Study on the Role of Core 3 O-glycans in Colorectal Cancer using Cell Models

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Abstract

O-GalNAc glycosylation, also known as mucin-type O-glycosylation (O-glycosylation) which is characterized by α-GalNAc linked to Serine, Threonine or Tyrosine residues in proteins is one major type of protein glycosylations. The O-glycans on glycoproteins play important roles in many biological processes. The common O-glycans are either Core-1, Galβ1-3GalNAc-α-R or Core-3, GlcNAcβ1-3GalNAc-α-R based structures. Core-1 O-glycans are the most predominant ones found in all animal cells, while Core-3 O-glycans appear to be restricted to proteins from epithelial cells of gastrointestinal tract. Notably, the Core-3 O-glycans were reportedly to play significant suppressive roles in colorectal tumor biology. The mechanisms underlying Core-3 O-glycans' tumor suppression, however, are not well understood. Core-3 N-acetylglucosaminyltransferase gene (*C3GnT*, *β3GnT6*) encodes the enzyme responsible for the initiation of Core-3 O-glycan biosynthesis. It is not known how *C3GnT* in intestinal epithelial cells is transcriptionally regulated. Furthermore, existing cell lines do not express *C3GnT*, and how the *C3GnT* is suppressed in colorectal cancer remains elusive. Herein, we firstly established and characterized three colorectal tumor cell lines with the expression of *C3GnT*. Ectopic expression of *C3GnT* eliminated the expression of Tn antigens in the *Cosmc*-deficient cells and led to synthesize Core-3 O-glycans as evidenced by CORA (Cellular O-glycome Reporter/Amplification). Furthermore, expression of *C3GnT* in *Cosmc*-deficient colorectal cancer cell lines caused inhibition of cell growth and migration. Moreover, exogenously expression of *CDX1* and *CDX2*, potential transcription factors for *C3GnT*, resulted in the elevated expression of *C3GnT* thereby decreased expression of Tn antigens in the *Cosmc*-deficient cells. Our ongoing studies will further address the mechanisms for how the suppression of *C3GnT* and loss of core-3 O-glycans lead to the progression and metastasis of human colorectal carcinoma. Overall, this study will lead to our better understanding of important role of *C3GnT* in colon cancer, and the development of potential therapeutics.

Summary

1. **Ectopic overexpression of *C3GnT* eliminated the expression of Tn antigens in the *Cosmc*-deficient cells, and led to synthesize an array of Core-3 O-glycans.**
2. **Expression of *C3GnT* in *Cosmc*-deficient colorectal cancer cell lines caused inhibition of cell growth and migration.**
3. **Exogenously expression of CDX1 and/or CDX2, potential transcription factors for *C3GnT*, resulted in the expression of C3GnT thereby decreased expression of Tn antigens in the *Cosmc*-deficient cells.**

Results

Overexpression of C3GnT in *Cosmc*-KO cells resulted in significantly reduced expression of Tn antigens

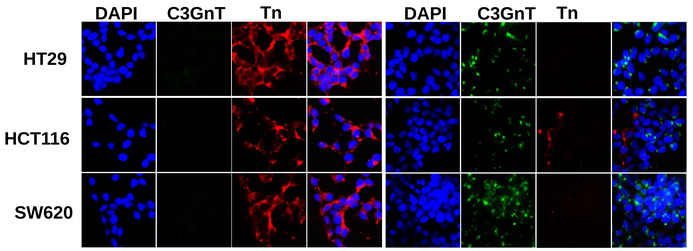

Figure 1: Ectopic overexpression of C3GnT resulted in significantly reduced cell-surface expression of Tn antigen. Expression of C3GnT (green) and Tn antigen (red) in sorted *Cosmc*-KO cells were immunofluorescently stained. Nuclei were counterstained with DAPI (blue).

