STEMNESS MARKER ALDEHYDE DEHYDROGENASE 1A1(ALDH1A1) PROMOTES TUMOR ANGIOGENESIS IN BREAST CANCER CELLS

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Introduction and aim: Angiogenesis is the process of growing new blood vessels from pre-existing vessels and an important hallmark of cancer. Cancer stem cells (CSCs) play pivotal role in the process of metastasis. In particular, they are able to secrete a number of angiogenic factors that activate endothelial cells to grow and migrate orchestrating the formation of a vascular pathological niche. Aldehyde dehydrogenase 1A1(ALDH1A1) catalyzes the oxidation of broad spectrum of toxic aldehydes and is a robust hallmark of breast CSCs. High levels of ALDH1A1in breast cancer patients are associated with cancer stem-like features, as cell self-protection, differentiation, expansion, and therapy resistance. In this work we determined whether ALDH1A1in breast cancer cells was involved in the output of angiogenic factors and whether it might influence tumor angiogenesis in a panel of in vitro and *in vivo* experimental settings.

Material and methods: To study the stem-like phenotype of breast cancer cells we used in vitro tumorspheres. To investigate the role of ALDH1A1in MCF-7tumor angiogenesisloss- and gain-of function strategies were applied. Under these conditions we analyzed the angiogenic factors release by tumor cells expressing different levels of ALDH1A1. Angiogenic features were studied using a transwell system. We co-cultured breast tumor cells (upper compartment) with endothelial cells (HUVEC, lower compartment) and HUVEC proliferation, migration and tube formation were assessed. Finally, *in vivo* tumor angiogenesis was studied performing xenograft nude mice model.

Results: We found that ALDH1A1expression correlated with stem-like features of breast cancer cells. In the same cells we observed a significant uperegulation of proangiogenic factors as RNA and protein levels. Co-culture with ALDH1A1expressing tumor cells promoted endothelial cell proliferation, migration and tube formation. Conversely, downregulation of endogenous ALDH1A1resulted in reduction of pro-angiogenic factors production and HUVEC recruitment. Finally, *in vivo* we found an increase of vascularization in tumors expressing ALDH1A1that was associated with a higher expression of VEGF (Ciccone V et al., J Exp Clin Cancer Res 2018; Ciccone V et al., J Exp Clin Cancer Res 2019).

Discussion and conclusion: These data indicate a correlation between ALDH1A1expression, stemness, angiogenic factor expression and tumor angiogenesis. Collectively, these data suggest that ALDH1A1in breast cancer regulates stemness and tumor angiogenesis and can be a putative target for therapeutic intervention.

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