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Research paper

Cadmium regulation of apoptotic and stress response genes in tumoral and immortalized epithelial cells of the human breast

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Abstract

Cadmium (Cd) is a widely-disseminated metal which can be imported and accumulated in living cells thereby drastically interfering with their biological mechanisms. Increasing interest has been recently focused on the elucidation of the cellular and molecular aspects of Cd-dependent regulation of gene expression and signal transduction pathways in different model system. Concerning breast cancer, very limited studies have been produced so far on the role played by Cd on estrogen receptor-negative human breast cancer cells, that are expected to be insensitive to the already-proven metallo-estrogenic effect exerted by Cd on the estrogen receptor-positive cell counterparts. Here, we have examined the effects of long-term (96 h) exposure of estrogen receptor-negative MDA-MB231 malignant adenocarcinoma cells to $CdCl_2$ at 5 μM concentration, corresponding to the IC_{50} for this time of incubation, by evaluating the expression levels of genes coding for stress response factors (e.g. heat shock proteins and metallothioneins), and for apoptosis-related factors and enzymes. In parallel, we tested the gene expression pattern of immortalized HB2 breast epithelial cells, taken as non-tumoral counterpart, after the same exposure to the metal which instead did not exert any change in their cell number with respect to controls. Our cumulative results indicate that, whilst HB2 cells appear to activate defense mechanisms against metal stress principally via *metallothionein* massive up-regulation and appearance of the spliced form of XBP-1 message, MDA-MB231 cells seem to couple the onset of a protective reaction (e.g. up-regulation of *hsp27* and *metallothioneins*) to the switching-on of new intracellular pathways directing cells to a kind of death which shares several aspects with the apoptotic program, such as down-regulation of *Bcl-2* and over-expression of *Dap kinase* and several *caspases*.

Keywords: Cadmium; Tumor cells; Gene expression; Stress response; Apoptosis

1. Introduction

Cadmium (Cd) is an underground mineral extracted as part of zinc deposits, which is widely found in plastics and as a component of batteries. It is an industrial and environmental pollutant released as air contaminant from fertilizers and, more prominently, in the form of wastewater. Food is at present the main source of daily exposure of humans to Cd, and sometimes drinking water may contain high levels of this heavy metal; also inhalation of smoke from cigarettes may lead to elevated Cd concentrations [1]. Cd has a very long biological half-life (about 25 years in humans), is not essential for the human body and is not involved in enzymatic processes as other metals (e.g. zinc, selenium, magnesium) are; on the other hand, due to its many chemical similarities to zinc, Cd may bind with high affinity to the zinc-binding domains of several metalloproteins, thereby drastically interfering with zinc-dependent cellular functions [2]. A great deal of experimental studies about the toxic effect of Cd have so far focused mostly on selected target organs such as liver, kidney, thymus, liver, prostate and testis [3–5]. Cd is in fact classified in group

Abbreviations: Cd, cadmium; MT, metallothionein; hsp, heat shock protein; ER, estrogen receptor; PDGF-A, platelet-derived growth factor A; IC_{50}, 50% inhibitory concentration; ANOVA, analysis of variance; SEM, standard error of the mean; PCR, polymerase chain reaction; RT, reverse transcriptase; SM, semi-quantitative multiplex; UPR, unfolded protein response; ERE, endoplasmic reticulum; ROS, reactive oxygen species; ERK, extracellular signal-regulated kinase.

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I of carcinogens by the International Agency of Research on Cancer [6], and studies reported in the literature indicate that it may play a role in both the initiation of cancer, by activating oncogenes, and in the progression of cancer, by increasing the metastatic potential of existing cancer cells, through mechanisms which see the potential involvement of multiple factors, from oncogene activation to perturbation of membrane junctional systems (e.g. [7,8]).

