Catalase overexpression in mammary cancer cells leads to a less aggressive phenotype and an altered response to chemotherapy

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A B S T R A C T

Because reactive oxygen species (ROS) are naturally produced as a consequence of aerobic metabolism, cells have developed a sophisticated set of antioxidant molecules to prevent the toxic accumulation of these species. However, compared with normal cells, malignant cells often exhibit increased levels of intracellular ROS and altered levels of antioxidant molecules. The resulting endogenous oxidative stress favors tumor growth by promoting genetic instability, cell proliferation and angiogenesis. In this context, we assessed the influence of catalase overexpression on the sensitivity of breast cancer cells towards various antitumor treatments. Our data show that catalase overexpression in MCF-7 cells leads to a 7-fold increase in catalase activity but provokes a 40% decrease in the expression of both glutathione peroxidase and peroxiredoxin II. Interestingly, proliferation and migration capacities of MCF-7 cells were impaired by the overexpression of catalase, as compared to parental cells. Regarding their sensitivity to antitumor treatments, we observed that cells overexpressing catalase were more sensitive to paclitaxel, etoposide and arsenic trioxide. However, no effect was observed on the cytotoxic response to ionizing radiations, 5-fluorouracil, cisplatin or doxorubicin. Finally, we observed that catalase overexpression protects cancer cells against the pro-oxidant combination of ascorbate and menadione, suggesting that changes in the expression of antioxidant enzymes could be a mechanism of resistance of cancer cells towards redox-based chemotherapeutic drugs.

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1. Introduction

Catalase is a tetrameric protein of 250 kDa, which consists of four similar subunits, each containing a heme group [1,2]. It is encoded by a single gene in mammalian species [3,4]. Catalase is highly expressed in some tissues, protecting cells against an excess formation of ROS. For instance, in red blood cells, catalase prevents the accumulation of hydrogen peroxide (H2O2) formed during oxygen transport [5]. Although the principal role of catalase is to detoxify H2O2 into water and oxygen, increasing evidence suggest that catalase is also involved in various other processes. Thus, an oxidase activity has been described for mammalian catalase, catalase is also involved in ROS generation in response to ultraviolet light (UVB) and it interacts with some proteins, such as the growth factor receptor-bound protein 2 (Grb2) or protein-tyrosine phosphatase Shp2, thereby participating in the integrin signaling [6–9].

The importance of catalase for human life is illustrated by the diseases that are associated with mutations of its gene. For example, acatalasemia is an autosomally inherited deficiency of erythrocyte catalase due to either guanine to adenine substitution (Japanese type A), threonine deletion (Japanese type B) or guanine–adenine insertion (Hungarian type) [10–12]. Acatalasemia is often benign but can sometimes be problematic, resulting in oral gangrene ulceration for Japanese patients or in essential hypertension [13–15]. Catalase polymorphism has also been associated to the occurrence of diabetes, vitiligo or Alzheimer’s disease [16]. It is noteworthy that mice deficient in catalase somehow recapitulate this phenotype, because they develop normally and are fertile, but show a differential sensitivity to oxidative tissue injury [17]. On the opposite, it has been observed that catalase overexpression targeted to mitochondria increases the lifespan of mice by about 20% [18] and protects cells from the deleterious effect of ionizing radiations [19].

Tumor cells frequently produce large amounts of reactive oxygen species [20]. This can be explained by the presence of mitochondrial defects and a decreased expression of antioxidant enzymes such as catalase or manganese superoxide dismutase (MnSOD) [21,22]. Thus, many reports have described decreased
catalase levels in a wide variety of tumors and cancer cell lines compared to normal cells [23–31]. The origin of this deficiency in cancer cells remains unclear but some hypothesis have been formulated such as hypermethyltion of the human catalase promoter or the implication of transcription factors [32,33]. Catalase is also down-regulated in healthy cells transformed with T-antigen of SV40 or Ras, although the underlying mechanisms of this down-regulation are still unknown [34,35]. Interestingly, it has also been observed that catalase levels are modified (increase or decrease) in cancer cell lines resistant to some chemotherapeutic agents or hydrogen peroxide [36–42].

