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Autocrine production of interleukin-6 confers ovarian cancer cells resistance to tamoxifen via ER isoforms and SRC-1

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ABSTRACT

Although 40–60% of ovarian cancer (OVCA)s express estrogen receptor (ER)α, only a minor proportion of patients respond to anti-estrogen treatment with ER antagonist tamoxifen (TAM). The mechanism underlying TAM resistance in the course of OVCA progression is incompletely understood. However, interleukin-6 (IL-6) plays a critical role in the development and progression of OVCA. Here we explore an association between IL-6 and TAM resistance. We demonstrate that both exogenous (a relatively short period of treatment with recombinant IL-6) and endogenous IL-6 (by transfecting with plasmid encoding for sense IL-6) induce TAM resistance in non-IL-6-expressing A2780 cells, while deleting of endogenous IL-6 expression in IL-6-overexpressing CAOV-3 cells (by transfecting with plasmid encoding for antisense IL-6) promotes the sensitivity of these cells to TAM. Further investigation indicates that TAM resistance caused by IL-6 is associated with the alteration of ERα, ERβ and steroid hormone receptor coactivator (SRC)-1 expression levels, the protein interactions between SRC-1 and ERα, but not ERβ, as well as blockage of estrogen-induced ER receptor nuclear translocation. These results show that IL-6 secreted by OVCA cells may contribute to the refractoriness of these cells to TAM via ER isoforms and SRC-1. Overexpression of IL-6 not only plays an important role in OVCA progression but also contributes to TAM resistance. Our studies suggest that TAM-IL-6-targeted adjunctive therapy may lead to a more effective intervention than TAM alone.

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1. Introduction

Ovarian cancer (OVCA) continues to be the most fatal gynecologic cancer, with an estimated 5-year survival of only 50% (Jemal et al., 2009). Ovarian carcinogenesis mechanisms have not yet been elucidated but appear to be different from those of breast tumor progression. Indeed, about two-thirds of breast cancer patients with estrogen receptor (ER)-positive tumors respond clinically to anti-estrogen treatment with ER antagonist tamoxifen (TAM). Though 40–60% of OVCAs express ERα (Rao and Slotman, 1991; Havrilesky et al., 2001), only a minor proportion of patients (ranging from 7% to 18%) respond to TAM therapy (Hatch et al., 1991; Scambia et al., 1995). Several studies have highlighted an increased risk of OVCA in patients receiving long-term estrogen replacement therapy (Beral et al., 2007; Glud et al., 2004; Lacey et al., 2006; Rossing et al., 2007). Estrogen exerts its effect through two receptors, ERα and ERβ, which are responsible for different biological functions, as indicated by their specific expression patterns and different effects in gene knockout experiments (Merchenthaler and Shugrue, 1999; Couse et al., 2000). A loss of ERβ expression or a decreased in ERβ/ERα ratio in epithelial OVCA as compared with normal tissues has been reported consistently by several groups (Brandenberger et al., 1998; Pujol et al., 1998; Rutherford et al., 2000; Bardin et al., 2004). Some studies have shown that ERβ expression might affect cellular proliferation, motility, and apoptosis of OVCA cells (Bardin et al., 2004; Treeck et al., 2007). Given that ERβ can counteract ERα signaling in some settings, loss of ERβ is thought to enhance ERα-mediated proliferation of hormone-dependent cancer cells (Lindberg et al., 2003). Bossard et al. have confirmed that ERβ can repress the expression, activity and signaling of ERα, thusly blocking its proliferative action (Bossard et al., 2012). Additionally, they have shown ERβ to both strongly reduce orthotopic ovarian xenograft development and decrease the presence of tumor cells at sites of metastasis,

