A glycosylation-dependent pathway of non-canonical VEGFR2 activation links tumor hypoxia to vascular remodeling and immunity. DO. Croci[1], M. Salatino[1], J. Ouyang[2*], N. Rubinstein[1*], ID. Mascanfroni[1], JP. Cerliani[1], JM. Ilarregui[1], V. Sundblad[1], MA. Toscano[1], CI. Domaica[1], S. Dergan-Dylon[1], MC. Croci[1], A. Albini[3], MA. Shipp[2] and GA. Rabinovich[1]. [1]Laboratorio de Inmunopatología, Instituto de Biología y Medicina Experimental (IBYME-CONICET), Argentina. [2]Department of Medical Oncology, Dana-Farber Cancer Institute, Boston, MA,USA. [3]Polo Scientifico e Tecnologico, IRCCS Multimedica, Milano, Italy.

The mechanisms linking tumor hypoxia, neovascularization and immunity are poorly understood. Resistance to VEGF-targeted antiangiogenic therapies suggests the contribution of non-canonical pathways to hypoxia-driven neovascularization. We previously demonstrated an essential role of galectin-1 (Gal-1) in the control of tumor growth by favoring tumor-immune escape. The present study was conducted to elucidate whether Gal1-glycan lattices link tumor hypoxia to neovascularization and to investigate whether disruption of these lattices using an anti Gal1 mAb, may contribute to remodeling tumor vascular networks and stimulation of anti-tumor immune responses. For this purpose, we first examined the 'glycosylation signature' of endothelilal cells (ECs) in resting conditions or exposed to proliferative, tolerogenic, inflammatory or hypoxic stimuli. In contrast to ECs stimulated with pro-inflammatory stimuli, ECs exposed to tolerogenic, proliferative or hypoxic microenvironment exhibited a substantial up-regulation of the repertoire of cell surface glycans that are critical for Gal-1 binding and angiogenesis ($p<0.01$). Screening of the phosphorylation status of a spectrum of growth factor receptors revealed a 2-fold increase in phosphorylation of VEGFR2, Akt and Erk1/2 upon exposure to Gal1, a pattern comparable to that induced by VEGF. In this regard, pharmacological inhibition of Akt or Erk1/2 or interruption of GnTV-mediated N-glycan branching (but not GCNT1-mediated core 2-O-glycan elongation) prevented Gal1 signaling and abrogated ECs proliferation ($p<0.01$), migration ($p<0.01$) and angiogenesis ($p<0.05$). Co-Ip experiments revealed specific association of Gal1 with VEGFR2 through N-glycan-dependent interactions. Consistently, VEGFR2 blockade prevented Gal1-induced ECs migration ($p<0.01$) and morphogenesis ($p<0.05$), whereas blockade of VEGFR1, VEGFR3, or VEGF had no effect, suggesting that signaling established between lectins and glycans might serve as alternative pathways by mimicking 'cognate ligands', thus preserving critical processes such as angiogenesis. Furthermore, hypoxia promoted ROS/NF-κB-dependent HIF-1α-independent up-regulation of tumor Gal1 ($p<0.01$). mAb- or shRNA-mediated disruption of Gal1-glycan lattices attenuated hypoxia-driven angiogenesis, while promoting pericyte maturation and vascular remodeling as shown by increased association of ECs with mature pericytes (αSMA+, desmin+ and RGS5-) (2-fold; $p<0.01$), decreased vessel diameter (2.7 fold; $p<0.01$) and alleviation of hypoxia in tumors treated with anti-Gal1 mAb. Moreover, anti-Gal-1 mAb-treated tumors showed a significant reduction in tumor growth ($p<0.01$) and evoked a T-cell specific immune response, as shown by increased T-cell proliferation ($p<0.01$) and augmented IFN-γ ($p<0.05$) and IL-17 ($p<0.05$) production compared to mice receiving control isotype. Moreover, tumor draining LN of mAb-treated mice had lower frequency of regulatory T cells ($p<0.05$) and lower IL-10 secretion ($p<0.05$) compared to mice receiving isotype control. Hence, disruption of lectin-glycan lattices, not only evokes an unleashed anti tumor immune response, but also reduces angiogenesis and favors remodeling of tumor vascular networks, highlighting the versatility of endogenous lectins and the dynamics of the 'glycome' during cancer progression.