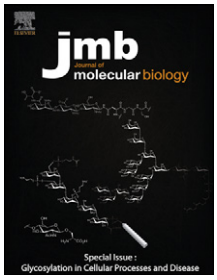

Shaping the Immune Landscape in Cancer by Galectin-Driven Regulatory Pathways

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Abstract

Along with the discovery of tumor-driven inflammatory pathways, there has been a considerable progress over the past 10 years in understanding the mechanisms leading to cancer immunosurveillance and immunoediting. Several regulatory pathways, typically involved in immune cell homeostasis, are co-opted by cancer cells to thwart the development of effective antitumor responses. These regulatory circuits include the engagement of inhibitory checkpoint pathways (CTLA-4, PD-1/PD-L1, LAG-3 and TIM-3), secretion of immunosuppressive cytokines (TGF-β, IL-10), and expansion and/or recruitment of myeloid or lymphoid regulatory cell populations. Elucidation of these pathways has inspired the design and implementation of novel immunotherapeutic modalities, which have already generated clinical benefits in an important number of cancer patients. Galectins, a family of glycan-binding proteins widely expressed in the tumor microenvironment (TME), have emerged as key players in immune evasion programs that differentially control the fate of effector and regulatory lymphoid and myeloid cell populations. How do galectins translate glycan-containing information into cellular programs that control immune regulatory cancer networks? Here, we uncover the selective roles of individual members of the galectin family in cancer-promoting inflammation, immunosuppression, and angiogenesis. Moreover, we highlight the relevance of corresponding glycosylated ligands and counter-receptors and the emerging function of these lectins as biological liaisons connecting commensal microbiota, systemic inflammation, and distal tumor growth. Understanding the molecular and cellular components of galectin-driven regulatory circuits, the implications of different glycosylation pathways in their functions and their clinical relevance in human cancer might lead to the development of new therapeutic approaches in a broad range of tumor types.

Galectin–Glycan Regulatory Pathways in Tumor-Associated Inflammation and Immunity

Inflammation is a hallmark of cancer [1]. Chronic inflammatory conditions such as ulcerative colitis or chronic infections are known to increase the risk of carcinogenesis. In addition, independent of the role of chronic inflammation in tumor initiation, an influx of inflammatory cells is a universal occurrence in the microenvironment of established tumors. Inflammation at tumor beds includes differentiated and immature hematopoietic cells (primarily of the myeloid lineage), cytokines produced by leukocytes, fibroblasts or tumor cells, and complement components. Overall, inflammation promotes, rather than blunting, malignant progression at multiple levels [2–4]. Firstly, inflammation fuels the proliferation and survival of malignant cells by activating transcription factors such as nuclear factor-κB and signal transducer and activator of transcription 3 (STAT3), which drive proliferative and anti-apoptotic pathways. Secondly, inflammatory cells, particularly myeloid leukocytes, are required for the generation of new blood vessels that support further tumor growth. In

addition, inflammatory cells contribute to the formation of pre-metastatic niches that promote malignant spreading. Inflammatory cells and their products also impair the effectiveness of chemotherapeutic agents, therefore representing a major target in understanding cancer initiation and malignant progression, as well as for the design of novel therapeutic interventions [1,2].