Overexpression of C3GnT in *Cosmc*-KO cells resulted in inhibition of cell growth and migration

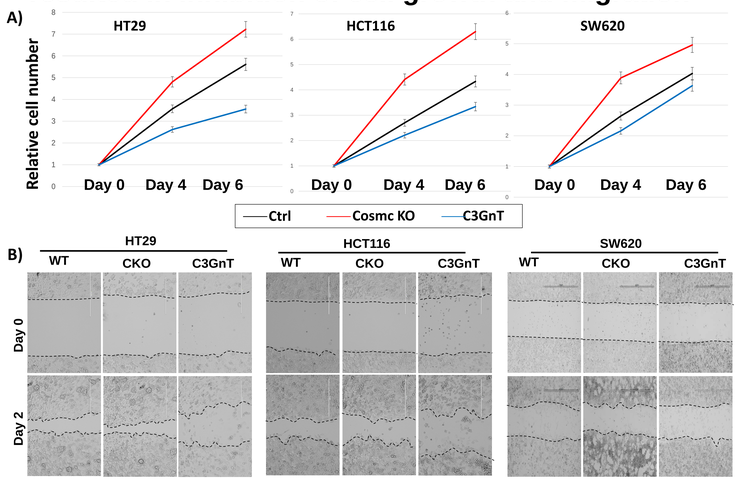

Figure 3: Effects of C3GnT overexpressing on the growth and migration of colorectal cancer cells in vitro. (A) Cell count assays were performed to analyze the effect of C3GnT overexpressing on colorectal cancer cell growth in vitro. (B) Wound healing assay was made in cell lines with 48 hours of recovery.

FDA Mission Relevance

Our research advances better understanding of important role of C3GnT in colon cancer that can help development of potential therapeutics to predict patient response to the targeted therapies. The acquired information also has implications in helping the FDA reviewers in making more informed regulatory decisions regarding specific drug combination strategies and interpretation of clinical safety and efficacy data.

Overexpression of C3GnT resulted in production of Core 3-based O-Glycans

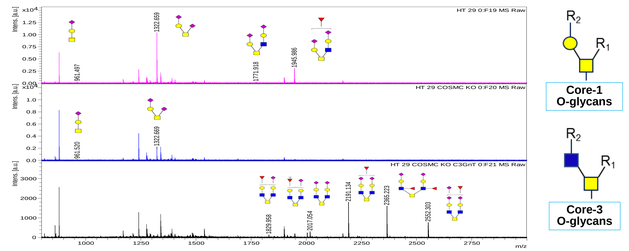

Figure 2: Ectopic overexpression of C3GnT led to synthesize an array of Core-3 O-glycans as confirmed by mass spectrometry (MS) analysis.

Overexpression of Transcription Factor CDX1 and/or CDX2 in *Cosmc*-KO cells resulted in expression of C3GnT

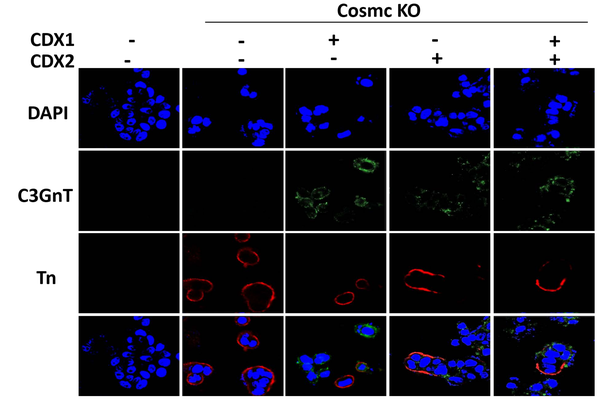

Figure 4: Ectopic overexpression of CDX1 and CDX2 in *Cosmc*-KO cells resulted in significantly increased expression of C3GnT. Expression of C3GnT (green) and Tn antigen (red) cells were immunofluorescently stained. Nuclei were counterstained with DAPI (blue).

Further Directions

1. **The role of *C3GnT* in human colorectal carcinoma:**
   - ❖ **The link between suppression of *C3GnT* and Tn antigen expression in the progression and metastasis of human colorectal carcinoma.**
   - ❖ **The expression status of *C3GnT* in human primary CRC samples.**
2. **The mechanisms for how the suppression of *C3GnT* and loss of core-3 O-glycans lead to the progression and metastasis of human colorectal carcinoma.**