Given the increasing development of pro-oxidant approaches for treating cancer, we investigated whether the modulation of the antioxidant capacities affects the survival of cancer cells exposed to such agents. To this end, the aim of this paper was to study the consequences of a catalase overexpression in a human breast cancer cell line (MCF-7), with regard to cellular sensitivity to the cytotoxic effects of standard or pro-oxidant chemotherapeutic agents, as well as resistance to radiotherapy.

2. Materials and methods

2.1. Cell culture

The breast cancer cell line MCF-7 CAT3, overexpressing catalase, was established from wild-type MCF-7 cells, which were purchased from ECACC (Salisbury, United Kingdom). Cells were maintained in DMEM supplemented with 10% fetal calf serum, in the presence of penicillin (10 000 U/ml) and streptomycin (10 mg/ml) from Gibco (Grand Island, NY, USA). Cultures were maintained at a density of about 5 × 10^4 cells/cm^2. The medium was changed at 48–72 h intervals. All cultures were maintained at 37 °C in 95% air/5% CO_2 with 100% humidity. Cultures were generally treated for 24 h either with 1 mM H_2O_2 or a H_2O_2-generating system currently used in our laboratory [25,26,43], namely sodium ascorbate and menadione sodium bisulfite (Asc/Men: 1 mM/10 μM, respectively). When indicated, the antioxidant N-acetyl cysteine (NAC) and the catalase inhibitor 3-amino-1,2,4-triazole (ATA) were added to cell cultures, respectively, at a concentration of 3 mM and 5 mM, for 1 h before the addition of ascorbate and menadione or H_2O_2. Alternatively, cultures were also treated for 48 h with the following drugs: 50 μM cisplatin, 100 μM 5-fluorouracil, 1 μM doxorubicin, 0.1 μM paclitaxel, 100 μM etoposide and 10 μM arsenic trioxide. Chemotherapeutic concentrations were selected on the basis of previous MTT results (not shown). They correspond to the cytotoxic concentrations generally found in the literature [44–48].

2.2. Chemicals

Menadione sodium bisulfite, sodium ascorbate, N-acetyl cysteine, 3-amino-1,2,4-triazole, hydrogen peroxide, cisplatin, 5-fluorouracil, doxorubicin, paclitaxel, etoposide and arsenic trioxide were purchased from Sigma (St. Louis, MO, USA). All other chemicals were ACS reagent grade.

2.3. Stable transfection

Plasmid construct pZeSV2(+) containing human catalase cDNA was a gift from Professor A. Cederbaum [49]. MCF-7 cells were transfected with 1 μg of plasmid using Fugene 6 reagent (Roche Applied Bioscience, Mannheim, Germany). The selection of successfully transfected cells was performed by adding 400 μg/ml of zeocin into the incubation medium for 3 weeks. Cell clones were then obtained and characterized by immunoblotting for catalase levels. Based on these results, the clone MCF-7 CAT3 was selected for this study.

2.4. Immunoblotting

At the indicated times, cells were washed twice with ice-cold PBS and then resuspended in an RIPA lysis buffer in the presence of protease (Protease Inhibitor Cocktail, Sigma, St. Louis, MO, USA) and phosphatase inhibitors (Phosphatase Inhibitor Cocktail, Calbiochem, Merck KGaA, Darmstadt, Germany). Samples were kept on ice for 5 min and then sonicated on ice at 100 W for 15 s with a Labsonic U sonicator (B Braun Biotech International, Melsungen, Germany). Sonicated samples were collected and stored at −80 °C. Equal amounts of proteins (20 μg) were subjected to SDS-PAGE (10 or 12% separating gel) followed by electroblot to nitrocellulose membranes. The membranes were blocked for 1 h in TBS buffer (pH 7.4) containing 5% powdered milk protein and then incubated overnight at 4 °C with the appropriate antibody. Rabbit polyclonal antibody against catalase was obtained from Calbiochem (Merck KGaA, Darmstadt, Germany); rabbit antibody against glutathione peroxidase; mouse antibody against superoxide dismutase were from Santa Cruz Biotechnology (Santa Cruz, CA, USA); mouse antibody against β-actin from Abcam (Cambridge, UK) and rabbit antibody against peroxiredoxin 2 was from Gentaur (Brussels, BE). Membranes were washed five times with TBS containing 0.1% Tween-20 (Sigma, St. Louis, MO, USA) and incubated for 60 min with a dilution of secondary antibody coupled to horseradish peroxidase. Protein bands were then detected by chemiluminescence, using the ECL detection kit from Pierce (Rockford, IL, USA). When appropriate, bands obtained via western blot analysis were quantified, using ImageJ software (http://rsb.info.nih.gov/ij) [50].

