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Understanding and overcoming chemoresistance in ovarian cancer: emerging role of the endothelin axis

A. Bagnato PhD and L. Rosanò PhD**

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Ovarian carcinoma, epithelial-to-mesenchymal transition, endothelin, chemoresistance, Snail

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Epithelial ovarian cancer (EOC) is the leading cause of gynecologic cancer mortality worldwide. The drug-resistant nature of EOC cells means that effective chemotherapies are lacking, which contributes to the high mortality in patients diagnosed with EOC [1]. This disappointing situation strongly suggests that improved understanding of the drug-resistance mechanistic underpinnings of EOC could lead to the development of novel therapeutic strategies for successful treatment of patients.

Epithelial–mesenchymal transition (EMT) has become prominently implicated as a means by which transformed epithelial cells can acquire the abilities to invade, resist apoptosis, and disseminate. This multifaceted EMT program can be activated transiently or stably, and to differing degrees, by carcinoma cells during the course of invasion and metastasis [2]. A set of pleiotropically acting transcriptional factors—including Snail, Slug, and Twist—orchestrate EMT and related migratory processes. Included among the cell-biology traits evoked by such transcription factors are loss of adherens junctions and associated conversion from a polygonal/epithelial to a spindly/fibroblastic morphology, expression of matrix-degrading enzymes, increased motility, and heightened resistance to apoptosis. Several of the transcription factors can directly repress E-cadherin gene expression, thereby depriving neoplastic epithelial cells of this key suppressor of motility and invasiveness [2]. Because EMT development is driven by key modulators that are directly controlled by numerous extracellular signals and pathways [3], it is becoming clear that blockade of these signalling pathways is critical for reverting EMT and related biologic effects, including drug sensitivity. Although cancer cells integrate multiple signalling pathways sustaining tumor progression, therapeutic interest in the endothelin-1 (ET-1)/endothelin A receptor (ET_AR) axis is supported by its central role in several human cancers, including EOC, in which its overexpression correlates with advanced stages [4].

In EOC cells, the autocrine loop mediated by the ET-1/ET_AR interaction has been implicated in the sustained activation of cell proliferation; escape from apoptosis; and angiogenesis, EMT, invasion, and metastasis [5]. In chemoresistant EOC cells, ET-1 and ET_AR are upregulated, paralleled by enhanced MAPK and Akt phosphorylation, cell proliferation, and reduced sensitivity to cytotoxic drugs, paclitaxel, and cisplatin [6].

It is becoming clear that EMT may reflect an ultimate adaptation of cancer cells to survive cytotoxic drug activity, and may thus be responsible for chemosensitivity [3]. In human EOC, changes in the expression of Snail, Slug, and Twist play an important role in ovarian tumorigenesis and progression; expression is significantly higher in advanced stages and in metastatic lesions [7,8]. Moreover, cellular morphology, motility, and molecular changes consistent with EMT, including enhanced expression of Snail and Twist, were reported to be related to paclitaxel resistance in EOC cells [9,10].

In our recent study, we reported that, compared with parental cells, resistant EOC cells showed enhanced expression levels of mesenchymal markers Snail, Slug, Twist, vimentin, and N-cadherin, and that the enhanced expression was associated with a concomitant decrease in E-cadherin expression [6]. Moreover, ET-1 enhanced the expression of Snail and Twist, and treatment with the selective ET_AR antagonist zibotentan inhibited the ET-1–induced effects, restoring E-cadherin expression. Remarkably, ET_AR blockade, either by zibotentan or by silencing, reverted the ET-1–induced suppression of E-cadherin promoter activity, suggesting that transcriptional regulation of E-cadherin may be important to ET_AR-driven EMT and acquisition of chemoresistance.

In parallel, ET-1 induced significant induction of Snail promoter activity, which was significantly inhibited by zibotentan, indicating that ET-1/ET_AR controls the transcriptional repression of E-cadherin through Snail in chemoresistant cells. Furthermore, upon treatment with ET-1, the E-cadherin promoter sequences were detected to be bound to Snail, suggesting that the ET-1–dependent and sustained binding of Snail in the E-cadherin promoter might account for EMT and the chemoresistant phenotype of those cells.

