Endothelin axis autocrine loop is positively regulated by the interplay between ET-1 and hypoxia-inducible factor-1 alpha in melanoma cells

Francesca Spinella, Valentina Caprara, Roberta Cianfrocca, laura Rosanò, Valeriana Di Castro, Pier Giorgio Natali, Anna Bagnato. Regina Elena National Cancer Institute, Molecular Pathology Laboratory, Italy.

Endothelin B receptor (ET_B), which is overexpressed in human cutaneous melanoma promotes melanoma progression and blood and lymphatic vessel growth upon activation by endothelin (ET)-1 or ET-3. We previously demonstrated that this mechanism occurs through a hypoxia-inducible factor (HIF)-1 alpha-mediated mechanism and that ET-1 may cooperate with hypoxia to increase this effect. In this study we show that in melanoma cells ETs increased ET-1 and ET_B mRNA and protein expression and their promoter activity that were completely blocked by the selective ET_B antagonist, BQ788, supporting the presence of a positive ET-1/ET_B-autocrine loop. Moreover when melanoma cells were exposed to hypoxia the induction of both ET_B and ET-1 was markedly enhanced demonstrating that hypoxia potentiates the ET-1-autocrine loop by reinforcing the expression of ET_B and the secretion of ET-1 that, in turn, increased simultaneously ET-1/ET_B expression. Silencing HIF-1 alpha or HIF-2 alpha significantly reduced the promoter activity and the expression of ET-1 and ET_B in response to hypoxia or ETs. Chromatin immunoprecipitation assay showed increased HIF-1 alpha and HIF-2 alpha binding to ET-1 and ET_B promoter in response to hypoxia or ET-1 stimulation. Interestingly, ET-1 stimulation rapidly induced an increase in nuclear localization of ET_B. Immunoprecipitation assay of nuclear extracts demonstrated that ET_B coimmunoprecipitates with both HIF-1 alpha and HIF-2 alpha suggesting that ET_B may associate with HIF- alpha to control ET-1 axis translation. Furthermore, we found that ET-1 or hypoxia induced vasculogenic differentiation of melanoma cell in tube-like structures. Silencing of HIF-1 alpha and HIF-2 alpha reduced the ET-1- and hypoxia-increased vasculogenic differentiation of melanoma cells concomitantly with a reduction in ET-1 and hypoxia-induced melanoma cell proliferation, and migration, providing that hypoxia and ET-1 axis share the same transcriptional machinery through which may regulate epigenetic control of genes involved in melanoma progression, such as ET-1 axis. Moreover, in melanoma xenografts, ET_B antagonist suppressed tumor growth, neovascularization-related effectors, indicating that targeting ET_B related signalling cascade may represent a novel treatment of melanoma by impairing the positive feedback loop between ET-1 axis and hypoxic melanoma microenvironment. Supported by AIRC.