2.5. Biochemical measurements

Catalase activity was measured using the TiSO_4 method [51]. Gpx and SOD activity were assayed spectrophotometrically by measuring, respectively, NADPH consumption and nitro-blue formazan formation inhibition as previously described [52,53].

The glutathione content was determined by using the Tietze enzyme recycling assay [54], with slight modifications [55]. Cells were washed twice with ice-cold phosphate buffered saline and then lysed with a solution of 5-sulfosalicylic acid (5%). After two freeze– thaw cycles, samples were centrifuged at 10,000 × g for 10 min and the resulting supernatants were kept at −80 °C until used. Ten microliters of the samples were then placed in a reaction mixture containing 0.2 U/ml of glutathione reductase, 50 μg/ml 5,5′-dithiobis(2-nitrobenzoic acid) and 1 mM ethylenediaminetetraacetic acid (EDTA) at pH 7. The reaction was initiated by the addition of 50 μM NADPH and changes in absorbance were recorded at 412 nm. Reduced (GSH) and oxidized (GSSG) glutathione were distinguished by the addition of methyl-2-vinylpyridine and their respective concentrations were determined from appropriate standard curves. Results were normalized to the protein content, using the method of Lowry.

The ATP content was assessed using the bioluminescence ATPLite kit (Perkin Elmer, Waltham, MA, USA) according to the procedure provided by the manufacturer. Results are expressed as nmol/10^6 cells.

2.6. Cell survival assays

The effects of Asc/Men, H_2O_2, some chemotherapeutic agents and different doses of X-rays on cell metabolic status were assessed by following the reduction of MTT (3-(4,5-dimethylthia-
zolyl)-2,5-diphenyltetrazolium bromide) to blue formazan [56]. Briefly, cells were seeded into 96-well plates at a density of 10,000 cells/well for 24 h and then incubated with the test compounds (4 wells were used for each condition). Cells were then washed with PBS and incubated with MTT (0.5 mg/ml) for 2 h
at 37 °C. Blue formazan crystals were solubilized by adding 100 μl DMSO/well, and the coloured solution was subsequently read at 550 nm. Results are expressed as % of MTT reduction compared to untreated control conditions.

Clonogenic assays were performed by seeding cells (500) in six-well plates at a single cell density. Cells were allowed to adhere overnight and then exposed to different doses of X-rays using a RT-250 device (Philips, Amsterdam, The Netherlands) X-ray irradiator with a dose of 0.8555 Gy/min. After 24 h, the incubation medium was removed; cells were washed with warm PBS, given fresh medium, and allowed to grow for 10 days. Clonogenic survival was determined by staining colonies using crystal violet.

2.7. Migration assay

Cellular migration was tested in wound-healing assays using culture inserts (Ibidi, München, DE) according to the manufacturer's instructions. After removal of culture inserts, pictures of the remaining gaps were taken every 24 h at different random points. Pictures were analyzed with the software Motic Image Plus 2.0 (Ted Pella Inc., Redding, CA, USA).

2.8. Intracellular ROS determination

Cells were seeded onto 96-well plates at a density of 15 000 cells per well in 100 μl of growth media. At 50% confluence, cells were then loaded with 10 μM 2',7'-Dichlorofluorescein diacetate DCFH-DA (Sigma, St. Louis, MO) in HBSS (Gibco, Grand Island, NY, USA) at 37 °C for 30 min. After loading, cells were washed twice with 200 μl HBSS in order to remove excess fluorescent dye. Cells were then treated with the test compounds, washed twice with 200 μl HBSS and then 100 μl HBSS/well was added. The fluorescence intensity of DCFH-DA was measured at an excitation wavelength of 485 nm and an emission wavelength of 520 nm. Relative changes in ROS concentration were determined by calculations of ΔF/F_0, where ΔF/F_0 = (F_t − F_0)/F_0. F_t represents the fluorescence reading at each time point and F_0 the initial fluorescence.

