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MECHANISTIC INSIGHTS ON THE ROLE OF AMBRISENTAN, AN ENDOTHELIN TYPE-A RECEPTOR ANTAGONIST, IN BREAST CANCER

by

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Abstract

The activation of the endothelin receptor type A (ETAR) by its ligand endothelin-1 (ET-1) is well known for its role in vasoconstriction. Interestingly, ET-1 and ETAR are over-expressed in various human tumors, including breast cancer. Several studies described an important role for ETAR in cancer progression and metastasis. The extensive network of interactions that exist between ET-1 axis and other signaling pathways can trigger an autocrine and paracrine signaling that modulates different tumorigenesis processes, such as cellular proliferation and survival, epithelial-to-mesenchymal transition (EMT) and chemoresistance. In this study, our main objective was to investigate the antitumor effects of Ambrisentan, a selective antagonist of ETAR, and FDA-approved for the treatment of pulmonary arterial hypertension (PAH), on tumor growth and metastasis using a syngeneic, orthotopic triple negative breast cancer (4T1) animal model. The results show a significant reduction in tumor growth and enhancement in overall animal survival in Ambrisentan-treated mice. This correlated with a significant decrease in the extent of tumor metastasis to the liver and lungs. Using luciferase-expressing 4T1 tumor cells (4T1-Luc2) and *in vivo* life imaging (IVIS) of animals, our studies revealed 5- and 18-fold decrease in the bioluminescence signal collected from the primary tumor site and distant organs, respectively, in Ambrisentan-treated mice. Using multi-color flowcytometry, accumulation of $CD11b^{+}Ly6G^{+}$ granulocytes in blood, peripheral lymphoid organs and lungs, a process driven by chemokines secreted by 4T1 cells, was inhibited by >50% following Ambrisentan treatment. Importantly, this was further confirmed by demonstrating >90% reduction in the number of 4T1-Luc2 tumor cells metastasizing to the lungs. Moreover, histological staining of liver tissue revealed a 43% decrease in the number of tumor foci compared to controls that was associated with Ambrisentan treatment. In an independent series of experiments, the effect of Ambrisentan on 4T1 cell growth was tested in the Chorioallantoic membrane (CAM) assay. The findings highlighted the capacity of Ambrisentan to inhibit tumor growth by ~25% independent of any effect on anti-tumor immune responses. To uncover the underlying mechanism for the anti-metastatic effect of Ambrisentan, we examined the extent of angiogenesis within the tumor tissue by immunohistochemical staining using CD31-specific mAb. The findings revealed that total tumor vascularity was reduced by ~50%, mainly due to a decrease in the size of blood vessels in animals treated with Ambrisentan. Analysis of gene expression in 4T1 cells treated with Ambrisentan revealed significant inhibition (40-50%) of CXCL1 and MMP9, which are essential factors for tumor progression and metastasis. Taken together, this study provides a rationale for using Ambrisentan as a potential adjuvant in cancer therapy.

Keywords: Endothelin type A receptor (ETAR), Endothelin-1 (ET-1), cancer metastasis, Ambrisentan, IVIS, Myeloid cells, lungs, Granulocytes, CAM, angiogenesis, CXCL1, MMP9