At cellular and molecular levels, a number of recent reports have provided increasing information on some aspects of Cd-dependent regulation of gene expression and signal transduction pathways ([9] for review). Intracellular import of Cd ions is likely to be mediated by voltage-sensitive calcium channels of the plasma membrane [10]; once accumulated, Cd is able to affect the pattern of transcriptional activity. In particular, at least in some of the cell lines tested, although elevated concentrations appeared to be inhibitory and cytotoxic [4,11], low concentrations of Cd appeared to up-regulate intracellular signalling pathways and cell proliferation, also stimulating DNA synthesis and enhancing the expression of several classes of genes; these include the immediate early genes *c-fos*, *c-jun* and *c-myc*, the onco-suppressor *p53*, and genes coding for the synthesis of protective molecules, such as *metallothioneins* (*MTs*), *glutathione* and *heat shock proteins* (*hsp*) (e.g. [12–14]). Also the expression of *TIF3* and *TEF-1δ* was found up-regulated in Cd-transformed BALB/c-3T3 cells [15], whilst Shin et al. [16] demonstrated the induction of orphan nuclear receptor *Nur77* expression in lung cells, proposing a correlation between the up-regulation of this apoptosis-related protein and the occurrence of pulmonary toxicity in response to exposure to Cd. In addition, other reports have appeared in the literature, contributing to expand the list of Cd-responsive genes in different model systems [4,11,16,17]. However, it is largely demonstrated that the sensitivity to Cd varies from one cytotype to another, with cancer cells mostly hypersensitive to treatment with the metal. Interestingly, experimental data have demonstrated that Cd may induce tumor suppression when administered at not overtly-toxic doses to tumor-cell bearing immuno-depleted mice (e.g. [18,19]). In light of the collective information obtained, it is now widely acknowledged that exposure to Cd evokes in cells a number of responses that involve not only death signalling reactions but also cellular protective reactions against toxicity [20]. Within the complex scenario of cell–metal interactions, a number of experimental investigations have also brought to light that the lethal effect exerted by Cd may in some cases be of apoptotic nature [20–24]. Thus, the molecular basis of the cytotoxic effect(s) exerted by Cd represents a field of research that still requires further and more detailed investigation.

As far as breast cancer is concerned, few data are available from the literature and most of them are restricted to the effect of metal administration to the estrogen receptor-positive (ER^+) cell line MCF-7. In particular, incubation with Cd was shown to stimulate the synthesis of hsp27 [12] and to impair p53 function by inducing phosphorylation of a serine residue and conformational changes in the protein [25,26]. More interestingly, Garcia-Morales et al. [27] found that Cd exerts an estrogen-mimetic effect on ER^+ MCF-7 cells, thereby stimulating the transcription of several estrogen-inducible genes, like *progesterone receptor*, *cathepsin D* and *pS2*, and the increase of cell growth rate; these effects were also observed in vivo by Johnson et al. [28]. Recently, Brama et al. [8] reported the ERα-dependent up-regulation of *c-fos*, *c-jun* and PDGF-A in Cd-treated MCF-7 cells, whilst Benbrahim-Tallaa et al. [29] demonstrated that aberrant estrogen signalling followed Cd treatment of prostate epithelial cells.

Taking into account that Cd–breast cancer biological interactions have been poorly investigated, and that to date there are no detailed studies on the molecular effects exerted by Cd on ER^- tumor cells, that are expected to be insensitive to the estradiol-like modulation by Cd, in the present study we wanted to examine the expression levels of stress response genes such as those coding for hsps and MTs and of genes coding for factors and enzymes involved in the onset of apoptosis (see Tables 1 and 2) in ER^- MDA-MB231 cells, obtained from mammary adenocarcinoma and endowed with high malignant potential, treated with a concentration of $CdCl_2$ corresponding to the IC_{50} after long-term incubation (96 h). The pattern of gene expression by the immortalized epithelial cell line HB2, obtained from non-tumoral breast, in response to the same metal concentration and time of exposure was tested in parallel and compared to that of the neoplastic model system under study.

Table 1
Stress response genes studied in the present work

Gene product	Function
hsp27	(27 kDa-**h**eat **s**hock **p**rotein) chaperonin endowed with protective activity against oxidative stress
hsc70/hsp75, grp78	(70 kDa-**h**eat **s**hock (**c**ognate) protein; 75 kDa-**h**eat shock **p**rotein; 78 kDa **g**lucose-**re**gulated stress **p**rotein) members of a chaperonin family whose over-expression protects cells from stress-induced apoptosis. Hsc70 is cytosolic and nuclear whereas hsp75 is localized in mitochondria and grp78 is endoplasmic reticulum-associated
hsp90α/β	(90 kDa-**h**eat **s**hock **p**rotein) isoforms of a cytosolic chaperonin involved in signal transduction, protein folding and cell proliferation, showing both pro- and anti-apoptotic effects
EDEM	(**E**ndoplasmic reticulum **d**egradation **e**nhancing α-**m**annosidase-like protein) acceptor of misfolded glycoproteins whose expression is induced by various types of endoplasmic reticulum stresses
XBP1	(**X**-box **b**inding **p**rotein-1) transcription factor that activates the unfolded protein response only when it is translated from the spliced form of its mRNA
MTIA, -B, -E, -F, -G, -H, -L, MTIIA, MTIII, MTIV	(**m**etallo**th**ionein isoforms) 6–7 kDa metal binding proteins that are induced upon exposure to heavy metals and participate in heavy metal regulation and detoxification. MTs I and II are the most widely expressed and are regulated coordinately in all tissues, MT III is brain specific, MT IV is expressed mainly in squamous epithelium