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Fucose is on the TRAIL of colon cancer

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Glycosylation is arguably the most abundant and complicated form of post-translational modification found in mammalian cells. Carbohydrate modifications on proteins play important roles in many aspects of biology, including development, protein folding, immune cell function, host-pathogen interactions, cellular communications, and signal transduction (for a comprehensive review of this subject, see the second edition of *Essentials of Glycobiology*¹ which is available on Bookshelf at NCBI). Of the ten different monosaccharide building blocks used to synthesize the thousands of oligosaccharide structures found in mammalian systems, fucose is gaining increasing importance. Fucose is a component of many different classes of glycan, including *N*-glycans, mucin-type *O*-glycans, and directly linked to the hydroxyl of serine or threonine residues as *O*-linked fucose (Figure 1)². It is typically found as a terminal modification of *N*- or mucin-type *O*-glycans. Examples of fucosylated structures in these contexts include the H antigen of the ABO blood group system, or the Lewis blood group antigens. Alternatively fucose is found at the core of *N*-glycans, linked to the *N*-acetylglucosamine directly attached to the protein (core fucosylation), or directly attached to the protein itself (*O*-fucosylation). Recent studies reveal functions for fucose in all of these contexts. For instance, fucose plays roles in selectin-mediated extravasation of leukocytes and lymphocyte homing, pathogen adhesion, and more recently modulation of signal transduction pathways (Figure 1)².

All fucosylation reactions in cells are catalyzed by fucosyltransferases, 13 of which are known to exist in the human genome². These enzymes are localized to the lumen of the endoplasmic reticulum (for POFUT1 and POFUT2) or the Golgi apparatus (for all others)^{2–4}. The donor substrate for all fucosyltransferases is GDP-fucose, which is synthesized in the cytoplasm and carried into the lumen of the ER/Golgi with the aid of one or more transporters. Mammalian cells have the capacity to synthesize GDP-fucose from other sugars (*de novo* pathway) or to utilize fucose provided from external sources (salvage pathway) (Figure 1). Two enzymes are required to convert GDP-mannose to GDP-fucose in the *de novo* pathway: GDP-mannose 4,6-dehydratase (GMD) and GDP-4-keto-6-deoxymannose 3,5-epimerase-4-reductase (FX). Mice lacking the *FX* gene (and thus all forms of fucosylation) display an embryonic lethal phenotype revealing the importance of fucose during development⁵. Much of the embryonic lethality can probably be explained by the *O*-linked fucose glycans (Figure 1). Elimination of the genes encoding either Pofut1 (*O*-fucosylates EGF repeats) or Pofut2 (*O*-fucosylates TSRs) in mice causes severe embryonic lethal phenotypes^{6, 7}. Thus, fucosylation obviously plays important roles in biology.

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Many of the effects of fucose are mediated through modulation of signal transduction pathways. The Notch receptor is modified at many sites with *O*-fucose, and loss of *O*-fucose in mammals abrogates function⁸. This explains the embryonic lethality of the Pofut1 knockout, since Notch function is essential for embryogenesis. Deletion of the gene encoding Fut8 (responsible for core fucosylation of *N*-glycans, Figure 1) revealed the importance of fucosylation in several other signaling pathways⁹. Although *Fut8*^{-/-} mice are born at normal rates, they display growth retardation and early death due to abnormal lung development and emphysema-like symptoms. The lung phenotype appears to be due to defects in TGF- β 1 signaling, suggesting that loss of core fucose on the *N*-glycans of TGF- β 1 receptors reduces their activity. Further examination of cells lacking Fut8 has revealed similar decreases in the activity of EGF receptor¹⁰, LDL-related protein 1¹¹, and α 3 β 1 integrin¹². In each case, loss of core fucosylation appears to affect ligand binding, not cell-surface expression of the receptors.

In this issue (pages **) Moriwaki and co-workers provide evidence that fucosylation affects another signaling pathway: TRAIL. TRAIL (for TNF-related apoptosis inducing ligand) is produced mainly by cells in the immune system where it functions in T cell homeostasis and NK cell-mediated killing of virally infected or oncogenically transformed cells¹³. Binding of TRAIL to TRAIL-receptors (Death receptors (DR) 4 or 5) induces oligomerization, resulting in the initiation of apoptosis and cell death. TRAIL also binds to decoy receptors (DcR1 or DcR2), which do not induce apoptosis and are believed to play a role in down regulation of the TRAIL signal. Because of their ability to kill tumor cells, soluble recombinant versions of TRAIL, as well as anti-TRAIL receptor antibodies, are in clinical trials in patients with several different types of tumors¹⁴. Although potentially a promising therapy, several different tumor lines are reported to be resistant to TRAIL-mediated killing.

Due to the importance of fucosylation in signaling pathways, Moriwaki *et al.* were examining global fucosylation levels in several colon cancer cell lines. They used *Aleuria aurantia* lectin (AAL) in Western blots of cell lysates to probe levels of fucosylation (AAL binds fucose in many contexts, but preferentially recognizes core fucosylation of *N*-glycans¹⁵). Surprisingly, extracts of HCT116 cells showed a dramatic reduction in AAL binding, suggesting a defect in fucosylation. To examine the reason for this reduction, they assayed both fucosyltransferase enzyme activities and GDP-fucose levels in the cells and could not detect GDP-fucose. These results suggested some defect in the *de novo* GDP-fucose pathway (Figure 1), and RT-PCR analysis revealed a truncated transcript for GMD. Western blots showed the absence of GMD protein, and transfection of the cells with a construct encoding full-length GMD restored fucosylation, demonstrating that HCT116 cells have a defect in fucosylation due to loss of functional GMD.

