Endothelin-1 acts as a survival factor in ovarian carcinoma cells

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ABSTRACT

The aim of this study was to evaluate the role of endothelin-1 (ET-1) in the sensitivity of ovarian carcinoma to paclitaxel, one of the most common drugs used for the management of this tumour histotype. ET-1 is a powerful mitogenic peptide produced by ovarian carcinomas and it acts as an autocrine growth factor, selectively through ETA receptor (ETAR), which is predominantly expressed in this tumour. OVCA 433 and HEY, two ovarian carcinoma cell lines, which produce elevated amounts of ET-1 and express abundantly high-affinity ETARs, were used. As demonstrated by sub-G1 peak in DNA content histograms and terminal transferase deoxytidyl uridine end labelling assay, we found that paclitaxel induces cytotoxic effect through the activation of apoptosis in both cell lines. When the treatment with paclitaxel was performed in association with ET-1, paclitaxel-induced apoptosis was inhibited. In order to evaluate which ET-1 receptor mediated the effect of ET-1 on protection from paclitaxel-induced apoptosis, we performed experiments using two selective antagonists for ETAR (BQ-123) and for ETBR (BQ-788). We showed that ETAR blockade inhibits the ET-1-induced survival activity against paclitaxel-mediated apoptosis. However, no effect was observed on blocking ETBR with BQ-788. Our results establish a novel role for ET-1 in determining survival of ovarian carcinoma cells and suggest that pharmacological ETAR blockade using a specific ETAR antagonist may provide a novel approach to the treatment of ovarian carcinoma in combination therapy.

INTRODUCTION

Endothelin-1 (ET-1) is produced by endothelial cells and by different tumour histotypes [1,2] and act through two distinct receptors, the ET\textsubscript{A} receptor (ET\textsubscript{A}R) and ET\textsubscript{B}R [3]. We previously demonstrated that ET-1 is highly expressed in ovarian cancer ascites, in primary and metastatic ovarian carcinomas [4,5] and that ET-1 acts selectively as an autocrine growth factor via ET\textsubscript{A}R in ET-1-producing ovarian carcinoma cells [6,7]. Recent evidence suggests that ET-1 modulates apoptosis induced by different stimuli in endothelial cells [8], fibroblasts [9], smooth muscle cells [10–12], colon carcinoma [13] and glioblastoma cells [14]. Several antineoplastic agents induce cytotoxicity through activation of apoptosis, and the inability of cancer cells to activate apoptosis is one of the mechanisms of resistance to cancer treatment [15]. Thus, the efficacy of anti-tumour therapy can be improved by increasing the susceptibility of the tumour to undergo apoptosis in response to cancer stimuli.

In order to evaluate whether ET-1 might be involved in tumour drug resistance, we analysed the in vitro response of two ovarian carcinoma cell lines, expressing elevated

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Key words: endothelin A receptor, endothelin A receptor antagonist, programmed cell death.
Abbreviations: DMEM, Dulbecco’s modified Eagle’s medium; ET-1, endothelin-1; ET\textsubscript{A}R, ET\textsubscript{A} receptor; TUNEL, terminal transferase deoxytidyl uridine end labelling.
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amounts of ET-1 and ET_\text{A} R [6], to paclitaxel, one of the most common drugs used for the management of advanced metastatic ovarian carcinoma [16].

**MATERIALS AND METHODS**

**Cell culture**

OVCA 433 and HEY human ovarian carcinoma cell lines were provided by Dr Giovanni Scambia (Catholic University School of Medicine, Rome, Italy) and were cultured in Dulbecco’s modified Eagle’s medium (DMEM) supplemented with 10% foetal calf serum, glutamine, penicillin and streptomycin sulphate. All culture reagents were from Gibco/BRL, Life Technologies, Inc. (Paisley, U.K.).

**Treatment**

Cells plated in supplemented medium and maintained for 24 h in serum-free DMEM were exposed to paclitaxel (Bristol-Myers Squibb, Latina, Italy) for 20 h at the dose of 60 nM. ET-1 (Peninsula Laboratories, Belford, CA, U.S.A.) was used at a dose of 100 nM. In combination experiments, cells were exposed simultaneously to ET-1 (100 nM) and paclitaxel (60 nM) for 20 h. When the two selective ET-1 receptor antagonists BQ-778 and BQ-123 (1 \mu M, Peninsula) were used, cells were preincubated with the inhibitor for 20 min before paclitaxel plus ET-1 treatment.

**Apoptosis**

The analysis of apoptotic cells was performed on adherent and floating cells by cytofluorimetric analysis of DNA content and terminal transferase deoxytidyl uridine end labelling (TUNEL) assay (Enzo Diagnostic, New York, NY, U.S.A.) on cytospin preparations as described previously [17,18].

**RESULTS**

To evaluate whether paclitaxel induces apoptosis in OVCA 433 and HEY ovarian carcinoma cell lines, exponentially growing cells were exposed to 60 nM paclitaxel for 24 h and apoptosis was analysed. Cytofluorimetric analysis of DNA content and TUNEL, performed after paclitaxel treatment, revealed the presence of sub-G_1 peaks and apoptotic cells respectively in both cell lines. As shown in Figure 1, the percentage of apoptotic cells, calculated by TUNEL assay, was approx. 25% and 30% for OVCA 433 and HEY cells respectively. Less than 10% of untreated cells were apoptotic. Cytofluorimetric analysis gives superimposable results (results not shown).

