

Disrupting the endothelin and Wnt relationship to overcome chemoresistance

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Abbreviations: APC, adenomatous polyposis coli; AXIN1, axis inhibition 1, CCND1, cyclin D1; ET_AR, endothelin A receptor; ET_BR, endothelin B receptor; EMT, epithelial to mesenchymal transition; EOC, epithelial ovarian cancer; ET-1, endothelin-1; GPCR, G-protein coupled receptor; GSK3, glycogen synthetase kinase 3; MMP-2, matrix metalloproteinase 2; TCF4, T-cell-specific transcription factor-4; β -TrCP, β -transducin repeat containing protein; VEGFR-2, vascular endothelial growth factor receptor-2

Knowledge of the mechanisms underlying chemoresistance is important in the development of novel targeted treatments for ovarian cancer. We recently reported that targeting endothelin A receptor/ β -arrestin-1, a binding partner of Wnt/ β -catenin, is sufficient to sensitize ovarian cancer to chemotherapy. This result highlights endothelin-1 receptor antagonists as potential anticancer therapeutics.

Epithelial ovarian cancer (EOC) is a disease plagued by recurrences and progressive chemoresistance. EOC cells assure their growth advantage and resistance to chemotherapy through the appropriation of key pathways, such as those controlled by G-protein coupled receptors (GPCRs). Accumulating molecular and in vivo evidence demonstrates that the activation of autocrine and paracrine signaling by the binding of endothelin-1 (ET-1, EDN1) to its GPCRs, endothelin A receptor (ET_AR, EDNRA) and endothelin B receptor (ET_BR, EDNRB), elicits pleiotropic effects in tumor cells and in the host microenvironment, modulating the epithelial to mesenchymal transition (EMT), chemoresistance, and the expansion of vascular networks.¹ Of particular interest, ET_AR was shown to be aberrantly activated in EOC and its expression has been correlated with platinum resistance and EMT marker expression.² This discovery was followed by analysis of EOC samples from The Cancer Genome Atlas (TCGA) that showed evidence for worse survival in EOC patients with ET_AR overexpression.³⁻⁴ In addition to ET_AR

overexpression in chemoresistant EOC cells, ET_BR also appears to have protumorigenic activity by promoting angiogenesis and lymphangiogenesis and evasion of the immune response.⁵ Hence, ET_AR and ET_BR, which are heterogeneously expressed in EOC cells, have emerged as key targets for cancer therapy.⁶⁻⁷ Emerging evidence demonstrates that acquisition of chemoresistance is highly dependent on contextual cues such as interactions with the tumor microenvironment and crosstalk with other signaling pathways. Noting that crosstalk between ET-1 signaling and other growth factor pathways drives tumor progression via the scaffold protein β -arrestin-1 (ARRB1) that serves as a co-pilot to organize complex signaling networks⁸⁻⁹, we hypothesized that an β -arrestin-1-mediated mechanism in ET-1 signaling may play a particularly important role in evasion of the drug response.

To investigate the mechanism underlying this resistance, we used resistant EOC cell lines generated by prolonged treatment with cisplatin or taxol. Upon ET_AR activation of these resistant cells,

β -arrestin-1 formed a nuclear complex with β -catenin and p300, resulting in histone acetylation that led to chromatin reorganization and enhanced transcription of genes such as ET-1 that are responsible for regulating the rate limiting step of the drug response¹⁰. Similarly, 13 of 13 platinum-resistant patients had increased recruitment of β -arrestin-1 and β -catenin on the *ET-1* promoter, providing further evidence that ET_AR/ β -arrestin-1 cooperates with Wnt signaling to acquire a chemoresistant phenotype through amplification of the ET-1 autocrine loop. This work expands what was previously known about the chemoresistance-associated functions of ET_AR, outlining a model in which ET-1 co-opts Wnt components for its own agenda, thus sustaining EMT, stemness features, cell invasion, and metastasis (Fig. 1).

The challenge that lies ahead is integrating our improved understanding of the interconnected molecular mechanisms promoted by the ET-1 axis in chemoresistant EOC cells with novel therapeutic options to improve patient outcomes. Can ET-1 receptors be

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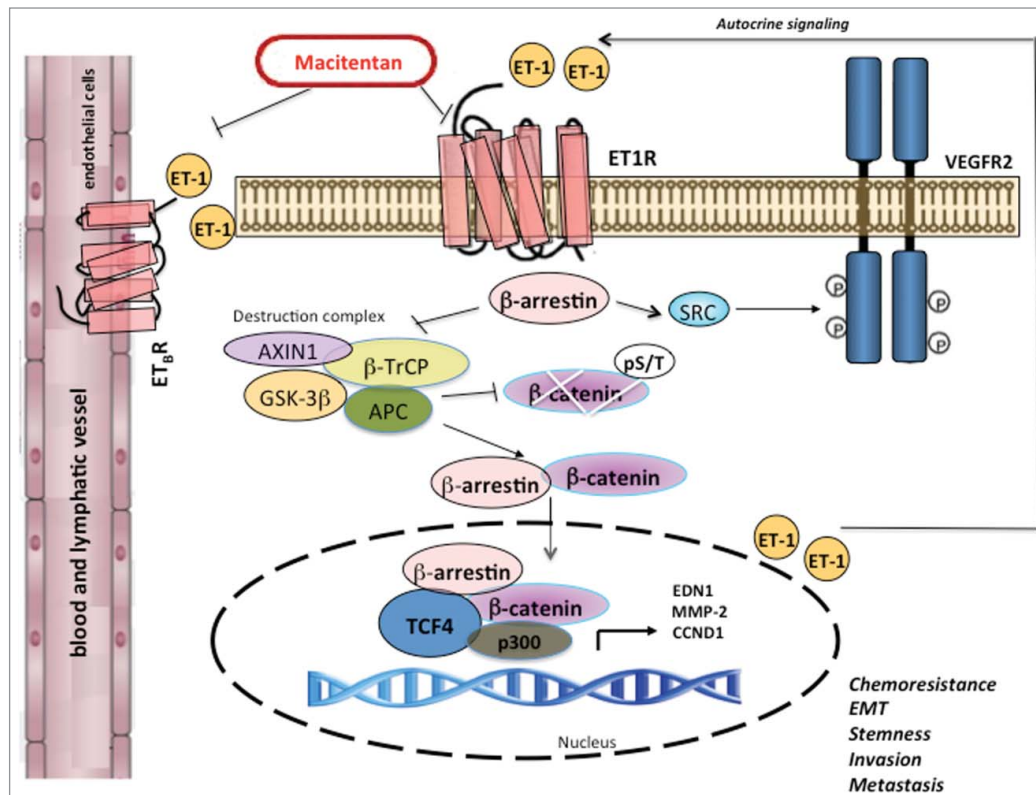