To examine the effects of fucosylation on tumor growth, the parental HCT116 and GMD-rescued cells were injected into athymic mice. Although the cells had similar growth rates *in vitro*, tumor growth and metastasis were substantially lower *in vivo* with the GMD-rescued cells. Subsequent studies revealed that the GMD-rescued cells were significantly more susceptible to TRAIL-mediated killing than the parental HCT116 cells, suggesting that TRAIL receptor activity is affected by fucosylation. Aberrant transcripts for GMD were found in two other human colon cancer cell lines, as well as a number of colon and ovarian cancer tissue samples. Thus, loss of GMD may be a common mechanism for cancer cells to evade TRAIL-mediated killing.

Little is known about how fucosylation is affecting TRAIL receptor function. In particular, it is not known whether fucose on an *N*-glycan, mucin-type *O*-glycan, or *O*-fucosylation (Figure 1) is mediating this effect. The lack of EGF repeats or TSRs in TRAIL receptors probably excludes *O*-fucosylation from playing a direct role, unless receptor cross-talk

somehow plays a role (a recent report suggests Notch activation sensitizes TRAIL-induced apoptosis in hepatocellular carcinoma cells¹⁶). A recent report demonstrated that mucin-type *O*-glycosylation of DR4 and 5 promotes ligand-induced receptor clustering¹⁷, indicating the importance of mucin-type *O*-glycans on the receptors. This same report showed that increased levels of transcripts for FUT3 and FUT6, two fucosyltransferases capable of forming Lewis blood group antigens (Figure 1), correlate positively with TRAIL activity. These results correlate nicely with the findings of Moriwaki *et al.*, suggesting that the presence of Lewis-type structures on mucin-type *O*-glycans on TRAIL receptors may enhance their activity. Although this idea deserves further study, Moriwaki and coworkers saw no effect on TRAIL activity in GMD-rescued cells when mucin-type *O*-glycosylation was inhibited using chemical inhibitors (data not shown), implying that the GMD-rescue of TRAIL activity is not dependent on mucin-type *O*-glycosylation. The other major candidate for mediating the effects of fucosylation on TRAIL receptor is core fucosylation. Interestingly DR5 does not contain any predicted *N*-glycosylation sites (Asn-X-Ser/Thr), but DR4 contains a single site at Asn156. Both of the decoy receptors contain several sites for *N*-glycosylation.

This study raises a number of interesting questions. As mentioned above, a major question is which type of fucosylated glycan is being affected. It is also important to identify which component of the pathway is modified with the fucosylated glycan (DR4 or 5, one of the decoy receptors, or something else). Preliminary results confirm the presence of *N*-glycans on DR4¹⁸. Determining the structure of this *N*-glycan in the HCT116 and GMD-rescued cells would help to resolve where it shows a change in fucosylation. Elimination of this site by mutagenesis may also reveal whether a fucose on the *N*-glycan is responsible for altering TRAIL activity. Ultimately the goal will be to understand the molecular mechanism by which a change in fucosylation affects this signaling pathway. The presence of the salvage pathway for GDP-fucose biosynthesis may also provide a potential therapy for cancers that have lost GMD. Addition of fucose to the medium of cells with defects in the *de novo* pathway can restore fucosylation¹⁹. Thus, taking advantage of the salvage pathway by providing fucose should make GMD-lacking tumors susceptible to TRAIL-mediated killing *in vivo*.

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Figure 1. Fucosylation pathways

GDP-fucose can be synthesized in the cytoplasm from GDP-mannose (*de novo* pathway) or from free fucose (salvage pathway). Once synthesized, GDP-fucose is transported into the lumen of the ER/Golgi (drawn as a single compartment for simplicity) through one or more transporters (the ER transporter has not been identified). Fucose is transferred from GDP-fucose to acceptor substrates in the lumen of the ER (Pofut1, Pofut2) or Golgi (all other fucosyltransferases). Pofut1 fucosylates serine/threonines in Epidermal Growth Factor-like (EGF) repeats, and Pofut2 fucosylates serine/threonines in Thrombospondin type 1 repeats (TSRs). Fucosylation leads to formation of cell-surface or secreted glycoproteins, some of which are shown on the surface of the cell or secreted. Although fucose occurs in many different types of structures, some specific examples are shown, including (from left to right): mucin-type *O*-glycans, *N*-glycans (both core and terminal fucosylation), *O*-fucose on TSRs, and *O*-fucose on EGF repeats. Note that Lewis type structures can occur on both *N*-glycan or mucin-type *O*-glycan cores. Known functions of fucosylated glycans are shown above each structure, including selectin recognition, pathogen binding, and a variety of signaling events (Notch, TGF-β1, EGF, integrins). For a more extensive review of fucosylation, see Becker and Lowe². Adapted from Smith *et al.*⁵ with permission ****note: permission pending****.