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Linking tumor hypoxia with VEGFR2 signaling and compensatory angiogenesis

Glycans make the difference

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Although blocking vascular endothelial growth factor (VEGF) signaling is clinically beneficial in certain cancers, tumor regrowth in treated patients suggests that compensatory angiogenic programs may limit the efficacy of anti-VEGF treatment. We found that association of galectin-1 with complex *N*-glycans on VEGFR2 links tumor hypoxia to VEGFR2 signaling and preserves angiogenesis in response to VEGF blockade.

In 1970 Judah Folkman proposed that angiogenesis -the process through which new blood vessels arise from pre-existing ones- could influence tumor progression.[1] These pioneer experiments have inspired the design of novel therapeutic modalities in cancer aimed at blocking abnormal angiogenesis. Later, the identification of vascular endothelial growth factor (VEGF) as a major pro-angiogenic factor and the elucidation of its specific receptors (VEGFRs) and downstream signaling pathways have facilitated the development of selective inhibitors that blocked the vascularization process and suppressed tumor growth.[2]

Blockade of VEGF-VEGFR2 signaling with bevacizumab (a neutralizing anti-VEGF mAb) or with receptor tyrosine kinases inhibitors have improved the clinical outcome of several types of cancers including metastatic colorectal cancer, non-small cell lung carcinoma, renal cell carcinoma and hepatocarcinoma.[2] However, patients respond to these therapies with varying degrees of sensitivity, and a number of these develop progressive resistance, suggesting that compensatory pathways may contribute to hypoxia-driven tumor angiogenesis.[3,4] These include the secretion of alternative pro-angiogenic factors such as fibroblast growth factor-2 (FGF-2), placental growth factor (PIGF) and interleukin-17 (IL-17), as well as the recruitment of Bv8-expressing myeloid-derived suppressor cells.[3-5]

Glycosylation, the process responsible for creating specific glycan structures in glycoproteins and glycolipids, is a posttranslational modification that involves the coordinated action of glycosyltranferases (enzymes that catalyze the transfer of a saccharide from a nucleotide sugar donor or a lipid sugar to a substrate) and glycosidases that catalyze the hydrolysis of glycosidic bonds in glycan structures. The glycosylation machinery represents >1% of the total genome and more than 100 glycosyltransferases and glycosidases have been identified to date.[6] Remarkably, differential glycosylation can control a variety of cellular processes by displaying or masking ligands for endogenous lectins which can subsequently activate or fine-tune receptor signaling.[6,7] Although the importance of protein glycosylation in immune-mediated processes has been largely appreciated in the past few years,[6] our awareness of the impact of glycosylation in vascular biology is much more limited.

Galectin-1 (Gal1), a member of a family of endogenous glycan-binding proteins is upregulated in a variety of tumors and contributes to tumor progression by influencing homotypic and heterotypic cell adhesion, tumor cell migration invasiveness and immune escape.[6,8] Interestingly, Gal1 expression is upregulated in tumor hypoxic microenvironments and influences the development of aberrant vascular networks.[8,9]

Recently we identified a mechanism based on the differential glycosylation of tumor-associated vessels that mimics VEGF signaling and preserves vascularization in anti-VEGF-refractory tumors.[10] In response to immunosuppressive cytokines and hypoxic microenvironments, endothelial cells expressed all the repertoire of glycans that are critical for Gal1 binding and angiogenesis, including increased β1,6 *N*-glycan branching, higher poly-*N*-acetyllactosamine extension, and lower α2,6 sialylation. At the molecular level, we found that Gal1 co-opts the VEGFR2 signaling pathway through binding to non-sialylated complex *N*-glycans on Ig3, Ig4, and Ig7 domains of VEGFR2. These glycosylation-dependent interactions

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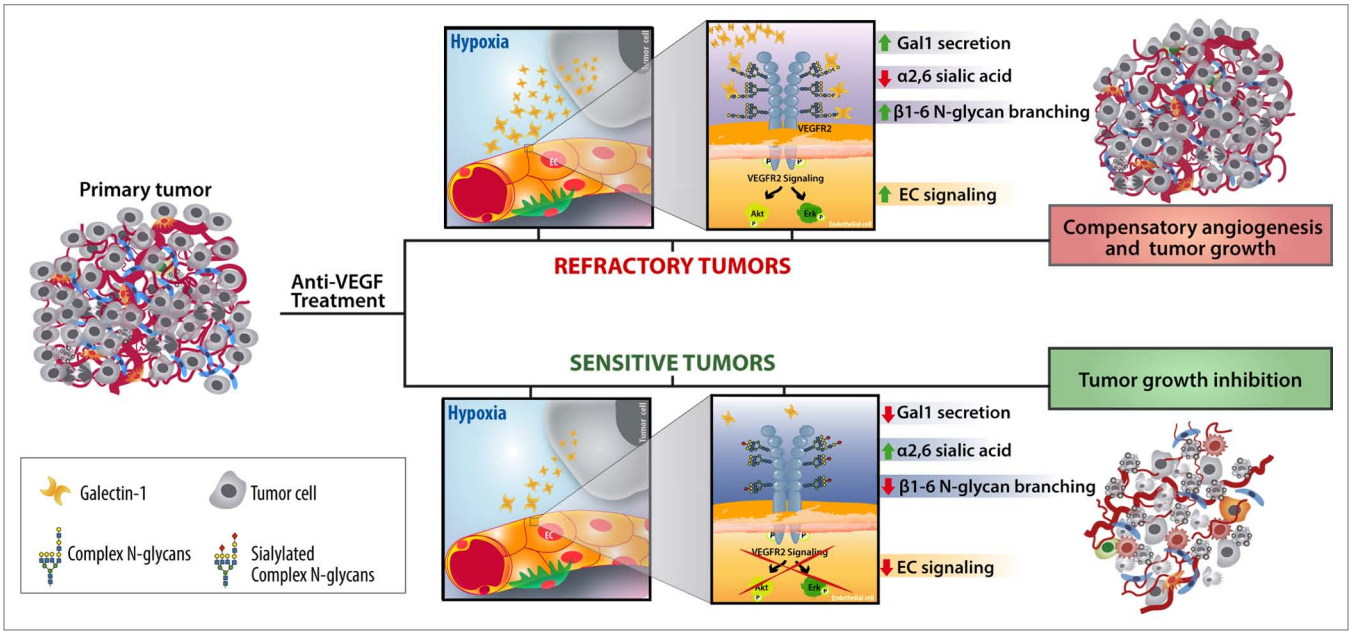

