Aberrant glycosylation in cancer: a novel molecular mechanism controlling metastasis

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ABSTRACT

Glycosylation alterations are involved in several steps of human tumors pathogenesis. In this issue of *Cancer Cell*, Agrawall et al. identified the glycosyltransferase FUT8 as a previously unrecognized mediator of melanoma metastasis, establishing core fucosylation as a potential therapeutic target for prevention and treatment of metastatic tumors.

Glycosylation is a tightly regulated cell and microenvironment specific process that is orchestrated by numerous glycosyltransferases and glycosidases. The advent of systemic approaches in Glycomics has contributed for the understanding of the glycans complex biosynthetic pathways and the definition of the structures and functional roles of glycans in physiological and disease conditions (Cummings and Pierce, 2014). At the molecular level, glycosylation actively regulates protein folding, activation, and degradation, as well as interferes with proteolytic cleavage.

Cumulative evidence shows that changes in glycosylation accompany the acquisition of the cellular features necessary for tumor growth and progression. The myriad of glycosylation alterations described in cancer (Figure 1) include the incomplete synthesis and expression of truncated glycan structures, increased expression of complex branched *N*-glycans, *de novo* expression of terminal sialylated glycans, and altered fucosylation (Pinho and Reis, 2015). Sialylation is a glycan modification frequently observed in tumors. An example is the simple truncated Sialyl-Tn antigen, which is rarely detected in healthy tissues but expressed by most carcinomas and associates with poor patient's prognosis. Other relevant sialylated structures include the selectins' ligand Sialyl-Lewis X, which increased levels correlate with patient's poor survival, and the widely used serological cancer-biomarker Sialyl-Lewis A (CA19.9). Glycan structures are also often decorated with fucose residues, that can be either linked to extended oligosaccharide chains, as it occurs within the Lewis antigens, or α1,6-linked to the innermost GlcNAc residue of *N*-glycans. The FUT8 enzyme is the only fucosyltransferase able to transfer α1,6-fucose generating the core fucosylation.

Glycans are important players in several processes involved in cancer progression, ranging from cell-cell and cell-extracellular matrix (ECM) adhesion to intracellular and extracellular communication. Moreover, there is compelling evidence that glycosylation interferes with inflammation and tumor immune surveillance. In addition to the extracellular glycosylation remodeling observed in cancer, modification of cytosolic proteins with *O*-GlcNAc is also a common event. This modification interferes with signaling cascades and impacts cancer cell cycle regulation, transcriptomic profiles and adaptability to the microenvironment (Slawson and Hart, 2011).

The majority of cancer related deaths can be attributed to metastasis. There are different glycan structures that have been associated with a pro-invasive phenotype. Increased expression of β1,6-linked branching oligosaccharide structures has been shown to associate with looser cell-cell adhesion and with a higher metastatic potential. Similarly, increased expression of sialylated structures favors cell detachment and increases interaction with ECM. Recently, terminal sialylation has also been shown to regulate oncogenic activation of tyrosine kinase receptors (Mereiter et al., 2016). The interaction between selectins and sialylated glycans, such as Sialyl-Lewis X, can also facilitate metastasis by promoting the adhesion of cancer cells to the vascular endothelium.

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Overall, increased sialylation interferes with several key molecular processes leading to tumor progression and patients' poor prognosis (Pinho and Reis, 2015).

Melanoma is one of the most lethal skin cancer worldwide and its poor clinical outcome derives from the high propensity to metastasize. In this issue of *Cancer Cell*, Agrawall et al. performed a systematic analysis of the melanoma glycome of clinical samples finding a correlation of α1,6-core fucosylation on *N*-glycans with a pro-metastatic behavior of the disease (Figure 1) (Agrawal et al., 2017). Melanoma samples showed significant changes in glycosyltransferases transcription, such as the increased expression of *N*-glycan branching enzymes (MGAT2, MGAT4A), polylactosamine extension enzyme (B3GNT2), sialyltransferases (ST6GAL1, ST6GAL2), and lower expression levels of enzymes responsible for α1,2 fucosylation (FUT1, FUT2). Interestingly, this study revealed that metastatic melanoma displays higher expression levels of fucosyltransferase FUT8 than primary tumors, explaining the increased expression of α1,6-core fucosylation (Agrawal et al., 2017). The increase in FUT8 expression levels was found to be transcriptional regulated by the TGFβ-Induced Factor Homeobox 2 (TGIF2) (Agrawal et al., 2017).