Along with the discovery of tumor-driven inflammatory pathways over the past 10 years, there has been a considerable progress in understanding cancer immunosurveillance and immunoediting based on the protection against the development of spontaneous and chemically induced tumors in animal models and the identification of targets for immune recognition in human cancer [5]. In fact, in the microenvironment of many established solid tumors, T cells can spontaneously exert clinically relevant pressure against malignant progression [5] and dramatically delay the progression of transplantable tumors [5]. Yet, in spite of these advances, a number of hurdles prevent the development of robust and durable antitumor responses. Thus, similar to inflammation, another independent although partially overlapping hallmark of cancer is the ability of tumor cells to elude or thwart antitumor immunity [6]. The mechanisms underlying these immune escape strategies involve: (a) impairment of antigen presentation; (b) activation of negative costimulatory signals- also called immune inhibitory checkpoints, including cytotoxic *T*-lymphocyte antigen 4 (CTLA-4), programmed death-ligand 1 (PD-L1), lymphocyte-activation gene 3 (LAG-3) and *T*-cell immunoglobulin and mucin-domain containing-3 (TIM-3); and (c) elaboration of a myriad of immunosuppressive factors such as transforming growth factor-β (TGF-β), interleukin (IL)-10 and indoleamine 2,3-dioxygenase (IDO). In addition, a number of regulatory cell populations, including $Foxp3^+$ and $Foxp3^-$ regulatory T cells (Tregs), natural killer T (NKT) cells, myeloid-derived suppressor cells (MDSCs), and mature immunosuppressive dendritic cells (DCs), contribute to undermine *T*-cell mediated tumor immunity [6]. In fact, accumulating evidence highlights the clinical benefit of blocking immune inhibitory checkpoints, either as monotherapies (e.g., anti-CTLA-4 or anti-PD-1 mAb) or in synergism with other immunotherapeutic modalities, to induce durable cancer regression and improve overall survival in patients with various malignancies by overcoming cancer-induced immunosuppression [5].

Glycans and Galectins in the Tumor Microenvironment

Although the complex regulatory pathways leading to tumor inflammation and immunosuppression have been largely studied at the gene, mRNA, and protein levels, the contribution of the glycome (the complete repertoire of cellular glycans) to these processes is poorly understood. Because of the non-template nature of carbohydrate synthesis, the macro- and microheterogeneity of glycosylation patterns of cell surface receptors, and the dynamic regulation of glycan structures in different physiologic and pathologic processes, deciphering the information encoded by the cellular glycome has proven a difficult task [7,8]. However, in spite of these limitations, endogenous glycan-binding proteins or lectins have been demonstrated to efficiently translate glycan-containing information into functional cellular responses including cell cycle progression, chemotaxis, differentiation, cytokine synthesis, and apoptosis by interacting with a discrete number of glycan structures [9,10]. In fact, lectins contribute to tumor growth and metastasis by influencing the signaling thresholds of glycosylated receptors or by modulating cell–cell interactions in the TME, leading to alterations of tumor cell migration, angiogenesis, inflammation, and immune escape [11–13]. However, in this regard, changes in protein glycosylation profiles have been largely observed not only in cancer cells themselves, but also in tumor-associated fibroblasts, endothelial cells, and immune cells [13,14]. Although the biological relevance of these changes is far from being completely understood, it has been demonstrated that the inflammatory cytokines typically up-regulated in cancer, particularly IL-6 and IL-1β, can change the glycosylation pattern of pancreatic and hepatocellular carcinoma cells, which contributes to the acceleration of malignant progression [15–17]. In addition, *O*-glycan branching also regulates the trafficking and effector activity of T cells, which are the major drivers of spontaneous antitumor immunity against established tumors, as well as memory *T*-cell differentiation [18,19]. The activity of enzymes that drive the elongation of branched structures on *O*-glycans is also influenced in T lymphocytes by a variety of cytokines such as IL-2, IL-4, or IL-15, with dissimilar activities [19].

Yet, what are the most prominent changes in glycosylation observed in the TME? One of the most notable hallmarks observed during tumor progression is the increased frequency of β1–6 branching of complex *N*-glycans, resulting from enhanced expression of *N*-acetylglucosaminyltransferase 5 (GnT5; encoded by MGAT5) [20], as well as augmented expression of the bisecting *N*-acetilglucosamine (GlcNAc) branch generated by the *N*-acetylglucosaminyltransferase 3 (GnT3; encoded by MGAT3) in malignant compared to healthy tissues [21]. Moreover, incomplete glycosylation has been reported to be a common feature of cancer-associated mucins, including expression of the T antigen (Galβ1–3GalNAc-α1-*O*-Ser/Thr), also called the Thomsen–Friedenreich antigen or