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therefore increasing the mouse survival rate (Bossard et al., 2012). In particular, recent clinical observations have documented that the loss of ERβ expression could correlate with a shorter overall survival of OVCA patients (Halon et al., 2011) and a metastatic lymph node status (Burges et al., 2010). A recently identified polymorphism (rs127572) of the ERβ gene has been associated with an increased risk of developing an OVCA (Lurie et al., 2011). However, it is still unknown whether this polymorphism affects ERβ expression. The intracellular location of ERβ in tumor cells seems to be important. Indeed, a recent study has shown ERβ to localize in the cytoplasm of tumor cells, while nuclear localization was primarily observed in normal epithelial cells (De Stefano et al., 2011). Moreover, cytoplasmic expression of ERβ was correlated to a poor outcome for patients with advanced serous OVCA (Drummond and Fuller, 2010). Combined with the aforementioned clinical correlations between ERβ and patient survival, these findings lead us to hypothesize that ERβ is a critical factor in ovarian tumor progression and to delineate the precise contribution of this receptor in the molecular pathways underlying OVCA carcinogenesis.

ERα is the marker of choice to decide endocrine treatment of breast cancer. However, despite an initial response to TAM therapy, one-third of patients will acquire resistance even though their ERα status remains unchanged (Osborne, 1998). ERβ has also been considered a marker of endocrine response. Emerging data support different functions for ERβ when it is expressed alone and when co-expressed with ERα (Murphy and Watson, 2006). With regard to the latter group (ERα+/ERβ+), the vast majority of retrospective clinical outcome studies strongly support the hypothesis that increased expression of ERβ is associated with an increased likelihood of response to endocrine therapy (Murphy and Watson, 2006). A more recent study has suggested a link between ERβ expression and endocrine sensitivity by increasing phosphatase and tensin homologue deleted on chromosome 10 (PTEN) levels and decreasing proto-oncogene *c-ErbB-2* (HER2)/Receptor tyrosine protein kinase erbB-3 (HER3) signaling, thereby reducing Akt signaling with subsequent effects on proliferation, survival and TAM sensitivity of breast cancer cells (Lindberg et al., 2011).

Nuclear hormone receptor coactivators are involved in enhancing the ligand-dependent transcriptional signal of numerous nuclear hormone receptors, including ER. Perhaps the most important of these coactivators is the p160 family, steroid hormone receptor coactivator (SRC)-1 (also called NCoA1), SRC-2 (also called TIF2, GRIP1 or NCoA2), and SRC-3 (also called AIB1, ACTR, p/CIP RAC3, TRAM1 or NCoA3) (Xu and Li, 2003). Elevated levels of SRC-1 and SRC-3 have been associated with decreased response to endocrine therapy and poorer clinical outcome, which may ultimately result in TAM resistance through enhancing its agonist behavior (Smith et al., 1997; Xu et al., 1998; Osborne et al., 2003; Myers et al., 2004).

Interleukin-6 (IL-6), a known mediator of immunological and inflammatory events, was elevated in serum and peritoneal fluid from patients with OVCA; high levels of IL-6 in body fluids were associated with poor prognosis and survival (Lane et al., 2011; Tempfer et al., 1997; Penson et al., 2000). IL-6 target cells express a low affinity receptor (IL-6Rα) devoid of transducing activity on their surface. The complex of IL-6 and IL-6Rα associates with the signal transducing membrane protein gp130, thereby inducing its dimerization and the initiation of signaling (Kishimoto et al., 1995). Multiple studies suggested a pathogenic role of this cytokine in the malignant transformation, progression and chemotherapy resistance of OVCA (Syed et al., 2002; Yang et al., 2009; Rabinovich et al., 2007; Nilsson et al., 2005; Wang et al., 2012, 2010). Our previous studies revealed that IL-6 was able to regulate two ER types expression and activate ER transcription through the mitogen activated protein kinase (MAPK) signaling pathway in the absence of estrogen. Human OVCA cell growth was also promoted, partly through activation of ER pathway (Yang et al., 2009).