2.9. Data analyses

All experiments were performed at least 3 times. Data were analyzed using an unpaired t-test, performed with GraphPad Prism software (GraphPad Software, San Diego, CA, USA). The level of significance was set at p < 0.05.

3. Results

3.1. Characterization of MCF-7 cells overexpressing human catalase

Catalase overexpression was evaluated by western-blot (Fig. 1A), showing a 10-fold overexpression of catalase in MCF-7 CAT3 cells compared to wild-type MCF-7 cells (MCF-7 CTL) (Fig. 1B). Accordingly, we observed that MCF-7 CAT3 exhibit 7-fold more catalase activity than parental MCF-7 cells (Fig. 1C).

Given the major role of catalase for the detoxification of hydrogen peroxide, we next assessed the effects of 1 mM H_2O_2 on both MCF-7 cells. As shown in Fig. 1D, MCF-7 cells overexpressing catalase were more resistant to H_2O_2 than their respective parental cells. Moreover, the catalase inhibitor 3-amino-1,2,4-triazole (ATA) completely abrogated the resistance of MCF-7 CAT3 to H_2O_2, demonstrating that the differential sensitivity between the two cell types is due to catalase overexpression. Other factors influencing cell survival such as energetic capacity or the maintenance of redox status seems to be not involved in the resistance of MCF CAT3 cells against H_2O_2. Indeed, similar levels of ATP (about 16 nmol ATP/10^6 cells) or GSH (about 60 nmol/mg prot) were observed in both cell types (data not shown).

3.2. Changes in the expression of other antioxidant enzymes following catalase overexpression

The levels of different antioxidant proteins were determined in order to characterize the antioxidant status of MCF-7 CAT3 with regard to parental MCF-7 cells. We focused on the three other canonical antioxidant enzymes, namely glutathione peroxidase (Gpx) and peroxiredoxin II (PRDX2), which also metabolize H_2O_2.

![Fig. 1](image-url) Characterization of MCF-7 cells overexpressing catalase. The histograms in white color represent MCF-7 CTL cells (□) and the histograms in black color represent MCF-7 CAT3 overexpressing catalase (■). (A and B) Wild-type MCF-7 cells (MCF-7 CTL) and MCF-7 cells overexpressing catalase (MCF-7 CAT3) were analyzed for catalase expression by western blot. (C) Catalase activity assay was performed as described in Section 2. (D) Cells were treated 24 h with 1 mM H_2O_2 and cell viability was determined by the measure of MTT reduction. Results are expressed as percentage of control. Data are means of three separate experiments. *p-value < 0.05, **p-value < 0.001.
and superoxide dismutase (SOD), which catalyses superoxide anion detoxification. Our results show that in cells that overexpress human catalase, the enzyme protein levels are rather decreased by about 40% for both Gpx and Prdx2 but it was not modified for SOD (Fig. 2A and B). The decrease in the amount of Gpx was further confirmed by a decrease of about 30% of the enzymatic activity (Fig. 2C). According to the unmodified amount of SOD protein, we did not observe any change in SOD enzyme activity (Fig. 2D).

3.3. Catalase overexpression induced a less aggressive phenotype in MCF-7 breast cancer cells

Since ROS are known to actively participate in signaling pathways that contribute to cell proliferation, we decided to investigate whether catalase overexpression in cancer cells could be associated to a change in cell proliferation and migration. As described in the experimental section, cell proliferation was evaluated by two techniques, the MTT reduction test and a

![Image](image1.png)

**Fig. 2.** Expression and activity of the other antioxidant enzymes. The histograms in white color represent MCF-7 CTL cells ([ ]) and the histograms in black color represent MCF-7 CAT3 overexpressing catalase ([ ]). (A and B) The protein levels of the different antioxidant enzymes were determined by western blot. (C and D) Gpx and SOD activities in homogenates of MCF-7 were determined by kinetic assays as previously described. Results were expressed in mU and U of enzyme activities, respectively. They were normalized for protein concentration. Data are means ± SEM, from three separate experiments. *p-value < 0.05, **p-value < 0.01.

