Regulation of mTOR signaling in colon cancer pathology

Abby Lund[1], Mansib Rehman[1], Robert Sticca[2,3], Donald A Jurivich[1], Ramkumar Mathur[1]
[1]Department of Geriatrics, [2]Department of Surgery, University of North Dakota, Grand Forks, ND
[3]Sanford Hospital, Fargo, ND

UND School of Medicine & Health Sciences
University of North Dakota

Abstract

Colorectal cancer (CRC) is the third leading cause of cancer deaths globally. Patients above the age of 65 had a 10–20% increased risk of colorectal cancer (1). The risk of tumor recurrence through metastasis remains a substantial challenge, regardless of early identification. Cellular or molecular mechanisms that affect gene regulation and cancer emergence are still unknown. Age-related alterations in epithelial and immune cell diversity make it challenging to examine tumor microenvironment transcriptomic and molecular features in the older adult. In our study, we found that aging immune cells exhibits tumorigenic pathways. Our published and preliminary research demonstrates that IL22 cytokines have a significant role in oncogenesis in aging. We identify that IL22/mTOR signaling controls aging CRC development and that understanding the tumor immune milieu may improve colorectal cancer treatment (CRC). *Based on we hypothesized that IL22/mTOR signaling promotes the growth of age-related colon cancer and that knowing the transcriptome and molecular components of the tumor immune microenvironment might improve the therapy of CRC in the elderly.* We investigate our hypothesis in two aims **Aim 1**. The role of mTOR function in tumor permissive environment. **Aim 2.** determine if blocking of mTOR to alleviate colon cancer in older adults.

IMPACT. Based on the planned study, we will be able to design personalized therapy for advanced CRC patients.

Background

The role of mTOR in colon cancer growth. mTOR, or the mammalian target of rapamycin, is a Ser/Thr kinase associated with several cancers owing to its capacity to enhance tumor cell growth, proliferation, and survival[23-27] [28-30]. Targeting mTOR is an effective anticancer treatment for a variety of malignancies, including colon cancer[28, 31-33]. Its mechanism of action against colon cancer remains unknown, including issues of rapamycin resistance. A deeper knowledge of the mTOR pathway is necessary to overcome this limitation and create novel medications for the treatment and prevention of colon cancer.

Epithelial fucosylation promotes a tumor-friendly milieu during aging. Fucosylation is a type of post-translational modification that involves the addition of fucose residues to oligosaccharides and proteins. The modification is enabled through the catalytic activity of the enzyme known as fucosyltransferase (FUT) (4). The linkage between FUT dysfunction and an elevation in gut fucosylation, as well as the facilitation of cell proliferation, metastasis, and tumor growth, has been established. Nevertheless, a comprehensive understanding the age-related alterations that augment fucosylation of gut epithelium participate in the development of colon cancer is yet to be elucidated. The production of Interleukin-22 is predominantly attributed to the innate lymphocyte subtype 3 (ILC3), which plays a regulatory role in fucosylation(5,6). The precise correlation between the signaling of IL22/IL22RA and FUT2 and FUT8 in the epithelium of colon cancer remains unclear.

Results

Increasing age is associated with the ubiquitous fucosylation of intestinal epithelial cells. First, we examined fucosylation levels in the intestinal epithelium of human and animal aging models to determine whether aging has a role in chronic fucosylation. We used fluorescein (488 nm) tagged Ulex-Europeans Agglutinin-1 (UEA-1) and rhodamine (594 nm) labeled Wheat Agglutinin to examine gut fucosylation in normal tissue from older persons (n = 4, 2M/2F) and compare it to colon biopsies from younger individuals (n = 11, 4M/7F) using a confocal microscope (WGA). Our findings showed that the levels of UEA-1 and WGA in the colons of older people significant than those in young people.

