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Original article

Chromatin remodeling regulates catalase expression during cancer cells adaptation to chronic oxidative stress

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ABSTRACT

Regulation of ROS metabolism plays a major role in cellular adaptation to oxidative stress in cancer cells, but the molecular mechanism that regulates catalase, a key antioxidant enzyme responsible for conversion of hydrogen peroxide to water and oxygen, remains to be elucidated. Therefore, we investigated the transcriptional regulatory mechanism controlling catalase expression in three human mammary cell lines: the normal mammary epithelial 250MK primary cells, the breast adenocarcinoma MCF-7 cells and an experimental model of MCF-7 cells resistant against oxidative stress resulting from chronic exposure to H_2O_2 (Resox), in which catalase was overexpressed. Here we identify a novel promoter region responsible for the regulation of catalase expression at −1518/−1226 locus and the key molecules that interact with this promoter and affect catalase transcription. We show that the AP-1 family member JunB and retinoic acid receptor alpha (RARα) mediate catalase transcriptional activation and repression, respectively, by controlling chromatin remodeling through a histone deacetylases-dependent mechanism. This regulatory mechanism plays an important role in redox adaptation to chronic exposure to H_2O_2 in breast cancer cells. Our study suggests that cancer adaptation to oxidative stress may be regulated by transcriptional factors through chromatin remodeling, and reveals a potential new mechanism to target cancer cells.

Abbreviations: AP-1, activator protein 1; APEX1, , DNA-(apurinic or apyrimidinic site) lyase; AP-MS, , affinity purification followed by mass spectrometry; ARID3A, , AT-rich interactive domain-containing protein 3A; Asc/Men, , ascorbate/menadione; ATRA, , all-trans-Retinoic acid; EF1, , elongation factor 1; DBHS, , Drosophila behavior/human splicing; Fosl2, , Fos-related antigen 2; Fox, , forkhead box protein; FUBP, , far upstream element-binding protein; GAPDH, , glyceraldehyde 3-phosphate dehydrogenase; GATA6, , GATA-binding protein 6; Gpx2, , glutathione peroxidase 2; HAT, , histone acetyltransferase; HDAC, , histone deacetylase; HIF-1α, , hypoxia-inducible factor; HMG, , high mobility group; HP1, , heterochromatin protein 1; JunB, , JunB proto-oncogene; MMP-1, , matrix metalloproteinase-1; mTOR, , mammalian target of rapamycin; NFIB, , nuclear factor 1 B-type; LMX1B, , LIM homeobox transcription factor 1-beta; NFκB, , nuclear factor kappa-light-chain-enhancer of activated B cells; NFY, , nuclear factor Y; NONO, , non-POU domain-containing octamer-binding protein; NURF, , nucleosome remodeling factor; PI3K, , phosphoinositide 3-kinase; PKB Akt, , protein kinase B; POU2F1Oct-1, , POU domain, class 2, transcription factor 1; PP1, , protein phosphatase 1; PPARγ, , peroxisome proliferator-activated receptor gamma; PSPC1, , paraspeckle component 1; RARα, , retinoic acid receptor alpha; RBPJ, , APCR-2 of Recombining binding protein suppressor of hairless; REST, , RE1-silencing transcription factor; ROS, , reactive oxygen species; RXRα, , retinoid X receptor alpha; SFPQ, , splicing factor, proline- and glutamine-rich; Sin3a, , paired amphipathic helix protein Sin3a; Sp1, , specificity protein 1; SWI/SNF, , switch/sucrose non fermentable; Tip60, , 60 kDa Tat-interactive protein; TSA, , trichostatin A; WICH, , WSTF-ISWI chromatin remodeling complex; WT1/Egr, , Wilms tumor 1/early growth response protein

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1. Introduction

Catalase is a key antioxidant enzyme which prevents the accumulation of hydrogen peroxide (H_2O_2) and has been implicated in many pathophysiological processes of human diseases, particularly during carcinogenesis [1,2].

Different levels of regulation have been reported in the last years to control catalase expression. They include transcriptional regulation, represented by the activity of transcription factors that induce or repress the transcriptional activity of human and rodent catalase promoters, post-transcriptional regulation (mRNA stability) and post-translational modification (phosphorylation and ubiquitination of the protein). In addition, epigenetic (DNA methylation, modifications of histones) and genetic (loss of heterozygosity, gene amplification) factors also play an important role in governing proper levels catalase activity in the cells. We have recently discussed these mechanisms in a review article [1].

Catalase is frequently downregulated in human tumor tissues compared to normal tissues of the same origin [3–5]. The Protein Kinase B (PKB/Akt)/Forkhead Box O (FoxO) transcription factors pathway is probably the best known regulator of catalase expression [6–8]. Indeed, FoxO3a has been reported to bind the rat *catalase* promoter [9], and in addition, the transactivating activity of this transcription factor is negatively regulated by the serine/threonine kinase Akt [10]. Interestingly, we have recently demonstrated that catalase expression in human MCF-7 breast cancer cells is repressed by the PI3K (phosphoinositide 3-kinase)/Akt/mTOR (mammalian target of rapamycin) signaling pathway while FoxO3a seems not to play a critical role in this gene regulation [11]. These results suggest the existence of diverse regulatory mechanisms involved in catalase expression in human cancer cells.

