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Autocrine human growth hormone increases sensitivity of mammary carcinoma cell to arsenic trioxide-induced apoptosis

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ABSTRACT

Human growth hormone (hGH) has been increasingly implicated in a variety of cancers; its up-regulation is observed in breast cancer and correlates with a poor outcome. Autocrine hGH promotes mammary carcinoma cell survival, proliferation, immortalization; it confers an invasive phenotype as a result of an epithelial–mesenchymal transition and contributes to chemoresistance and radioresistance. Arsenic trioxide (ATO) is being successfully used as a first and second line therapy for the treatment of patients with acute promyelocytic leukemia. It also inhibits tumor cell growth and induces apoptosis in a broad range of solid tumors. In the present study, we investigated the effect of hGH on sensitivity of a mammary adenocarcinoma cell to ATO, using a stable hGH-transfectant MCF-7 cell line, MCF7-hGH. Our results demonstrated for the first time that the overexpression of hGH increased sensitivity of the breast cancer cell line MCF-7 to ATO through apoptotic and anti-proliferative mechanisms. The effect of ATO on the transcriptional level of genes involved in survival (Bcl-2, Bax and Survivin), self-sufficiency in growth signals (c-Myc, ARF, Cdc25A, p53 and Bax), immortalization (hTERT) and invasion and metastasis (MMP-2 and MMP-9, uPA and uPAR and E-cadherin) was more pronounced in MCF7-hGH compared with its parental MCF-7 line. Our study may highlight the potential application of ATO for the treatment of patients with breast cancer, especially in those who have metastatic and chemoresistant tumor phenotype possibly due to the over expression of hGH.

1. Introduction

Breast cancer is the most prevalent malignancy found in women (Siegel et al., 2012). Despite considerable advances in early detection, diagnosis, and treatment, breast cancer is among the leading causes of cancer-related deaths in women because of the development of resistance to a wide variety of drugs and metastatic spread of cancer cells to distant organs. Virtually, all the roughly 40,000 annual breast cancer related deaths in the United States can be said to have occurred because the chemotherapy failed. Therefore, understanding the molecular factors responsible for drug resistance and metastasis of breast cancer is urgently needed to develop novel therapeutic approaches.

An accumulating number of evidence reveals that human growth hormone (hGH) has a pivotal role in the development and progression of tumors of the female reproductive system. In a recent pathway analysis of mammary cancer genome-wide association study, the growth hormone signaling pathway was identified as the pathway third most significantly correlated with susceptibility to develop mammary carcinoma (Menashe et al., 2010). In addition to the secretion of hGH from the pituitary, it is now widely accepted that this hormone is also produced locally in the mammary gland and functions in an autocrine or paracrine manner (Harvey, 2010) for the proliferation and differentiation of cells and tissues. Pathological roles of autocrine/paracrine hGH has been postulated in mammary carcinoma (Thijssen, 2009; Perry et al., 2008). It has been demonstrated that autocrine hGH promotes mammary carcinoma cell growth and survival; migration and invasion; induces tumor angiogenesis; chemoresistance and radioresistance; protects against oxidative cell stress; and its overexpression is sufficient to oncogenically transform the human mammary epithelial cell to a mesenchymal morphology which is associated with acquisition of migratory and invasive phenotypes during carcinoma progression (Brunet-Dunand et al., 2009; Thiery, 2002; Perry et al., 2008; Mukhina et al., 2004).

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Up-regulation of hGH is observed in breast cancer and correlates with a poor outcome. Also, a high level of hGH expression has been reported in metastatic breast cancer [26]. In a recent study investigating the potential association of hGH expression with the clinicopathological features of mammary carcinoma, hGH was expressed in a large number of tumor specimens (52.8%), and over expression level of hGH was significantly associated with lymph node metastasis, tumor grade, tumor stage, and proliferative index in mammary carcinoma. Also, the upregulation of hGH was associated with a significant worse relapse-free survival and overall survival in patients with mammary or endometrial carcinoma (Wu et al., 2011). So, it seems reasonable to establish different therapeutic agents for improving the outcomes of treatment and for long-term survival of breast cancer patients.

Arsenic trioxide (ATO) is a highly efficacious agent for the treatment of both newly diagnosed and all-trans-retinoic acid (ATRA)-refractory acute promyelocytic leukemia (APL) patients (Ghavamzadeh et al., 2006; Ghavamzadeh et al., 2011). An overwhelming number of studies imply that ATO induces apoptosis in a variety of tumor cells including acute myeloid leukemia (Xu et al., 2009; Momeny et al., 2010; Ghaffari et al., 2012b), multiple myeloma (Gazitt and Akay, 2005), glioblastoma (Dizaji et al., 2012) and neuroblastoma (Pettersson et al., 2007). The pleiotropic effects of ATO include modulation of the intracellular glutathione redox system and oxidative injury (Jing et al., 1999), induction of mitotic arrest due to inhibition of spindle apparatus and microtubuline formation (Li and Broome, 1999), DNA damage and inhibition of DNA repair (Yoo et al., 2009) which finally leads to apoptosis. Additionally, there are evidences that ATO might suppress growth and proliferation of tumor cells through the inhibition of telomerase and shortening of telomere length (Zhang et al., 2003; Ghaffari et al., 2012b). Moreover, a recent study shows that ATO by modulation of tumor and metastatic suppressor miRNAs may elicit cell cycle arrest and apoptosis in APL cell (Ghaffari et al., 2012a). In the present study, we investigated the effect of hGH on sensitivity of a mammary adenocarcinoma cell to ATO, using a stable hGH-transfectant MCF-7 cell line, MCF7-hGH. Our results showed for the first time that overexpression of hGH in a mammary adenocarcinoma cell line not only did not have inhibitory effects against the cytotoxicity of ATO, but also it increased the sensitivity of the breast cancer cell line MCF-7 to ATO through apoptotic and anti-proliferative mechanisms. The result of this study designates that ATO treatment may be beneficial for the treatment of patients with hGH positive tumors.