In this study, we investigated the role of IL-6 expression in modulating cellular sensitivity to TAM in human OVCA cells. We also explored the potential mechanisms involved in IL-6-mediated TAM resistance.

2. Materials and methods

2.1. Cell lines and cell culture

Human OVCA cell lines A2780, CAOV-3 and ES-2 were obtained from the American Type Culture Collection. A2780 and ES-2 cells were cultured in RPMI 1640 (Life Technologies, Inc., Gaithersburg, MD) containing 10% fetal bovine serum (FBS) (Life Technologies, Inc.), CAOV-3 cells were grown in DMEM (Life Technologies, Inc.) with 10% FBS.

Recombinant human IL-6 (R&D Systems, Minneapolis, MN) was used to pretreat A2780 cells. The cells were cultured in the presence of exogenous IL-6 (50 ng/ml) for 10 days. IL-6 was added to the culture every 2 days (Conze et al., 2001). After the pretreatment period, the cells (A2780/preIL-6) were harvested, washed, and replated in the presence of IL-6, and their resistance to TAM (Sigma, St. Louis, MO, USA) was determined by the MTT assay.

2.2. Enzyme-Linked Immunosorbent Assay (ELISA)

The cells were cultured for 48 h in 1 ml of medium containing 1% charcoal-stripped FBS (sFBS) (Hyclone Laboratories, Inc., Logan, UT). The supernatants were collected and clarified by centrifugation. The level of IL-6 was determined by ELISA (R&D Systems) according to the manufacturer's instructions.

2.3. Semiquantitative RT-PCR

Total RNA was isolated from cells with TRIzol (Invitrogen, San Diego, CA) according to the manufacturer's instructions. Primer sequences were designed by Vector NTI 8 software and synthesized by TaKaRa Biotechnology Co., Ltd. (Dalian, China). The primer sequences were as follow: IL-6, 5′-TTGACAAACAAATTCGGTACA-3′ (forward) and 5′-GAGGTGC CCATGCTACA-3′ (reverse), for ERα, 5′-AACAAAGGCATGGAGCATCTGT-3′ (forward) and 5′-GTGATGTAA-TACTTTTGCAAGG-3′ (reverse), for ERβ, 5′-GCGCTGTCTGCAGC GATT ACGC-3′ (forward) and 5′-CACCATTCCCACTTCGTAACAC-3′ (reverse), for SRC-1, 5′-CCACCCCTGAATGCTCAAATGT-3′ (forward) and 5′-CTGCTCCTGGATACTG GAAGAC-3′ (reverse), for β-actin, 5′-TGGAATCCTGTGGCATCCATGAAAC-3′ (forward) and 5′-TAAAACGC AGCTCAGTAACAGTCC-3′ (reverse). One Step RNA PCR Kit (AMV) (TaKaRa Biotechnology) was used to do RT-PCR. PCR products were fractionated on 1.5% agarose gel and analyzed with Quantity One-4.5.6 software (Bio-Rad, Hercules, CA). The results were normalized against β-actin, and presented as target mRNA: β-actin ratio.

2.4. Total cell lysate and nuclear extract preparation

Total cell lysates were obtained as previously described (Yang et al., 2009). To prepare nuclear extracts, cells were harvested, washed with PBS once, and resuspended in a hypotonic buffer (10 mM HEPES-KOH pH 7.9, 10 mM KCl, 0.1 mM EDTA, 0.4% NP-40, 0.5 mM PMSF, 0.5 mM DTT, 1 mM NaV, 20 mM NaF, and 1 μg/ml aprotinin) and incubated on ice for 20 min. Nuclei were precipitated by centrifugation at 6000 rpm at 4 °C for 10 min. After washing once with hypotonic buffer, the nuclei were lysed in a high-salt buffer (10 mM HEPES-KOH pH 7.9, 400 mM NaCl, 1 mM EDTA, 1 mM DTT, 0.5 mM PMSF, 1 mM NaV, 20 mM NaF, 20%

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