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Hsp90 cleavage by an oxidative stress leads to its client proteins degradation and cancer cell death

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ABSTRACT

The heat shock protein 90 (Hsp90) plays a crucial role in the stability of several proteins that are essential for malignant transformation. Hsp90 is therefore an interesting therapeutic target for cancer therapy. In this paper, we investigated whether an oxidative stress generated during ascorbate-driven menadione redox cycling (ascorbate/menadione), affects Hsp90 leading to the degradation of some critical proteins and cell death. Unlike 17-AAG, which inhibits Hsp90 but enhances Hsp70 levels, ascorbate/menadione-treated cells present an additional Hsp90 protein band of about 70 kDa as shown by Western blot analysis, suggesting Hsp90 cleavage. This Hsp90 cleavage seems to be a selective phenomenon since it was observed in a large panel of cancer cell lines but not in non-transformed cells. Antibodies raised against either the N-terminus or the C-terminus domains of Hsp90 suggest that the site of cleavage should be located at its N-terminal part. Furthermore, antibodies raised against either the α- or the β-Hsp90 isoform show that Hsp90β is cleaved while the α isoform is down-regulated. We have further shown that different Hsp90 client proteins like Bcr-Abl (a chimerical protein expressed in K562 leukemia cells), RIP and Akt, were degraded when K562 cells were exposed to an oxidative stress. Both Hsp90 cleavage and Bcr-Abl degradation were observed by incubating K562 cells with another H_2O_2-generating system (glucose/glucose oxidase) and by incubating KU812 cells (another leukemia cell line) with ascorbate/menadione. Due to the major role of Hsp90 in stabilizing oncogenic and mutated proteins, these results may have potential clinical applications.

1. Introduction

The heat shock protein 90 (Hsp90) plays a crucial role in the stability of several proteins (like Bcr-Abl, Akt, RIP, mutated p53, etc.) that are essential for malignant transformation [1,2]. Hsp90 is a conformational flexible protein that associates with a distinct set of co-chaperones depending on nucleotide (ADP or ATP) occupancy of an amino-terminal binding pocket. Nucleotide exchange and ATP hydrolysis drive the so-called Hsp90 chaperone machine to bind co-chaperones as well as client proteins, protecting these latter from proteasomal degradation [3]. The ability of Hsp90 to interact with multiple signaling networks is exploited by cancer cells, in which the expression of Hsp90 is increased [4]. Given the number of key nodal proteins that are Hsp90 clients, its inhibition represents an interesting target for cancer therapies. For instance, the

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safety evaluation of a small molecule inhibitor of Hsp90, the benzoquinone ansamycin antibiotic 17-allylamino-17-demethoxygeldanamycin (17-AAG), has been recently completed in four phase I clinical studies [5–8]. In fact, 17-AAG appears to act by suppressing the chaperone function of Hsp90, which causes the degradation of several client proteins.

On the other hand, it has been recently reported that the chaperoning function of Hsp90 may be disrupted by protein cleavage induced by hydrogen peroxide [9,10] or other reactive oxygen species (ROS) generated by arsenate [11]. The mechanisms underlying such a protein cleavage are still elusive, but due to the major role of Hsp90 in stabilizing key proteins involved in cancer cell survival, we decided to investigate whether an oxidative stress affects Hsp90, inducing the degradation of its client proteins and leading to cancer cell death. The rationale of our approach was that in K562 cells, a human leukemia cell line expressing Bcr-Abl (an Hsp90 client protein), oxidative stress generated by ascorbate-driven menadione redox cycling (ascorbate/menadione), inhibits glycolysis leading to an ATP depletion [12]. Since the assembly of the Hsp90–client protein complexes requires ATP [13,14], this could represent a new strategy to inhibit Hsp90.

The main finding of this work is that ascorbate/menadione induces an oxidative stress that causes a cleavage of Hsp90, which appears to preferentially affect cancer cells rather than normal cells. Indeed, by comparing a large panel of normal and cancer cell lines, the Hsp90 cleavage by ascorbate/menadione was observed in all tumor cell lines tested but in none of the non-transformed cells. This partial proteolysis disrupts the chaperoning function of Hsp90 leading to degradation of its client proteins: Bcr-Abl, RIP and Akt. The major role of oxidative stress in this process is supported by the fact that both Hsp90 cleavage and Bcr-Abl degradation were observed in K562 cells incubated with another H_2O_2-generating system (glucose/glucose oxidase) and by the effects of redox-modulators (N-acetylcysteine and aminotriazole). Due to the critical function played by Hsp90 in stabilizing oncogenic proteins in cancer cells, these data suggest that ascorbate/menadione might be of interest in anticancer therapy.

