Targeting glycosylation-dependent non-canonical VEGFR2 signaling promotes vascular remodeling and augments tumor immunity

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Increased resistance to antiangiogenic therapies suggests the contribution of non-canonical pathways to hypoxia-driven neovascularization. The aim of this study was to evaluate whether Galectin-1-glycan interactions link tumor hypoxia to neovascularization and to investigate whether disruption of these lattices using an anti Galectin-1 mAb, may contribute to remodeling tumor vasculature and potentiate anti-tumor immune responses. For this purpose, we first examined the glycosylation signature of endothelial cells (ECs) in different microenvironments. In contrast to ECs stimulated with pro-inflammatory stimuli, ECs exposed to tolerogenic, proliferative or hypoxic microenvironment exhibited a substantial up-regulation of cell surface glycans that are critical for Galectin-1 binding (p<0.01). Accordingly, we found VEGF-independent activation of the VEGFR2 signaling pathway upon exposure of ECs to Galectin-1. Co-immunoprecipitation experiments revealed specific association of Galectin-1 with VEGFR2 through N-glycan-dependent interactions. Consistently, VEGFR2 blockade prevented Galectin-1-induced angiogenesis (p<0.01), whereas blockade of VEGFR1, VEGFR3, or VEGF had no effect. Interestingly, exposure to hypoxic microenvironments also up-regulated Galectin-1 expression through mechanisms mediated by activation of the NF-κB pathway. In vivo disruption of Galectin-1-glycan lattices, using a anti Galectin-1 mAb (F8.G7) abrogated hypoxia-driven angiogenesis and promoted remodeling of tumor vasculature, as shown by increased coverage of mature pericytes (p<0.01) and decreased tumor vessel diameter (p<0.01). Administration of the F8.G7 mAb in the B16, LLC1 or EL4 tumor models promoted a reduction in tumor growth (p<0.01) and evoked a T cell-mediated immune response, as shown by increased T-cell proliferation (p<0.01) and IFN-γ (p<0.05) production. Moreover, tumor-draining LN of F8.G7-treated mice had lower frequency of CD4+CD25+Foxp3+ regulatory T cells (p<0.05) and lower IL-10 secretion (p<0.05). Our findings highlight the versatility of endogenous lectins and the dynamics of the ‘glycome’ during cancer progression and provide the first evidence of a multitargeted agent capable of promoting vascular remodeling and overcoming tumor immunosuppression through specific interruption of lectin-glycan interactions.