Figure 1. Association of Gal1 with complex *N*-gycans on VEGFR2 compensates for the absence of cognate ligand in anti-VEGF refractory tumors. In response to VEGF blockade, anti-VEGF refractory tumors (upper pannel) secrete higher amounts of Gal1 and their associated endothelial cells (ECs) express all the repertoire of glycans that are critical for Gal1 binding (increased β1,6 *N*-glycan branching, augmented poly-*N*-acetyllactosamine extension and lower α2,6 sialylation). This inducible EC glycophenotype facilitated Gal1 signaling, compensatory angiogenesis and tumor growth. In contrast, blood vessels associated with anti-VEGF sensitive tumors (lower panel) displayed higher amounts of α2,6-linked sialic acid which prevented Gal-1-VEGFR2 interactions.

promoted segregation of VEGFR2 into membrane microdomains and prolonged residency of this receptor on the surface of endothelial cells.[10]

In vitro, serum-free conditioned medium from anti-VEGF refractory, but not anti-VEGF sensitive tumors, induced endothelial cell exposure of Gal1-specific ligands. More importantly, tumor-associated vessels from mice inoculated with tumors that were sensitive to anti-VEGF (B16-F0 melanoma and CT26 colon carcinoma), expressed high amounts of α2,6-linked sialic acid in response to VEGF blockade, which prevented Gal1 binding and angiogenesis (**Fig. 1**). In contrast, mice inoculated with anti-VEGF refractory tumors (Lewis lung adenocarcinoma; LLC1 and R1.1 T cell lymphoma) secreted increased Gal1 and their associated vasculature expressed higher amounts of β1–6GlcNAc-branched complex *N*-glycans and decreased α2,6 sialylation in response to VEGF blockade (**Fig. 1**). Programmed remodeling of the EC glycome facilitated Gal-1-*N*-glycan interactions and promoted compensatory angiogenesis in tumors with limited sensitivity to anti-VEGF.[10] Lack of β1–6GlcNAc-branched *N*-glycans in endothelial cells or silencing of tumor-derived Gal1 converted refractory into anti-VEGF-sensitive tumors, whereas elimination of α2,6-linked sialic acid limited the efficacy of anti-VEGF treatment in sensitive tumors. This effect involved Gal1-VEGFR2 interactions, as it was prevented when Gal1 was silenced in tumor cells or when mice were treated with anti-VEGF monoclonal antibodies plus axitinib, a receptor tyrosine kinase inhibitor that preferentially perturbs VEGFR signaling.[10]

Finally, we validated the therapeutic efficacy of an anti-Gal1 neutralizing monoclonal antibody that selectively inhibits the function of Gal1 but not other members of the galectin family.[9,10] Administration of the anti-Gal1 antibody promoted tumor growth inhibition and circumvented compensatory angiogenesis induced by VEGF blockade. Interruption of Gal1-*N*-glycan interactions promoted transient normalization of the tumor-associated vasculature in vivo. This effect was reflected by decreased vessel diameter, fewer dilated and tortuous vessels and greater coverage by mature pericytes in vessels from anti-Gal1-treated tumors.[10] Moreover, disruption of complex *N*-glycans or antibody-mediated Gal1 blockade contributed to alleviate tumor hypoxia and facilitated influx of immune cells into the tumor microenvironment early after treatment. This effect resulted in augmented T-cell proliferation and enhanced IFN-γ and IL-17 production by tumor-draining lymph node cells.[10] These results emphasize the dual effects of blocking Gal1-*N*-glycan interactions, which influence tumor growth by attenuating aberrant angiogenesis and evoking T cell-mediated responses. Our findings offer novel opportunities for circumventing resistance to VEGF-targeted therapies and potentiating cancer immunotherapeutic modalities.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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