![Image](image2.png)

**Fig. 3.** Effect of catalase overexpression on proliferation and migration. (A) Cells were seeded (500 cells) in 100-mm dishes and grown for 10–14 days. Colonies with more than 50 cells were counted. Data are represented as the mean number of cell colonies ± SEM, (B) Cells were grown in 96-well plate at a density of 50 000 cells/ml and an MTT assay was performed each 24 h for 72 h. Results are expressed as a percentage of control at time zero. Data are means of three separate experiments. (C) MCF-7 cells were seeded into two different reservoirs of a migration insert and allowed to adhere overnight (Ibidi München, DE). The insert was then removed and pictures were collected every day. Pictures are representative of three separate experiments. *p-value < 0.05, **p-value < 0.01.
clonogenic assay. A significant decrease of cell proliferation was observed whatever the procedure utilized, suggesting that decreased intracellular ROS levels are tightly associated to cell growth (Fig. 3A and B). Although cell proliferation and cell migration are not necessarily linked processes, some data from the literature suggest an inverse correlation between catalase expression and cell migration capacity [57]. Therefore, we decided to evaluate this parameter by using a wound-healing assay. As shown in Fig. 3C, MCF-7 CAT3 cells lost their ability to migrate, whereas their parental MCF-7 cells induced gap closure in approximately 8 days.

3.4. Catalase overexpression is not inducing either chemotherapy or radiotherapy resistance

Although our data showed that catalase overexpression did not result in any benefit for cancer cell growth and migration of cancer cells, we addressed the question about the behavior of these cells towards chemotherapeutic agents or a standard free radical-mediated process such as exposure to ionizing radiations. For that purpose, we used several classical chemotherapeutic agents, acting by different pathways, namely cisplatin, 5-fluorouracil, doxorubicin, paclitaxel, etoposide and arsenic trioxide. As shown in Fig. 4A, catalase overexpression did not protect MCF-7 cells against the toxicity of these agents. Interestingly, we observed a significant increase of cell death when MCF-7 CAT3 cells were treated with paclitaxel, etoposide and arsenic trioxide, when compared to their parental cells. Interestingly, it should be noted that a ROS-mediated process leading to cytotoxicity has been proposed for all these compounds [58–60]. However, no effects were observed when cells were treated with cisplatin or doxorubicin.

In addition to direct DNA damage, ionizing radiations are also known to generate ROS by water radiolysis [61]. We therefore checked whether catalase overexpression could protect cells against different doses of X-rays. However, we did not observe any difference between MCF-7 CAT3 cells and wild-type cells, either by using a clonogenic assay (Fig. 4B) or the reduction of MTT (data not shown).

3.5. Catalase overexpression protects against pro-oxidant drugs

Since cancer cells generally exhibit more intracellular ROS than non-transformed cells [21,62], pro-oxidant therapies represent an interesting approach to promote cancer cell killing. One of these promising therapies is the combination between ascorbate and menadione (Asc/Men). This association generates a redox cycling that induces ROS production (mainly H$_2$O$_2$) and kills cancer cells by a necrotic, poly(ADP-ribose) polymerase (PARP)-mediated cell death [25,43]. As shown in Fig. 5A, ROS production induced by Asc/Men combination is higher in wild-type MCF-7 cells than in MCF-7 cells overexpressing catalase. Interestingly, the catalase inhibitor ATA induces an increase of intracellular ROS in both cell lines but ROS levels are significantly higher in MCF-7 control cells than in MCF-7 CAT3 cells. On the contrary, the antioxidant N-acetylcysteine (NAC) abrogated ROS production generated by Asc/Men (Fig. 5A). We also observed that catalase overexpression protected cancer cells against Asc/Men-mediated cell death, and that the use of ATA decreased the viability in both cell lines when cells were exposed to this H$_2$O$_2$-generating system (Fig. 5B).

