Not so sweet malignant transformation

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A critical requirement for the success of a multicellular organism is the ability to exquisitely regulate the growth of its component cells. Tumors, also called neoplasias, occur when that regulation is disturbed; the term neoplasia simply means “new growth.” The pathology community classifies neoplasias as benign or malignant based on morphologic features of the cells in the tumor, the presence of metastases, and more recently immunologic and genetic features, as a way to predict how a particular neoplasia will behave and impact the patient with time. Within malignant tumors, generally understood as “cancer,” the constituent cells will show a pattern of changes denoted as dysplasia, such as altered nuclear/cytoplasmic ratios, hyperchromatic nuclei, variable cell size and shape, disrupted polarity and stratification, lack of differentiation, abnormal mitotic figures, and multiple nuclei. In general, cancers that have more extensive dysplastic features will be more aggressive and more likely to metastasize.

Research into the mechanisms by which cancer originates has developed various *in vitro* models for comparing the features of nontransformed versus malignantly transformed cells. Properties, such as loss of contact inhibition, changes in adhesive properties, override of senescence, matrix invasion, and inappropriate migration are thought to reflect the dysplastic features observed in malignant tumors *in vivo*. Recent advances in genomic sequencing have revealed that multiple genes must be mutated before a tumor takes on a malignant phenotype and that many mutations involve various aspects of growth regulation. How these mutations explicitly lead to the oncogenic or dysplastic features of malignant tumors is not yet well understood.

A long-noted characteristic of malignant transformation has been the aberrant glycosylation of both proteins and lipids. Buck et al. (1) reported this in 1970 for N-glycans from virus transformed cells. A number of immunologic epitopes identified as characteristic of malignant cells proved to be carbohydrate structures on lipids and proteins (2). However, the mechanistic relationship between altered glycosylation and the socially inappropriate cell behaviors that typify malignancy remained a mystery for decades.

Among the most characteristic and widespread of these carbohydrate epitopes, particularly in epithelial cell cancers, are Tn and STn (Fig. 1) that arise when mucin-type O-linked glycans, which are normally more complex structures, are truncated so that only a single *N*-acetylgalactosamine (GalNAc) (Tn) or GalNAc modified with the sugar sialic acid (STn) remains attached to a protein via the hydroxyl of Ser or Thr. The correlation of Tn antigen presence with poor prognosis and lower survival rates for breast cancers suggested that truncation of these glycans contributes to the more aggressive behavior of Tn-positive tumors (3) but gave no indication of how this might occur.

The ground-breaking work by Radhakrishnan et al. (4) in PNAS has taken advantage of a novel technique they developed to identify sites of O-glycan addition to proteins (5, 6), to provide evidence that truncation of O-glycans induces oncogenic features, such as enhanced growth and invasion. The results are remarkable not only because they indicate that O-glycans play a critical role in regulating growth and adhesive properties of cells, but because they also demonstrate that multiple different properties associated with an enhanced malignant phenotype are affected when O-glycans are truncated. In addition, the accessibility of these O-glycans at the plasma membrane as parts of short peptide sequences makes them attractive as markers for malignant cells and as targets for immunotherapeutic agents.

Mucin-type O-linked glycans are highly complex, heterogeneous structures that are assembled in a nontemplate process as they pass through the endoplasmic reticulum and Golgi. The initial step in their synthesis is the addition of GalNAc to Ser or Thr residues by one or more of 20 different peptide-specific GalNAc-transferases (Fig. 1). The peptide specificity of these GalNAc-transferases results in addition of O-linked glycans to specific locations on a large number of different glycoproteins, depending on which GalNAc-transferases are expressed in a given cell (7). In the vast majority of cases, the GalNAc is modified with a β 1,3-linked galactose (Gal). Additional sugars and branch points can be added to this structure to form complex and,

Fig. 1. Tn antigen arises by truncation of mucin-type O-glycans. Synthesis of mucin-type O-glycans is initiated by the transfer of GalNAc from UDP-GalNAc to Ser or Thr residues by one or more of 20 known peptide-specific GalNAc-transferases. In most instances Gal is transferred from UDP-Gal to the GalNAc by C1GalT1. Other sugars and branch points can then be added by a series of additional glycosyltransferases to form a variety of structures. The private chaperone COSMC is essential for expression of active C1GalT1. In the absence of COSMC no C1GalT1 activity is expressed and the O-linked GalNAc is not further modified and remains “truncated.” Although GalNAc-Ser/Thr is the essential feature of Tn antigens, the peptide surrounding the GalNAc-Ser/Thr is also a part of the epitope. As a consequence, antibodies can be raised that distinguish Tn antigen on peptide A from Tn antigen on peptide B.

