperone function. Hsp16.2 also formed oligomeric complexes in aqueous solution. Hsp16.2 was found to be expressed at different levels in cell lines and tissues, and was mainly localized to the nucleus and the cytosol, but to a smaller extent, it could be also found in mitochondria. Hsp16.2 could be modified covalently by poly(ADP ribosylation) and acetylation. Hsp16.2 over-expression prevented etoposide-induced cell death as well as the release of mitochondrial cytochrome *c* and caspase activation. These data suggest that Hsp16.2 can prevent the destabilization of mitochondrial membrane systems and could represent a suitable target for modulating cell death pathways.

Keywords: Chaperone; sHsp; Apoptosis; Cytoprotection, Tumor genesis

Introduction

The small heat shock proteins (sHsps) are found in most organisms where they are induced upon stress (Van Montfort et al., 2001). They function as molecular chaperones since they are able to prevent thermal and chemical-induced aggregation of a variety of targets both in vitro and in vivo (Basha et al., 2004; Ehrnsperger et al., 1997; Franzmann et al., 2005; Haslbeck, 2002; Stromer et al., 2003). The sHsp family is characterized by a highly conserved C-terminal domain of about 90 amino acids, the "α-crystallin domain", and a variable N-terminal domain (De Jong et al., 1998; Horwitz, 2003; Mornon et al., 1998; Narberhaus, 2002). They are considered to be ATP-independent chaperones that are not involved in regulating protein folding. However, under conditions of cellular stress, they interact with and so stabilize partially folded target proteins to prevent their aggregation and precipitation. For this reason, it is considered that sHsps

Abbreviations: GAPDH, glyceraldehyde-3-phosphate dehydrogenase; GST, glutathione *S*-transferase; sHsps, small heat shock proteins

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can create a reservoir of denatured proteins, which could be refolded in the presence of ATP-dependent chaperones (Ehrnsperger et al., 1997; Lee et al., 1997; Franzmann et al., 2005; Haslbeck, 2002; Stromer et al., 2003).

sHsps have monomeric masses between 12 and 43 kDa. These monomers associate into oligomeric structures with mostly 12 or 24 subunits. Even larger complexes with up to 50 subunits were observed for α-crystallin (Horwitz, 1992, 2003). The large variability in the length of the N-terminal regions appears to be responsible for the significant variations of the sizes of the oligomers (Narberhaus, 2002).

sHsps interact with various components of the programmed cell death machinery upstream and downstream of the mitochondrial apoptotic events, and can prevent apoptosis in different lethal stress situations (Arrigo, 2005; Kamradt et al., 2005). Furthermore, they are over-expressed in malignant tumors where they are involved in tumor cell growth and can induce cytostatic resistance (Kang et al., 2004; Rocchi et al., 2004). Altogether, these properties suggest that these sHsps are promising targets for modulating cell death pathways. The human sHsp family is comprised of 10 known members (Haslbeck et al., 2005; Kappe et al., 2003). Some of them are ubiquitously expressed such as HspB1, HspB5, HspB6, and HspB8, the others are expressed in a tissue-restricted manner like HspB2, HspB3, HspB4, HspB7, HspB9, and HspB10 (Taylor and Benjamin, 2005). It has also been shown that sHsps are involved in some disorders, such as cataract and neuropathy (Horwitz, 2003; Mackay et al., 2003; Sun and MacRae, 2005).

Searching the NCBI database, we could identify similarity between the amino acid sequences of sHsps and C1orf41 protein, a protein of yet uncharacterized function. Thus, the protein encoded in C1orf41 may have a physiological role similar to that of sHsps. To indicate this putative physiological role, we use the name small heat shock-like protein (Hsp16.2) for the C1orf41 protein further on.

Materials and methods

Materials

Human tissues from patients were provided by the Department of Pathology and Oncotherapy, Medical University of Pécs. All tissue samples were obtained after approval by the local Ethics Committee. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was obtained from Roche Diagnostics. Aldolase, albumin, Hsp27, Histone, isopropyl-β-D-thiogalactopyranoside, protease inhibitor mixture, glutathione Sepharose 4B, and all of the chemicals for cell cultures were purchased from Sigma. We used pQE30, PQE60 vectors, Ni-NTA columns (Qiagen Inc., Valencia, CA, USA), pcDNA3.1, protein G-Sepharose, Sephadex G25 column (Pharmacia, Uppsala, Sweden), human placental RNA (Ambion, TX, USA), and etoposide (Chemicon International Inc.). The following antibodies were used: anti-poly (ADP-ribose), anti-acetylated lysine monoclonal antibody (Alexis Biotechnology, London, UK), anti-cytochrome *c* monoclonal and anti-caspase-3 polyclonal antibodies (Pharmingen, San Diego, CA), anti-actin rabbit antibody, anti-histone H3 rabbit antibody, anti-mouse IgG and anti-rabbit IgG, monoclonal anti-phosphoserine mouse antibody (Sigma), anti-GAPDH rabbit antibody, anti-AKT rabbit antibody (Cell Signaling), and anti-citrate synthase rabbit IgG (Santa Cruz Biotechnology).

Isolation of full-length Hsp16.2 cDNA

Full-length Hsp16.2 cDNA was isolated by PCR amplification using 4 μg total placental RNA and specific primers designed based on the complete cDNA sequence of C1orf41 protein. The resulting PCR fragments were inserted into pUC57-T (Gibco BRL, USA), and insert-containing clones were selected. Plasmid preparation was carried out with a QIAprep Spin Miniprep kit (Qiagen, Valencia, CA, USA). Sequencing was performed by Taq BigDye terminator cycle sequencing using an automated ABI PRISM 310 Genetic Analyzer (Perkin-Elmer, USA).

Databank search

The cDNA of C1or41 protein (Hsp16.2) was compared to different expressed sequence tags (ESTs) and genomic databases by BLAST algorithm. Alignment of the Hsp16.2 cDNA and related ESTs to genomic sequences was performed with LocusLink and the UniGene EST ProfileViewer all provided by NCBI (Bethesda, MD, USA). Multiple amino acid sequence alignment of Hsp16.2 to its homologues was carried out with Multalin (Corpet, 1988). Phosphorylation prediction search was performed by using the NetPhos 2.0 Server program of the Technical University of Denmark.

Construction of bacterial expression plasmids and purification of recombinant proteins

The whole open reading frame (ORF) of the Hsp16.2 cDNA was cloned into pQE30, pGEX-4T-1, and pcDNA3.1 (Invitrogen). Protein expression and purification was performed according to the manufacturers' protocol. The primary structure and purity of recombinant Hsp16.2-GST (GST—glutathione *S*-transferase)