Pleiotropic effects of O-glycosylation in colon cancer

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Edited by Eric R. Fearon

Changes in the O-glycosylation of proteins have long been associated with the development of cancer, but establishing causal relationships between altered glycosylation and cancer progression remains incomplete. In this study, the authors perform comparative analyses of the cellular phenotypes, transcriptional changes, and alterations in the glycoproteome in colon cancer cells that differentially express one glycosyltransferase. Their results provide a wealth of data on which future studies can be based.

The search for factors involved in the development and progression of cancer has revealed a multitude of genetic, epigenetic, and phenotypic changes that distinguish malignant cells from their normal counterparts. One such change typically seen in tumors is the alteration of protein glycosylation (1). In particular, changes in mucin-type O-linked glycosylation have been associated with cancer development and poor prognosis—and many diagnostic markers are based on these changes (1). Mucin-type O-linked glycosylation is initiated by a family of enzymes (known as the GalNAcTs or ppGalNAcTs) that catalyze the addition of GalNAc onto the serine or threonine residues of proteins transiting the Golgi apparatus (Fig. 1A) (2). In mammals, other enzymes add additional sugars to the extant GalNAc in a stepwise process to form what are known as core structures, which can then be further elongated and branched by other glycosyltransferases (Fig. 1A) (2). However, in cancerous tissues, these structures are typically truncated, with many existing as only a single GalNAc (Tn antigen) or GalNAc capped with sialic acid (STn antigen) (1). The reasons for O-glycan truncation in cancerous tissues remain largely unknown, although many studies have implicated the enzymes involved in O-glycan extension in these alterations (1, 3, 4). Likewise, whether the changes in O-glycan structure are contributing to neoplastic transformation or are a by-product of the transformation process itself remains an ongoing area of inquiry.

In this issue of JBC, Wandall and colleagues (5) report new insights into the potential role of one member of the enzyme family that initiates O-linked glycosylation (GalNAc-T6) in the cellular transformations typically seen in cancerous cells and tissues. GalNAc-T6 is one of the 20 family members in mammals that are responsible for the initiation of O-linked glycosylation (2). Members of this family are essential in Drosophila and are involved in various cellular and developmental processes (6, 7). Aberrant GALNT6 expression has previously been observed in several cancer types, but the biological role of GALNT6 in vivo is still unknown.

To initiate their study, Wandall and colleagues (5) examined the expression profiles for all 20 GALNT isoforms in 288 colon adenocarcinomas and 41 healthy colon samples, observing that GALNT6 expression is largely absent in healthy colon tissue but up-regulated in many colon adenocarcinomas. The authors then used the colon adenocarcinoma cell line LS174T (which expresses GALNT6) to create an edited version where GALNT6 has been deleted (LS174TΔT6) to interrogate the resultant cellular and transcriptional changes. Wandall and colleagues found that loss of GALNT6 in the LS174T colon cancer cell line led to apparent enhanced differentiation and reduced proliferation (Fig. 1B). By performing transcriptional profiling, the authors found that cells lacking GALNT6 had decreased expression of stem cell markers (such as LGR5) and increased expression of genes associated with both differentiation and cell adhesion, phenotypes that more closely reflect those of normal colon epithelial cells (Fig. 1B). In line with this, LS174TΔT6 cells formed crypt-like structures more readily than LS174T cells. Moreover, these effects appear to be unique to GALNT6 as deletion of GALNT3 (a closely-related member of this family) did not result in similar phenotypes.

As the primary effect of the GalNAc-T6 enzyme is expected to be on protein glycosylation, the authors examined changes in the O-glycoproteome using a version of LS174T cells in which the COSMC gene is deleted (which allows for more facile identification of O-glycosylated proteins because of the truncated structure of the O-glycans). These cells (also known as SimpleCells or LS174TΔSC) were compared with a version with GALNT6 ablated (LS174TΔSCΔT6) to identify the proteins that are specifically glycosylated by GalNAc-T6. Interestingly, they found a small number of proteins that appear to be GalNAc-T6-specific targets, including MIA3 (also known as Tango1), which had previously been associated with colon cancer development (8) and is essential for secretory apparatus structure and secretion (7, 9).

The findings of Lavrsen et al. (5) provide a robust characterization of how the expression (or loss) of a single enzyme can produce a multitude of cellular changes. Like many studies, the work raises additional questions regarding the complexities of

This work was supported by the Intramural Research Program of the National Institutes of Health, NIDCR Grant Z01-DE-000713 (to K. G. T. H.). The authors declare that they have no conflicts of interests with the contents of this article. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

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3 The abbreviation used is: GalNAc-T, N-acetylgalactosaminyltransferase.
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Figure 1. The O-glycosylation pathway and a summary of the effects of GALNT6 expression in LS174T colon adenocarcinoma cells. A, shown are the major pathways and enzymes involved in the synthesis of mucin-type O-linked glycans. O-Glycan structures typically seen in cancerous tissues are in the orange-shaded boxes. B, summary of the changes observed in LS174T colon adenocarcinoma cells with and without GALNT6 expression.

cellular transformation. Are the observed GALNT6–specific effects unique to this particular colon cancer cell line (whose transformation may be due in part to the loss of the cyclin-dependent kinase inhibitor p21WAF1) or could GALNT6 expression induce neoplastic phenotypes in normal colon cells? Does GalNAc-T6–specific glycosylation of the six proteins identified affect their biological function? Further, does differential glycosylation of these six proteins contribute to the broad phenotypic changes observed in LS174T cells? How might the role of GALNT6 be related to that of GALNT12, another member of this family previously shown to be mutated in colon cancer patients (10)? And finally, what is the normal biological function of GALNT6 in vivo? Understanding its normal function may provide additional insights into how its mis-expression could disrupt normal cellular homeostasis.

The study by Lavrsen et al. (5) offers comprehensive information about the cellular, transcriptional, and glycoproteomic changes resulting from expression of a single GalNAcT. This work also provides a platform for investigating the detailed mechanisms of GalNAc-T6 action in cells. For example, it will be interesting to investigate whether GalNAc-T6 influences secretory apparatus structure (through its action on MIA3) and/or glycan structure, which has been previously identified as a factor contributing to changes in cell proliferation and invasion in other cell culture systems (4). More broadly, this study serves to emphasize that there is still much to be discovered about the roles of O-glycosylation in both health and disease.

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