Due to the altered expression of antioxidant enzymes in cancer cells [1,3–5,12], targeting the redox status of cancer cells is an important approach to potentiate chemotherapy [13–25]. In this context, the elucidation of molecular mechanisms regulating catalase expression as well as understanding the biological consequences of chronic exposure of cancer cells to ROS (reactive oxygen species) leading to cell adaptation are issues of utmost importance. Therefore, the aim of this work was to elucidate the transcriptional regulatory mechanism controlling catalase expression in breast cancer cells. To this end, three human mammary cell lines were studied: the normal mammary epithelial 250MK primary cells, the breast adenocarcinoma MCF-7 cells and an experimental model of MCF-7 cells resistant to oxidative stress (namely the Resox cells) resulting from chronic exposure to H_2O_2 [24,26]. These Resox cells display overexpression of catalase compared to the parental cell line [11,27].

In this study, we discovered a new regulatory region of the human catalase promoter ($-1518/-1226$) in which some transcription factors may be bound regulating catalase expression. We found that the AP-1 family member JunB may mediate transcriptional activation of *catalase* gene. Indeed, the AP-1 transcription factors Jun (c-jun, JunB, JunD) and FOS (c-FOS, FOSB, FOSL1, FOSL2) form dimers that bind to specific binding sites in target genes to regulate their expression [28]. On the other hand, we also found that the retinoic acid receptor alpha (RARα) transcription factor represses the expression of catalase. Interestingly, RARα which forms dimers with RXR proteins that bind to RAR/RXR specific response elements (RARE) in target genes to regulate their expression [29], has been reported to antagonize with AP-1 transactivation members [30,31], thus providing a mechanistic explanation regarding the opposite effects of both transcription factors. Moreover, we have identified numerous proteins involved in gene transcription including multifunctional nuclear proteins, ATP-dependent chromatin remodeling complexes, histone-modifying complexes and proteins modifying the chromatin structure. In such regulatory processes of catalase expression an underlying histone deacetylases (HDAC)-dependent mechanism was also unveiled. All together, these findings indicate that chromatin remodeling promoted by AP-1 and RARα transcription factors tightly regulates catalase expression in breast cancer cells.

2. Materials and methods

2.1. Cell culture and chemicals

A Resox cell line was established from wild-type human breast cancer MCF-7 cells (ATCC, Manassas, VA, United States) [27]. Briefly, a MCF-7 cell line resistant to oxidative stress (namely Resox cells) was established by chronic exposing cells to the pro-oxidant association of Ascorbate/Menadione (Asc/Men) for 6 months, starting with 0.5 mM ascorbate/5 μM menadione to a final concentration of 1.5 mM ascorbate/15 μM menadione. Cells were first treated at 50% confluence by replacing their media with fresh media containing Asc/Men. When surviving cells reached 50% confluence, they were washed with warm PBS and treated again (every 1–7 days, depending on the level of resistance). To avoid the development of islets of resistance, which could arise from cooperation between cells, the cells were trypsinized approximately every 2 weeks and subcultured into new flasks. After selection, the cell line was stabilized in drug-free medium for 1 month.

The following human cell lines, MDA-MB-231, T98, U87MG, K562, KU812, T24, DU145, KHOS-240, AG1523, HepG2 and Ishikawa, were purchased from ATCC. Cells were maintained in RPMI (K562 and KU812 cells) or DMEM (other cancer cell lines) medium supplemented with 10% fetal calf serum, in the presence of penicillin (100 U/ml) and streptomycin (100 μg/ml) from Gibco (Grand Island, NY, USA). Human mammary epithelial cells 250MK were provided by Martha Stampfer and James Garbe (Lawrence Berkeley National Laboratory, Berkeley, California, USA). They were maintained in a M87A+CT+X medium and used between passage 8 and 10 [32]. Trichostatin A (TSA), R_0 41–5253, *all-trans*-Retinoic acid (ATRA), BMS-493, aminotriazole, sodium-ascorbate and menadione bisulfite were purchased from Sigma (St Louis, MO, USA).

2.2. Immunoblotting

The procedures for protein sample preparation from cell cultures, protein quantification, immunoblotting and data analyses were performed as previously described [11,33]. For JunB and RARα immunoblotting, nuclear extracts were prepared as previously described [11]. Rabbit polyclonal antibody against catalase (AB1212) was obtained from Millipore (Merck KGaA, Darmstadt, Germany); mouse monoclonal antibody against β-actin (ab6276) and rabbit polyclonal antibody against lamin B1 (ab133741) were from Abcam (Cambridge, UK). Rabbit polyclonal antibody against RARα (sc-551) and mouse monoclonal antibody against JunB (sc-8051) were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Protein bands were then detected by chemiluminescence, using the ECL detection kit (Pierce, Thermo Scientific, Rockford, IL, USA). When appropriate, bands obtained via Western blot analysis were quantified, using ImageJ software (http://rsb.info.nih.gov/ij/). Nuclear protein expression was normalized to that of lamin B1 and cytoplasmic protein expression was normalized to that of β-actin.

2.3. Real-time PCR

Total RNA were extracted with the TriPure reagent from Roche Applied Science Diagnostics (Mannheim, Germany). Reverse