4. Discussion

Given the increasing development of pro-oxidant approaches in cancer treatment [63], we investigated to which extent the survival of cancer cells exposed to such agents is influenced by the modulation of the cellular antioxidant capacities. Indeed, it has long been known that cancer cells present less antioxidant defenses than healthy cells. This could be explained by the fact that cancer cells require higher ROS amounts than healthy cells, which have normal antioxidant capacities. Despite the absence of
reference values for catalase activity in normal human breast tissue, it may be considered that overexpressing catalase by genetic manipulations almost restores a physiological level of enzymatic activity. On the other hand, the fact that there was no difference in GSH or ATP levels between the two cell types, leads to conclude that resistance against H2O2 in MCF-7 CAT3 cells is mainly due to catalase overexpression. This is also supported by the observation that MCF-7 CAT3 cells are also resistant against Asc/Men, a well known hydrogen peroxide-generating system.

Our results showed that catalase overexpression results in a decreased ability of MCF-7 CAT3 cells to proliferate and migrate, a phenotype that may be linked to the lower ROS levels present in these cells, as compared to the parental MCF-7 cells. Indeed, it is well-known that basal ROS levels greatly participate in cancer cell proliferation, notably through the activation of mitogenic signaling pathways such as the ERK/MAPK pathway [64]. It may be argued that cells showing a decreased capacity to proliferate and migrate should be more sensitive to anticancer drugs or to the exposure to ionizing radiations. However, MCF-7 CAT3 cells were not more sensitive to X-rays, cisplatin, doxorubicin or 5-fluourouracil than wild-type MCF-7 cells. The situation was different when cells overexpressing catalase were incubated in the presence of paclitaxel, etoposide or trioxen (As2O3), cell toxicity being more important than for the parental cell line. Interestingly, few authors have reported changes (either a decrease or an increase) in the expression of catalase in cancer cells resistant to some chemotherapeutic agents, such as doxorubicin or cisplatin but these authors did not provide an explanation to the origin and the consequences of these changes [36,38–40].

Given that MCF-7 CAT3 cells are less sensitive against H2O2 than wild-type MCF-7 cells, the absence of resistance of these cells to anticancer drugs and ionizing radiations may appear contradictory. To avoid misleading conclusions, it should be noted that in the former case, the main oxidizing agent is H2O2, a specific substrate of catalase, while in the latter case (anticancer drugs and ionizing radiations), several free radical species can be produced, including oxygen free radicals and, in a less extent, hydrogen peroxide. The absence of protection against cisplatin, doxorubicin and ionizing radiations can be explained by these theoretical explanations, coupled to the fact that ROS generation is not the main cytotoxic mechanism of these agents (i.e. direct DNA damage or topoisomerase inhibition). It should be noted that some authors have reported that cells overexpressing a mitochondria-targeted catalase were protected against X-rays [19]. Considering that we did not use a mitochondria tagged catalase cDNA, the different subcellular localization could explain the differences between the results obtained by Epperly et al. and ours.

The increased sensitivity of MCF-7 CAT3 cells towards paclitaxel, etoposide and trioxen, is more difficult to understand. Indeed, if the effects of these drugs are ROS-mediated, one could expect that cells overexpressing catalase would be less sensitive to these agents. What we observed was just the opposite, suggesting that other ROS or free radicals, different from H2O2, are likely involved in the molecular mechanisms leading to the cytotoxicity of these agents. In addition, it should be reminded that MCF-7 CAT3 cells present less Gpx and Prdx2 than MCF-7 cells, a characteristic that could also explain an increased sensitivity towards other types of radicals.

In conclusion, our results show that overexpressing catalase in breast cancer cells leads to an increased resistance of these cells against pro-oxidant drugs (mainly H2O2-mediated processes). Nevertheless, no particular cell resistance to standard anticancer drugs or ionizing radiations was observed. Further studies will be required to identify the molecular pathways which are affected by catalase overexpression, and their influence on the ability of cells to proliferate and migrate.

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References