Epithelial cells also gain increased activity of matrix metalloproteinases, which leads to an invasive phenotype [2]. Significant upregulation and activity of matrix metalloproteinases 2 and 9, associated with increased invasive capability, were observed in chemoresistant cells, confirming the association between the invasive phenotype and the chemoresistant properties of those cells [6]. Our results provide evidence that ET-1/ET_AR overexpression, by regulating EMT and invasive behaviour, endows EOC cells with an increased survival capacity and resistance to chemotherapeutic agents.

Blockade of ET_AR by zibotentan reverted EMT, inhibited invasiveness, and restored drug sensitivity, enhancing the susceptibility of these cells to chemotherapy. *In vivo,* zibotentan inhibited growth of sensitive and resistant EOC tumor xenografts and sensitized them to chemotherapy, suggesting that a combination therapy might be effective at inducing apoptotic death and overcoming resistance in EOC cells. Analysis of human EOC tissues validated the preclinical results, revealing that ET_AR is overexpressed in chemoresistant tumors and is associated with expression of EMT markers [6].

Cancer stem cells (CSCs) have a critical role in drug resistance, which might explain why cancer is difficult to eradicate completely. Recent work has suggested that may be a link between the CSC phenotype and that induced by the process of EMT [11]. Cells that have an EMT phenotype share many molecular characteristics with CSC. The CSCs isolated from EOC samples express markers associated with stem cells and EMT, including Snail and Slug, suggesting that EOC cells, by going through EMT, acquire "stemness" characteristics qualifying them to acquire chemoresistance [12,13]. In that context, recent data suggest that Twist may also be an important regulator of "stemness" in EOC cells, indicating that initiation of the EMT program may be critical for the acquisition of stem-cell-like characteristics resulting in chemoresistance.

Because EMT and CSCs could have important roles in the regulation of sensitivity and resistance to anticancer drugs, antagonizing a specific receptor to target CSCs or cells that have an EMT phenotype could, therefore, become a novel strategy for increasing the sensitivity of EOC cells to conventional chemotherapeutics. In this regard, a recent pathway analysis revealed that ET-1 signalling was among the canonical pathways significantly associated with resistance to platinum-based chemotherapy, whose several links to EMT and "stemness" reinforce the correlation of both processes with therapy resistance [14].

Under this scenario, signals initiating EMT would be an ideal target because they are the seeds for metastasis and recurrence. Accumulating evidence clearly suggests that CSCs and EMT-type cells have critical roles in drug resistance. Thus, inhibitors of EMT or agents that could either reverse the EMT phenotype or kill CSCs would be a novel strategy for the treatment of cancers. Of clinical relevance, knockdown of ET_AR levels by small interfering RNA or blockade by zibotentan reverted the EMT phenotype, inhibited invasive behaviour, and increased susceptibility to chemotherapeutic agents, suggesting that ET_AR-mediated EMT signalling can represent a "salvage pathway" during chemoresistance development. The significant association between ET_AR overexpression and the resistant phenotype for the first time identified ET_AR as a predictor of chemoresistance in human EOC tissues and highlighted its relationship with EMT marker expression in the context of resistant tumors [6].

Improved understanding of the molecular mechanisms underlying drug resistance in EOC cells could be useful for devising targeted therapeutic approaches using an ET_AR antagonist in combination with conventional therapeutics to treat human EOC by preventing EMT-associated escape signalling. These new treatments are expected to result in better outcomes than can currently be achieved using conventional approaches alone.

CONFLICT OF INTEREST DISCLOSURES

The authors have no financial conflicts of interest to disclose.

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Correspondence to: Anna Bagnato, Molecular Pathology Laboratory A, Regina Elena National Cancer Institute, Via delle Messi D'Oro 156, Rome 00158 Italy.
E-mail: bagnato@ifo.it

* Molecular Pathology Laboratory A, Regina Elena National Cancer Institute, Rome, Italy.