Eps8: a common target for endothelial and tumoral cells

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Angiogenesis is essential for tumor growth and metastasis, and is governed by tumor cells themselves through the local production of pro-angiogenic factors that attract and activate endothelial cells (ECs) in the peritumoral microenvironment. Importantly, vessels also represent the main route used by invasive tumor cells to metastasize.

There are significant similarities in the mechanisms which enable tumor cells to invade into surrounding tissues, and ECs to generate new capillaries. In particular, a common requirement for angiogenesis and metastasis is the ability of endothelial and tumor cells respectively, to undergo movement and invasion. In this regard, the reorganization and reassembly of the actin cytoskeleton are key steps in both processes which are regulated by a large variety of actin-binding proteins with specialized functions. Among these proteins, we focused our attention on Epidermal growth factor receptor Pathway Substrate 8 (Eps8) which has been already critically involved in the regulation of cell motility and invasion in some human tumors of epithelial origin.

Recently, the crucial role of Eps8 in the regulation of tumor invasiveness has been extended to human glioblastoma (GBM) cell lines where we showed the critical requirement of Eps8 for the maintenance of their intrinsic invasive behavior[1]. In particular, we found that silencing of the protein by small interfering RNAs (siRNAs) fully abrogated the migratory and invasive capacities of three different human GBM cell lines both in 2-dimensional (2-D) and 3-dimensional (3-D) *in vitro* assays. The inhibitory effect on invasion was maintained independently by the migration mode utilized by the cells in our 3-D model, and was accompanied by an impaired formation of actin-based cytoskeletal protrusive structures that correlate with the loss of migratory phenotype.

Until now, nothing much is known about the contribution of Eps8 to the invasive properties of ECs, and more generally to angiogenesis. For these reasons, we studied the expression and function of Eps8 in human umbilical vein endothelial cells (HUVECs). We found the expression of a 97 kD band (corresponding to the most studied component of the Eps8 family) that was however significantly lower when compared to the Eps8 expression in human tumor cells such as GBMs. The silencing of Eps8 did not affect in HUVECs neither growth nor signaling in response to Vascular Endothelial Growth Factor (VEGF), the main growth factor acting on ECs. Importantly, also the phosphorylation on Ser1177 responsible for the endothelial Nitric Oxide Synthase (eNOS) activation was fully conserved in the absence of Eps8, indicating the maintenance of the ability of ECs to produce nitric oxide. When our analysis was shifted towards morphological tests *i.e.* tubulogenesis on Matrigel and 3-D spheroid sprouting assay, we observed that Eps8 silencing totally abrogated the ability of HUVECs to form tubular structures or sprouts, respectively. These results demonstrated a crucial role for Eps8 in the *in vitro* processes responsible for capillary sprouting and vessel network organization.

In conclusion, our data demonstrated for the first time the involvement of Eps8 in the control of the invasive and morphological processes required for the establishment of new blood vessels. Importantly, these results suggest that Eps8, acting at the same time on both cancer and endothelial cells, might represent an innovative target for the design of new drugs for the treatment of tumors, especially for those cancers crucially dependent on angiogenesis such as GBMs.

[1]Cattaneo MG et al Exp Cell Res 2012 318:1901-12.