![Figure 1](image)

Figure 1: ET-1 protects OVCA 433 and HEY cells from paclitaxel-induced apoptosis via ET_\text{A} R

The percentage of apoptotic cells was evaluated by TUNEL assay. Each value represents the mean ± S.D. of three determinations. The results shown are representative of four experiments. Cells were serum-starved for 24 h and treated with 60 nM paclitaxel for 20 h in the absence or presence of 100 nM ET-1. Where reported, cells were preincubated with 1 \mu M BQ-123 or 1 \mu M BQ-788 before ET-1/paclitaxel treatment.

To investigate whether ET-1 protects ovarian carcinoma cells from paclitaxel-induced apoptosis, OVCA 433 and HEY cells were exposed to 60 nM paclitaxel in the presence of 100 nM ET-1 for 20 h. The presence of 100 nM ET-1 inhibited the paclitaxel-induced apoptosis in both lines. In fact, the percentage of apoptotic cells decreased from 25% to 5% when HEY cells were treated with paclitaxel in the absence or presence of ET-1 respectively. Similarly, the percentage apoptosis of OVCA 433 cells induced by paclitaxel (32%) decreased to 15% when the drug was administered in the presence of ET-1.

Since the effects of ET-1 on mammalian cells are mediated by two distinct subtypes of G-protein-coupled receptors, ET_\text{A} and ET_\text{B} [3], we also characterized the ET-1 receptor subtype mediating the survival effect observed. To this purpose, OVCA 433 cells were exposed for 20 min to two different ET-1 receptor antagonists before the combined treatment with paclitaxel and ET-1. In particular, BQ-123 and BQ-788, ET_\text{R} and ET_\text{B} specific antagonists respectively, were used at a dose of 1 \mu M. As shown in Figure 1, only ET_\text{R} is involved in the ET-1 effect of protection from paclitaxel-induced apoptosis. In fact, the pretreatment with the ET_\text{R}-specific antagonist, BQ-123, abrogates the protection by ET-1 from paclitaxel-induced apoptosis. In contrast, addition
of the ET_{A}R antagonist, BQ-788, at the same concentration, did not show significant effect on ET-1-induced protection against apoptosis. Neither BQ-123 nor BQ-788 alone affected the apoptotic frequency of OVCA 433 cells, the percentage of apoptotic cells being similar to that observed after exposure to paclitaxel alone.

**DISCUSSION**

The apoptosis pathway, which is regulated at various levels within the signalling cascade, plays an important role in cancer growth and sensitivity to treatments. A number of anticancer drugs exert their action by inducing apoptosis, and the ability of cancer cells to activate programmed cell death is a critical factor in the therapeutic response [15]. Several growth factor and growth factor receptors have been shown to modulate apoptosis in ovarian carcinoma cells.

The ability of ET-1 to inhibit apoptosis induced by different stimuli, such as serum deprivation, Fas-L and paclitaxel, has been demonstrated in several systems including fibroblast, endothelial, vascular and prostatic smooth muscle and tumour cells [8–14].

We have demonstrated previously a novel mechanism in the growth control of ovarian carcinoma mediated by the ET-1 autocrine loop that selectively occurs via ET_{A}Rs [4]. However, the role of ET-1 as a regulator of paclitaxel-induced apoptosis in ovarian carcinoma has not been described to date.

In this paper, we showed that ET-1 confers resistance to paclitaxel-induced apoptosis in two ovarian carcinoma cell lines. A marked inhibition by ET-1 of paclitaxel-induced apoptosis is demonstrated by the reduced percentage of cells in the sub-G_{1} peak in the cytofluorimetric analysis of DNA content and by the reduced number of apoptotic cells in the TUNEL assay.

Experiments to define the mechanism by which ET-1 protects specific cell types from paclitaxel-induced apoptosis are now in progress. Since paclitaxel induces apoptosis through phosphorylation and inactivation of Bcl-2 [19], it is possible that ET-1 protects ovarian cancer cells from paclitaxel-induced apoptosis by a Bcl-2-dependent mechanism.

We also found that ET_{A}R mediates the anti-apoptotic effect of ET-1 observed in ovarian carcinoma. In fact, we found that the addition of a specific ET_{A}R antagonist, BQ-123, but not of a specific ET_{A}R antagonist, BQ-788, blocked the ET-1-induced resistance to paclitaxel-mediated apoptosis.

Controversial results were reported concerning the ET-1 receptor involved in mediating cell survival. In fact, both ET, R and ET_{A}R have been found to mediate ET-1-dependent protection in different cellular contexts [8,9,13,14], thus, indicating that the receptor involved in the protecting effect of ET-1 strictly depends on the cell type. Interestingly, receptors that are highly expressed on the cells are those mediating ET-1 effects. In fact, in human glioblastoma and rat endothelial cells, expressing a high level of ET_{A}R, ET-1 mainly acts via the ET_{A}R receptor [8,4], while it functions as a survival factor through ET_{B}R in rat fibroblasts, human smooth cells and colon carcinoma cells, showing high levels of ET_{A}R [9,13].

Our findings, demonstrating that ET-1 protects human ovarian epithelial tumour cells against apoptosis via ET_{A}R, are particularly relevant to understand the function of ET-1 receptors in ovarian cancer and add further information on the overall importance of ET-1/ET_{A}R system in regulating ovarian carcinoma cell proliferation and survival [4,5,7].

Our findings suggest that new therapeutic strategies, using a specific ET_{A}R antagonist combined with conventional chemotherapy, might provide an additional approach to the treatment of ovarian carcinoma.

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