Autocrine VEGFC-VEGFR2 signaling is of importance for the growth of Glioblastoma Multiforme

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Background

- The Vascular Endothelial Growth Factor (VEGF)-A can together with a number of other VEGF variants activate the VEGF Receptor 2 (VEGFR2)

- VEGFR2 has in addition to be expressed on endothelial cells (where it is linked to angiogenesis through paracrine stimulation), by VEGF produced by the tumor cells) recently been shown also to be expressed by Glioblastoma Multiforme (GBM) tumor cells.

- However, although autocrine VEGFA-VEGFR2 signaling has been identified in GBM cells, contradicting results exist for the effects of inhibiting VEGFA and VEGFR2 respectively in GBM cells.

- This indicates that activation of VEGFR2 in GBM cells is not solely dependent on VEGFA.

Methods

- CPH017p4 and CPH036p6 are two different GBM cell cultures originating from two different primary tumors from GBM patients and their subsequent xenografts. Cells were cultured in Neurobasal-A media supplemented with B-27, L-glutamine, NGF, and EGF.

- Cells were treated with SU1498 for inhibition of VEGFR2 phosphorylation, recombinant VEGF-A165 protein, Bevacizumab for inhibition of VEGFCA, recombinant VEGF protein and VEGFC-specific siRNA or non-specific scrambled (sc.) siRNA as control. The siRNA was delivered by plasmid transfection 24h prior to further experiments.

- Cell viability profiles were measured by MTT assay.

- Protein expression was determined by Western blot (WB) analysis while mRNA expression was measured by real-time quantitative PCR (Q-RT-PCR). Lysates from human dermal microvascular endothelial cells (HDMEC) and human dermal lymphatic endothelial cells (HDLEC) were used as controls.

- For establishment of tumor xenografts 100,000 CPH017p4 cells, which had been stably transduced for Luc2 expression, were injected orthotopically into the brain of NMRI nude female mice. Cells had one day prior to injection been transfected with either VEGFC-siRNA or sc.-siRNA. Tumor development was followed by Bioluminescence imaging. Mice were sacrificed when they presented tumor related symptoms or 20% weight loss.

- Patient tumor material was collected during surgery at Rigshospitalet, Denmark, under approval of the Scientific Ethical Committee for Copenhagen and Frederiksberg (KF 01-034/04). Tumors were diagnosed as GBM according to the WHO 2000/2007 guidelines.

- Statistics: One sample t-test, setting the hypothetical value to 1, was used for analysis of Q-RT-PCR and luminescence measurements. General linear model analysis was used for evaluation of in vitro growth curves, while survival analysis was performed using the Kaplan-Meier method and the log-rank test for comparisons between the treatment and the control group.

Aim

To examine if VEGFA is the only regulator of VEGFR2 in GBM tumor cells

Results

- VEGFR2 and VEGFA expression

- Role of VEGFA and VEGFR2 for the viability of GBM cells

- VEGFC expression and its effect on p-VEGFR2 and cell viability

- Effect of VEGFC knockdown on in vivo growth of GBM cells

- VEGFC expression in GBM patient tumors

Conclusions

- Like VEGFA, VEGFC can stimulate autocrine VEGFR2 activation in GBM cells

- VEGFC is of importance for cell viability and tumor growth in GBM cells

- VEGFC is a potential target for future GBM therapy