Dissecting the complex crosstalk network between endothelin-1 axis and vascular endothelial growth factor system in melanoma cells

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Phenotypic and genotypic analyses of cutaneous melanoma have identified endothelin B receptor (ET_B) as tumor progression marker, thus representing a potential therapeutic target. We previously reported that the binding of ET-1 to ET_B stimulates angiogenesis and lymphangiogenesis directly on blood and lymphatic endothelial cells and by stimulating vascular endothelial growth factor (VEGF)-A and VEGF-C production. In this study we investigated as to whether in melanoma cells ET-1 axis may interact with VEGF-A and VEGF-C signaling pathways to heighten cellular responsiveness. We found that primary and metastatic melanoma cell lines expressed besides VEGF-A, also VEGF-C and its selective receptor VEGFR-3, at mRNA and protein level. Following ET-1 stimulation, VEGF-A and VEGF-C mRNA were upregulated, showing a 4.5 and 2.5 fold increase compared to the control, respectively, and VEGFR-3 and downstream signaling intermediates, such as p42/44 MAPK and Akt, were rapidly phosphorylated. The use of selective ET_B antagonist, BQ788, completely blocked the VEGFR-3 phosphorylation and down-stream signaling pathways, demonstrating that ET-1-induced VEGFR-3 activation progress through ET_B. Inhibition of c-Src activity by PP2 reduced ET-1-induced VEGFR-3 phosphorylation, demonstrating that ET-1 transactivates VEGFR-3 through an intracellular mechanism mediated by c-Src to expand the signaling network. Stimulation with ET-1 in combination with VEGF-A or VEGF-C increased p42/44 MAPK and AKT phosphorylation, and resulted in a greater degree, 4 fold increase, of migrated melanoma cell compared to a single factor. Furthermore, this combination significantly ($p< 0.005$) enhanced (3.5 fold increase compared to ET-1 alone) the ET-1-induced vasculogenic differentiation of melanoma cell in tube-like structures, a phenomenon defined as *vasculogenic mimicry* and associated with high aggressive phenotype, indicating that crosstalk between ET-1/ET_B axis with VEGFR-3/VEGF-C system may enhance cancer cell motility and invasiveness and contributes to the promotion of cancer metastasis. Finally, in melanoma xenografts, ET_B antagonist induced a 60% inhibition of tumor growth compared to control mice, and a reduction in neovascularization-related effectors, indicating that targeting ET_B related signalling cascade may represent a novel treatment of melanoma by impairing the crosstalk between ET axis and VEGF in melanoma. Supported by AIRC.