Glycosylation changes in cancer cells can be found on several proteins, from adhesion molecules to transmembrane receptors, including signaling and inflammatory molecules (Figure 1). The biological function of a specific glycan largely depends on the carrying protein displaying this structure, as well as on the specific amino acid glycosite. Systematic glycoproteomic approaches have recently allowed the discovery of protein glycosylation sites occurring in cancer (Campos et al., 2015; Steentoft et al., 2011). Analysis of metastatic melanoma cells revealed that the neural cell adhesion molecule L1 (L1CAM), a glycoprotein associated with cancer cell dissemination and metastasis (Altevogt et al., 2016), displayed α1,6-core fucosylation of *N*-glycans (Agrawal et al., 2017). This core fucosylation on L1CAM can have major molecular and biological implications. L1CAM is known to be cleaved by plasmin and this proteolytic cleavage has been previously demonstrated to inhibit its ability to mediate spreading and metastatic growth (Valiente et al., 2014). Agrawall et al. demonstrated that core fucosylation precludes L1CAM cleavage by plasmin (Figure 1). The presence of an *N*-glycosylation site in L1CAM in close proximity to the plasmin cleavage site, together with the demonstration that plasmin-mediated cleavage of L1CAM is inhibited in FUT8 overexpressing cells, further supports this mechanism. The ability of uncleaved L1CAM to interact with the vasculature at distal organs explains how FUT8 expression contributes for melanoma metastases.

Previous studies have also shown that altered fucosylation can affect the biology of melanoma metastasis. The transcription factor ATF2 has been shown to suppress metastasis by altering α-1,2 fucosylation (Lau et al., 2015). Overall, these findings clearly demonstrate that glycosylation can control key mechanisms in the biology of melanoma progression.

The biosynthesis of core fucosylation occurs in complex *N*-glycans that concomitantly differ in other glycan structures, such as branch number, composition, length, and capping arrangements (Pinho and Reis, 2015). These structural glycan variations can also affect the binding of carbohydrate-recognition proteins, such as galectins, selectins, and siglecs. Such glycosylation variations can ultimately exert significant functional effects on the cancer cell interaction with ECM and with other cells, including those from the immune system and at metastatic sites.

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Moreover, the study by Agrawal et al., together with several recent studies in the field, points for the need for systematic approaches using appropriate cell and animal models to address the various biological processes controlled by glycosylation (Agrawal et al., 2017; Campos et al., 2015; Mereiter et al., 2016; Steentoft et al., 2011). These strategies contribute for the understanding of the role of primary and metastatic tumor glycome and glycoproteome, as well as their clonal and temporal variations during disease progression and cancer therapy. Furthermore, these approaches foster the identification of novel therapeutic strategies in cancer, such as the modulation of the glycosylation status of L1CAM in metastatic melanoma by targeting fucosyltransferase FUT8 (Agrawal et al., 2017).

Taking into consideration that glycans and glycoconjugates intervene in various steps of tumor progression, the cellular biosynthetic machinery involved in glycan biosynthesis and modification constitutes a promising target for cancer treatment. Future efforts should focus on the definition of specific glycosylation inhibitors that can either target a pro-metastatic biological function, or interfere with the modulation of the immune response. These approaches hold great potential in cancer therapy.

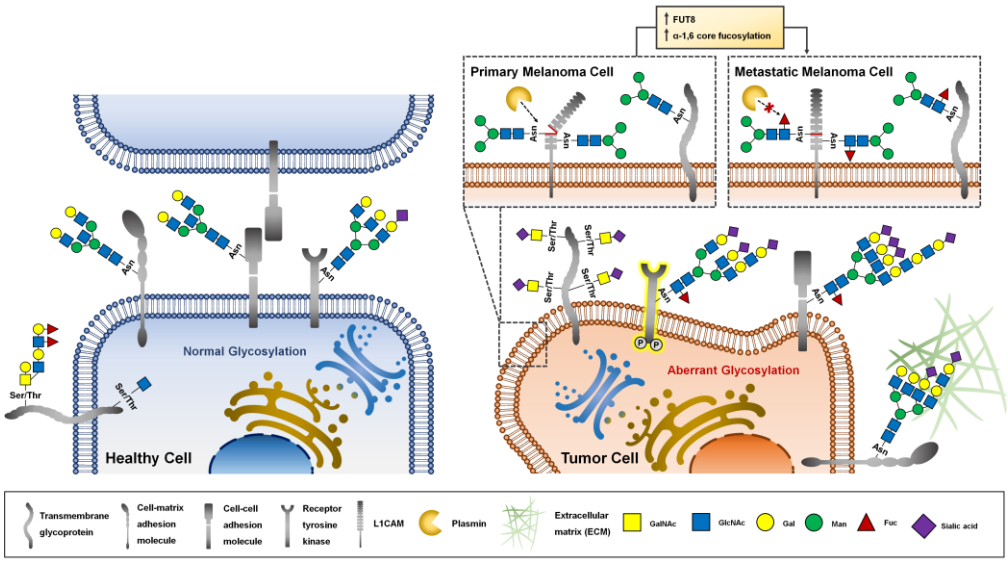

Figure 1. Glycosylation alterations in cancer. Cancer development and progression encompasses major alterations in cell glycosylation, including incomplete synthesis of *O*-glycan structures, increased expression of branched *N*-glycans, expression of terminal sialylated glycans, and altered fucosylation. Agrawal et al. identified FUT8-mediated α1,6-core fucosylation as a molecular driver of metastasis in melanoma, by precluding L1CAM protein proteolytic cleavage by plasmin.

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