in many cases, very large glycans. Expression of the single β 1,3Galactosyltransferase (C1GalT1) that mediates this addition is dependent on the expression of a private chaperone, core 1 β 3-Gal-T-specific molecular chaperone (COSMC) (8). To date, the only known function of COSMC is to act as a chaperone for C1GalT1.

Radhakrishnan et al. (4) have used zinc-finger nuclease disruption of COSMC to generate cells that are not able to elongate the O-linked GalNAc, and thus express only the Tn antigen. Mass-spectrometric methods are used to identify the proteins modified with O-linked GalNAc and to map the locations of the O-linked GalNAc moieties. Disruption of COSMC expression in a pancreatic tumor cell line, T3M4, and keratinocyte cell line, HaCat, induced changes in behavior

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characteristic of malignant cells in in vitro model systems and in xenografts that could be reversed by reintroduction of COSMC. These include changes in proliferation rate, loss of contact inhibition of growth, loss of tissue architecture, changes in basement membrane adhesion, and invasive growth. Performing phosphoproteomics in HaCat cells with and without COSMC disruption revealed changes in phosphorylation of many proteins associated with adhesion and formation of tight junctions.

What is remarkable is that truncation of mucin-type O-linked glycans affects so many features that are characteristic of oncogenic changes associated with malignant behavior. HaCat cells take on many of the features of malignant tumor cells upon disruption of COSMC. Radhakrishnan et al. (4) identified 1,471 O-glycosites on 446 O-glycoproteins in HaCat cells. Truncation of the O-glycan structures will have an impact on numerous surface O-glycoproteins, potentially altering proprotein processing, modulating ligand-binding properties and signal transduction by receptors, and changing access to surface proteins by proteases. Precisely which O-glycans on which O-glycoproteins are critical and what the mechanisms are for the impact of O-glycan truncation remain to be determined; however, these findings represent an exciting advance in our understanding of the changes seen in malignant cells.

Another consequence of O-glycan truncation is that the numerous Tn antigens formed are each unique because each one consists of the Ser or Thr O-linked GalNAc and the surrounding peptide sequence (Fig. 1). Thus, it is possible to generate immunoreagents, such as monoclonal antibodies that recognize specific O-glycans. These have great potential as diagnostic and therapeutic agents (9). The

ability to identify the sites of O-glycan addition will markedly enhance generation of such reagents.

Nearly all epithelial tumors express Tn antigens at their surface. Some 200 genes encode the glycosyltransferases that are responsible for the synthesis of all glycans on proteins and lipids. Nucleotide sequence analysis of 201 glycosylation-related genes in pancreatic tumors revealed very few mutations in glycosyltransferases themselves.

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Mutations of C1GalT1 and COSMC were also rare. Instead, Radhakrishnan et al. (4) found that in 38% (13 of 34) pancreatic cancer tissue samples the COSMC promoter was methylated. Furthermore, promoter methylation correlated with loss of C1GalT1 expression and the presence of Tn antigen. Methylation of the COSMC promoter thus accounts for loss of C1GalT1 expression in

nearly 40% of pancreatic tumors. It is of course possible that COSMC has other as yet unknown functions aside from being a private chaperone for C1GalT1; however, the strong correlations between altered O-glycans and changes in cellular properties at multiple levels makes it highly likely that COSMC promoter dysregulation and its role in the truncation of mucin glycans represent a critical function for oncogenic transformation.

Future studies building on this platform promise further insights into the precise mechanistic basis for all of the changes observed following truncation of mucin-type O-glycans. However, the observation that truncation of O-glycans contributes to a number of distinct oncogenic features of malignant cells provides a strong impetus for future development of a mechanistic understanding of the role of mucin-type O-glycans in both normal and transformed cells. This latest chapter in the carbohydrate structure-function epic validates concepts that were introduced four decades ago using technical advances undreamed of then, and a generous measure of persistence to progress toward contemporary fruition.

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