Figure 1. Interplay between ET_AR/β-arrestin-1 and Wnt/β-catenin drives chemoresistance in ovarian cancer. In chemoresistant ovarian cancer cells, binding of endothelin-1 (ET-1, EDN1) to its receptors leads to recruitment of β-arrestin-1 (ARRB1). β-arrestin inhibits the destruction complex composed of glycogen synthetase kinase 3 (GSK3), axis inhibition 1 (AXIN1), β-transducin repeat containing protein (β-TrCP), and adenomatous polyposis coli (APC), and thus promotes the accumulation of a non-Ser/Thr phosphorylated active form of β-catenin. β-arrestin-1 shuttles with β-catenin into the nucleus, where it interacts with p300 histone acetyltransferase to enhance β-catenin/T-cell-specific transcription factor-4 (TCF4)-transactivation, thus promoting the transcription of genes such as *EDN1*, *matrix metalloproteinase 2* (*MMP-2*), or *cyclin D1* (*CCND1*) and leading to enhanced chemoresistance, epithelial to mesenchymal transition (EMT), stemness, cell plasticity, invasion, and metastasis. As a signal transducer, endothelin A receptor (ET_AR, EDNRA)/β-arrestin-1 initiates transactivation of the vascular endothelial growth factor receptor-2 (VEGFR-2) through SRC. In parallel, paracrine production of ET-1 activates endothelin B receptor (ET_BR, EDNRB) expressed on endothelial cells, promoting expansion of vascular networks. The dual ET_AR and ET_BR antagonist macitentan targets not only cancer cells (which express ET_AR and ET_BR) but also tumor-associated stromal elements (which express ET_BR).

directly targeted to resensitize EOC cells to cisplatin or taxol? Treatment of resistant cells with the approved small molecule macitentan, a dual ET_AR/ET_BR antagonist that prevents formation of the β-arrestin-1/β-catenin/p300 complex on the target, caused a strong reduction in growth and invasiveness. *In vivo*, macitentan significantly inhibited tumor growth, neovascularization, intravasation, and peritoneal dissemination in chemoresistant EOC xenografts by interfering with ET_AR expressed on EOC cells and ET_BR expressed on endothelial cells. One striking observation is that the combination of macitentan and chemotherapy restored sensitivity to cisplatin and taxol. Consistent with the *in*

vitro results, analysis in human platinum-resistant EOC tissues showed that ET_AR overexpression is significantly associated with chemoresistance and poor prognosis, emphasizing ET_AR as a potential predictive marker of drug response. Taken together, the results of our study provide novel mechanistic insights into how Wnt/β-catenin signaling is wired to ET_AR/β-arrestin-1 to enable EOC cells to become unresponsive to chemotherapeutics. In the nucleus, the β-arrestin-1-β-catenin connection represents the initial scaffold on which transcriptional regulatory complexes could be built to regulate epigenetic modifications. Furthermore, in the cytosol, distinct β-arrestin-1 complexes can recruit factors that activate crosstalk

with tyrosine kinase receptor, such as vascular endothelial growth factor receptor-2 (VEGFR-2), indicating that β-arrestin-1 might control an intriguing spectrum of pathway interactions that regulate drug sensitivity.

Although these findings reveal the interplay between ET_AR and Wnt/β-catenin signaling, a number of questions remain. For example, does nuclear β-arrestin-1 have a common role in mediating β-catenin transcription in other ET-1-driven tumors? How does β-arrestin-1 translocate to the nucleus in response to ET_AR activation and is the receptor part of the nuclear complex? What causes the relatively high level of ET_AR in chemoresistant EOC? Does miRNA regulation play a role in

promoting chemoresistance by upregulating ET_AR? These and other issues, such as whole-genome ChIP-seq analysis to identify new β-arrestin-1 partners, are currently being addressed to understand the early events leading to the acquisition of chemoresistance in EOC and other ET-1-driven malignancies.

Targeting multiple networks using the dual ET_AR and ET_BR antagonist macitentan to overcome compensatory mechanisms of therapy escape provides the

potential advantage of targeting not only cancer cells (which typically express ET_AR) but also microenvironment-associated elements, such as vascular, lymphatic, and inflammatory cells and fibroblasts, which all express ET_BR. The present study therefore has biological and clinical relevance for the development of prognostic tools and novel treatments to overcome chemoresistance, and offers a strong rationale for translation of the experimental approach of combining

macitentan with chemotherapy into immediate clinical evaluation in this disease setting.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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