2. Materials and methods

2.1. Stable cell line production, cell culture and ATO treatment

Stable MCF7-hGH cell line expressing hGH was constructed as previously report. Briefly; the coding region of hGH was amplified by reverse transcription PCR of human cDNA. PCR products were cloned into pCDNA3.1 (+) expression plasmid. MCF-7 cell was transfected with pCDNA-hGH, and transfected cells stably expressing transgene were selected by G418. The expression of hGH protein was confirmed using immunocytochemistry. An empty pcDNA3 vector was used as a control.

All cell lines were cultured in RPMI 1640 medium supplemented with 10% fetal bovine serum (Invitrogen, Gaithersburg, MD, USA), 2 mM L-glutamine (Invitrogen), 100 U/ml penicillin G (Invitrogen), and 100 μg/ml streptomycin (Invitrogen). Cells were incubated at 37 °C in a humidified atmosphere containing 5% CO2. For ATO treatment, the cells were cultured in the absence and presence of varying concentrations of ATO, ranging 1, 2, 3 and 4 μM concentrations.

2.2. Microculture tetrazolium test (MTT assay)

The inhibitory effect of ATO on cell viability was measured by uptake of thiazolyl blue tetrazolium bromide (MTT, Sigma). Cells were seeded into a 96-well plate at a density of 7000 cells/well; then they were treated with ATO at 1, 2, 3 and 4 μM for 24, 48 and 72 h. The media were replaced by MTT solution (0.5 mg/ml), and after 3 h, with DMSO. The color absorbance was measured at a wavelength of 570 nm in an ELISA reader. The intensity of dissolved formazan crystal was measured at 570 nm. The percentage cell viability was calculated as $(OD_{exp}/OD_{con}) \times 100$, where OD_{exp} and OD_{con} are the optical densities of treated and untreated cells, respectively.

2.3. Colony formation assay

Colony formation assay was used to assess in vitro cell survival. Cells were seeded onto 6-well plates with a density of 100 cells/well. After treatment with desired concentrations of ATO for 48 h, the media were replaced by fresh media with no drug and followed for 3 weeks. Thereafter, the plates were rinsed with phosphate-buffered saline (PBS) solution and stained with crystal violet solution containing crystal violet (0.5% w/v) and glutaraldehyde (6% v/v). Ultimately, colonies were counted by naked eye and the survival fraction (SF) was estimated as: (mean colony counts)/(cells plated) × (plating efficiency), where plating efficiency (PE) was determined as (mean colony counts)/(cells plated for untreated controls).

2.4. BrdU cell proliferation assay

The DNA synthesis of cells was measured by quantification of incorporated BrdU via the colorimetric bromodeoxyuridine (BrdU) ELISA kit (Roche, Germany) according to the manufacturer's instructions. Briefly, cells (4000 cell/well) were treated with various concentrations of ATO for 48 h and then were incubated with the BrdU labeling solution at 37 C for 8 h. The cells were then fixed and DNA was denatured using 200 μl of FixDenat solution. Following incubation with the peroxidase-conjugated anti-BrdU antibody (anti-BrdU-POD) at room temperature for 1 h, plates were exposed to 100 μl of substrate tetramethyl-benzidine (TMB). Lastly, plates were read at 370 nm in an ELISA reader. The effect of ATO on the DNA synthesis was measured applying the succeeding formula: BrdU incorporation (%) = $OD_{exp}/OD_{con} \times 100$, where OD_{exp} and OD_{con} are the optical densitometries of the treated and untreated control cells, respectively.

2.5. Quantification of apoptosis using flow cytometry

For the detection of apoptosis induced by ATO, the Hoechst 33342/Propidium iodide (PI) double stain apoptosis detection was used by a flow cytometry analysis. Briefly, about 1×10^6 cells were treated with desired concentrations of ATO for 48 h, collected, washed twice with cold PBS, and fixed in 70% ethanol overnight. The cells were then incubated with 1 ml PBS containing Hoechst 33,342 stock solution (5 mg/ml) and 1 μl of PI stock solution (1 mg/ml). The fluorescence intensity of Hoechst 33,342 and PI were analyzed by a flow cytometry instrument (Partec PasIII, Germany), using excitation/emission ~350/461 and ~535/617 nm for Hoechst 33,342 and PI, respectively. Then the data was analyzed using the FlowMax software.

2.6. Analysis of gene expression by quantitative real-time RT-PCR

Total RNA was extracted using TriPure Isolation Reagent (Roche Applied Science, Germany) from cultured cells and was quantified