Neuro-Oncology
20(11), 1421–1422, 2018 | doi:10.1093/neuonc/noy127 | Advance Access date 21 August 2018

Revival of the VEGF ligand family?

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See the article by Michaelsen et al. pp. 1462-1474.

The failure of anti-angiogenic drugs to increase the survival of glioblastoma (GBM) patients was certainly one of the most disappointing results of the last 10 years in neuro-oncology. A meta-analysis of available clinical trials with various compounds and encompassing more than 4300 patients confirmed the lack of survival benefit either as first- or second-line treatment.[1] Despite this, the use of bevacizumab, a monoclonal antibody against vascular endothelial growth factor A (VEGF-A) and the most promising anti-angiogenic agent on the market, remains a matter of debate. Bevacizumab is approved for recurrent GBM in several countries, including the US, Australia, and Japan,[2] and although not approved by the European Medical Agency, it continues to be used in some European countries as a salvage therapy. There is lingering hope that a subpopulation of patients benefiting from the drug may be identified and/or that a combination treatment may be effective.

The original concept of anti-angiogenic treatment focused on blocking tumor vascularization, thereby interfering with oxygen and nutrient supply. Later, a vascular normalization window was proposed during which blood perfusion is increased, allowing for better drug delivery. Although morphological normalization of blood vessels is regularly seen with anti-angiogenic drugs, normalization at the functional level is not a general phenomenon.[3,4] Over time, it became clear that VEGF is not only important for endothelial cells, but has vessel-independent autocrine effects on tumor cells.[5,6]

The VEGF ligand family is composed of 5 polypeptides: VEGF-A, VEGF-B, VEGF-C, VEGF-D, and placenta growth factor (PlGF). Complexity is enhanced by alternative splicing and proteolytic processing. While VEGF-A is the main pro-angiogenic factor released by tumor and endothelial cells, the expression of VEGF-C in human glioma and its correlation with tumor grade was described more than 10 years ago.[7,8] The recent paper by Michaelsen et al (this issue) identifies VEGF-C rather than VEGF-A as being mainly responsible for autocrine VEGF receptor 2 (VEGFR2) activation and sustained GBM cell viability and proliferation.[9] The authors confirm significant VEGF-C expression in patient samples and find that VEGF-C knockdown in patient-derived GBM cell lines impacts survival and induces apoptosis. Intriguingly, this effect is seen despite high levels of VEGF-A present in the medium and could be recapitulated with a VEGFR2 inhibitor, but not with bevacizumab. VEGF-C knockdown also significantly reduced tumor growth in vivo, resulting in a survival benefit in mice, although it is not clear whether this is due to reduced tumor take based on the compromised viability of implanted cells. The study further shows that bevacizumab treatment increases VEGF-C expression in vitro, suggesting that VEGF-C may act as a compensatory pro-angiogenic and pro-tumorigenic factor in response to bevacizumab. This led to the clinically important question of a potential synergistic effect of VEGF-C knockdown and bevacizumab: although a minor additive effect was observed in vitro, the combination treatment was disappointing in vivo.

These data raise several questions, the first one related to the receptor(s) involved. VEGFs bind to their cognate receptor tyrosine kinases (RTKs) VEGFR1, VEGFR2, and VEGFR3 and to additional cell surface–expressed co-receptors, such as heparan sulfate proteoglycans, neuropilins, integrins, and ephrin B2. Although VEGF ligands display differential affinities for VEGFRs, there is considerable promiscuity. While PlGF and VEGF-B specifically bind VEGFR1, VEGF-A binds both VEGFR1 and VEGFR2. VEGF-C and VEGF-D bind VEGFR2 and VEGFR3.[10] Occurrence of receptor heterodimers allows for further fine tuning of functional responses. In a previous study, VEGFR2 activation was shown to maintain GBM cell viability as demonstrated by genetic and chemical interference.[6] Expression of VEGFR2 protein in clinical GBM samples was found to be restricted to a subpopulation of tumor cells (ranging from 0.5 to 60% of cells). Strong heterogeneity is also reflected in the cellular GBM models used by Michaelsen et al, where strong (1 out of 7), intermediate (3 out of 7), and no VEGFR2 expression (3 out of 7) was observed, suggesting that dependence on VEGFR2 signaling may identify a clonal subpopulation of tumor cells. On the other hand, the authors have not firmly demonstrated that VEGFR2 is the (only) mediator of VEGF-C activity.

The most difficult conundrum is the finding that VEGF-C has a strong pro-survival effect on GBM cells, while VEGF-A does not. Since both ligands activate VEGFR2, as indicated by increased receptor phosphorylation, this implies the induction of separate downstream signaling. VEGFR2 activity is regulated not only by its ligands, but also by the presence or absence of co-receptors and specific extracellular matrix proteins. Moreover, membrane

localization, internalization, and trafficking have been recognized as important means to regulate receptor activation.[11,12] For instance, phosphatidylinositol-3 kinase/Akt signaling is induced at the plasma membrane, whereas extracellular signal-regulated kinase (ERK) activation occurs predominantly in endosomal compartments. Endosomal trafficking maintains active VEGFR2 signaling over longer time periods and is thus a major regulatory factor, considering that more than 80% of VEGFR2 is localized in the cytosolic compartment.[11] These multiple adjustment levels might explain differential downstream signaling and variable functional effects despite activation of the same receptor. Interestingly, although not discussed by the authors, Michaelsen et al show increased ERK phosphorylation in the presence of VEGF-C, but not with VEGF-A (see Figure 4A in Michaelsen et al[9]).

From a scientific point of view, the highly complex VEGF-VEGFR biology is exciting and its dissection generates new insights into molecular and cellular mechanisms; however, this also raises the challenge of translation into successful therapeutics. Indeed the enthusiasm for clinical application is limited. Considering VEGF-A as the main pro-angiogenic factor in GBM and VEGF-C a major pro-tumorigenic factor, the application of small molecule inhibitors of the receptor should allow equal interference with endothelial and tumor cell signaling, in a ligand-independent manner. This should translate to a better treatment response by RTK inhibitors compared with inhibiting either ligand alone. Unfortunately, the reality has shown that multiple VEGFR inhibitors, such as cediranib, sunitinib, vatalanib, sorafenib, vandetanib, and axitinib, have been unsuccessful in clinical trials for GBM. A more thorough understanding of the differential signaling of VEGF family members and of the involved receptors will be necessary to change the paradigm toward a more optimistic view for VEGFR pathway inhibition in GBM.

Authorship statement. The author confirms that the text is the sole product of the author and that no third party had input or gave support to its writing.

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