Efficacy of the Eph-ephrin system antagonism in tumor angiogenesis and growth: *in vitro* and *in vivo* evidence.

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The Eph receptors represent the largest family of receptor tyrosine kinases (RTK) in humans with 14 members identified so far and divided in 2 classes, EphAs and EphBs, based on their amino acid sequence homology and on their affinity for cell-bound ephrin ligands. In the last 20 years several evidence showed a deregulated expression and/or function of these proteins in many solid tumors and a prominent role for Eph-ephrin signaling in tumor angiogenesis and in the tumor-microenviroment communications was demonstrated as well. Despite of these evidence, that make Eph-ephrin system an interesting new target in the tumor therapy, research programs aimed at developing pharmacological tools targeting this system are still in their initial stage as available compounds suffer for chemical or pharmacological drawbacks, limiting their application both *in vitro* and *in vivo*. Since 2009 our research group discovered and developed molecules able to interfere with this system and here we report the pharmacological characterization of UniPR1331 a new potent and selective amino acid conjugate of 3β-hydroxy-?^{5}-cholenic acid. UniPR1331 was able to inhibit Eph-ephrin binding in a ELISA binding assay and the ephrin dependent Eph phosphorylation in human prostate adenocarcinoma cells (PC3 cells) in the low micromolar range. Similar results were obtained when UniPR1331 was tested as antagonist of Eph receptors activation in human glioblastoma cells (U87GM) and in human umbilical vein endothelial cells (HUVEC). Moreover, UniPR1331 was inactive when tested as enzymatic inhibitor of the EphA2 kinase domain confirming to be a protein-protein interaction inhibitor (i-PPI). When tested in *in vitro* angiogenesis and vasculogenic mimicry UniPR1331 inhibited HUVEC and U87MG tubes formation respectively. Notably the chicken embryo chorioallantoic membrane assay confirmed the anti-angiogenic activity of UniPR1331. Finally, UniPR1331 oral bioavailability and blood brain barrier penetration capability allowed us to test its efficacy both in xenograft and orthotopic model of glioblastoma growth after oral administration (30mg/kg/day). In particular UniPR1331 was as effective as sunitinib and bevacizumab in inhibiting the tumor growth and in improving the time to progression (TTP) when tested in subcutaneous xenograft of U87MG cells. Moreover UniPR1331, when tested on luciferase-tagged U87MG intra-brain orthotopic model, mimicking a small tumor which regrows after surgery, was more effective than sunitinib and bevacizumab in improving the disease free survival and the overall survival of mice. In conclusion our data suggest that targeting of Eph-ephrin protein-protein interaction may be a new and efficacy strategy in the therapy of solid tumors.