2. Materials and methods

2.1. Chemicals and antibodies

Menadione sodium bisulfite, sodium ascorbate, dimethylsulfoxide, N-acetyl-cysteine, glucose oxidase, 3-methyladenine, pepstatin, 17-allylamino-17-demethoxygeldanamycin (17-AAG), nitro-blue-tetrazolium (NBT), 5-bromo-4-chloro-3-indolyl phosphate (BCIP), hydrogen peroxide, antipain and cycloheximide were purchased from Sigma (St. Louis, MO). Iodoacetate was purchased from Acros Organics (Geel, Belgium). Calpeptin was purchased from Calbiochem (San Diego, CA). Polyclonal rabbit primary antibodies against c-abl (#2862) and Akt (#4685) were purchased from Cell Signaling Technology (Danvers, MA). Mouse monoclonal primary antibodies against Hsp90α/β C-terminus were purchased from either Santa Cruz Biotechnology (F-8) (Santa Cruz, CA), Pharmingen (clone 68) (San Jose, CA), or StressGen (clone AC-88) (Ann Harbor, MI). Unless otherwise indicated, the Santa Cruz antibody was used for detection of Hsp90. Mouse monoclonal antibodies against specific isoforms of Hsp90 were from Abcam (Cambridge, UK) for Hsp90α (clone D7a) and Zymed (San Francisco, CA) for Hsp90β (clone H9010). Goat polyclonal antibody directed against Hsp90 N-terminus (N-17) was purchased from Santa Cruz Biotechnology (Santa Cruz, CA). Mouse monoclonal primary antibody against β-actin (clone AC-15) was purchased from Abcam (Cambridge, UK), mouse monoclonal primary antibody against Hsp70 (clone C92F3A-5) was purchased from StressGen (Ann Harbor, MI). Mouse monoclonal primary antibody against RIP (clone 38) was purchased from Pharmingen (San Jose, CA). Mouse monoclonal primary antibody against LC3 (clone 5F10) was purchased from Nanotools (Teningen, Germany). Rabbit secondary antibodies were purchased from Chemicon (Billerica, MA). Mouse secondary antibodies were purchased from Dako (Glostrup, Denmark). Complete Mini protease inhibitor cocktail was purchased from Roche Applied System (Mannheim, Germany). All other chemicals were ACS reagent grade.

2.2. Cell culture conditions

The CML cell line K562 was a gift of Dr. F. Brasseur (Ludwig Institute for Cancer Research-LICR-Brussels) and maintained in RPMI medium supplemented with 10% foetal calf serum, streptomycin 100 μg/ml, penicillin 100 IU/ml, and gentamicin (50 μg/ml) at 37 °C in humidified 5% CO_2. Cells were treated with ascorbate (2 mM) and menadione (10 μM) either separately or in association for the indicated length of time.

K562 cells were incubated at a concentration of 1 million cells per ml. Inhibitors and/or ascorbate/menadione were added directly to the incubation medium at the indicated times. When required, preincubation of 1 h was used for some of the inhibitors. Glucose oxidase was added directly (0.25 U/ml) to the medium, which was supplemented with 25 mM of glucose to avoid its depletion.

The additional cancer cell lines FSAII (human fibrosarcoma), LLC (human lung adenocarcinoma) and B16 melanoma (murine melanoma), were a gift of Dr. O. Feron (FATH, UCL, Belgium). The MCF7 (human breast carcinoma) cell line was a gift of Dr. F. Brasseur (Ludwig Institute for Cancer Research-LICR-Brussels). The Ishikawa (human endometrial adenocarcinoma) cell line was obtained from the European Collection of Cell Cultures (ECACC, UK). The KU812 (chronic myelogenous leukemia) cell line was purchased from the German Collection of Microorganisms and Cell Cultures (DSMZ, Braunschweig, Germany). In addition, we also employed the TLT (murine hepatoma) cell line. The panel of non-transformed cell lines includes Balb/c3T3 (mouse fibroblasts) from the European Collection of Cell Cultures (ECACC, UK), HUVEC (human endothelial cells), freshly isolated mouse hepatocytes and human peripheral blood leucocytes.

2.3. Cell survival and ATP measurement

Cellular viability was estimated by measuring the activity of lactate dehydrogenase (LDH) both in the culture medium and