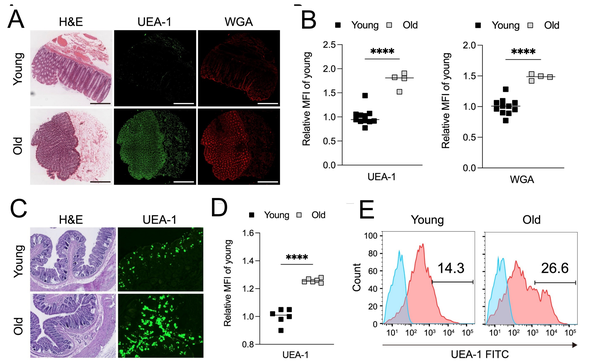

Fig-1. A: Hematoxylin and Eosin (H&E) staining (left) IHC with human UEA-1 and WGA. B: Young and old UEA-1 and WGA relative MFI. C-D: H&E and UEA-1 and UEA-1 MFI from eight-week (young) and two-year-old C57BL/6 mice colons (n=6). E: UEA-1 flow cytometry histograms in eight-week-old and two-year-old C57BL/6 mice colon.

Increased gut epithelium fucosylation is linked to risk of colon cancer in elder human. To identify if aging is linked to amplify gut epithelial fucosylation, we evaluate Tissue Microarray of young and old individual colon biopsies obtained from Biomax corporation and stained for fucosylation, *Ulex-europaeus* agglutinin-1 (UEA-1). We found 3-fold UEA staining detected in the old individuals compared to the young. On the other hand, we found 5-fold increased epithelial fucosylation in adenocarcinoma patient compared to the healthy young individual.

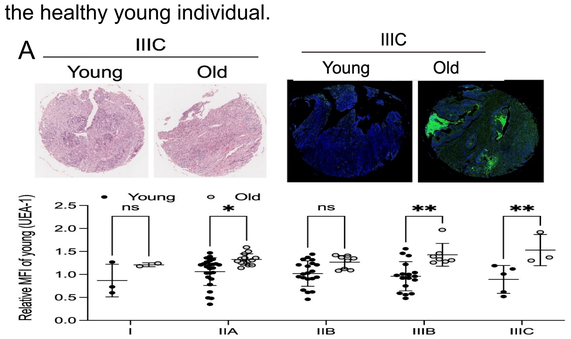

Fig2. Colon tissue microarray analysis from 106 adenocarcinoma and 2 signet ring cell tissue sections in young (n=74) and old (n=34) groups. A: H&E (top) and IHC (middle and bottom) staining of human UEA-1.. B: UEA-1 relative MFI in young cancer patients. ns, non-significant; *, 0.05; **, 0.01; ****, 0.0001 using two-way ANOVA

Results

IL22 signaling human colon cancer cell line DLD1. We next test effect of IL22 on the DLD1 cell for IL22 downstream signaling. DLD1 human colon cancer cell line were invitro cultured and treated with human recombinant IL22 with the indicated amount of IL22 for two hours. RNA isolated and qPCR done for indicated TGFβR, IL22, and p19 primers and graph plotted using graph pad software.

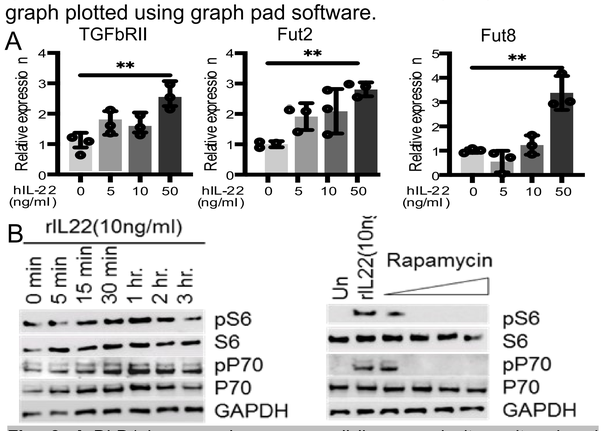

Fig 3. A DLD1 human colon cancer cell line were invitro cultured and treated with human recombinant IL22 with the indicated amount of IL22 for two hours. RNA isolated and qPCR done for indicated TGFβR, Fut2 and Fut8 expression measured by qPCR. B. pS6, pp70 , S6 and p70 and GAPDH probed to detect mTOR signaling detected by western blot

Resveratrol inhibits fucosylation genes linked with colon cancer cell migration. Resveratrol, a plant-derived polyphenol, inhibits cell death by scavenging free radicals and repairing DNA damage in healthy and cancerous cells. Our results showed that resveratrol reduced the activity of *FUT2* and *FUT8* genes in DLD1 cells. The proliferation and migration of tumor cells were substantially inhibited by resveratrol *in vitro,* which have been linked to cancer.

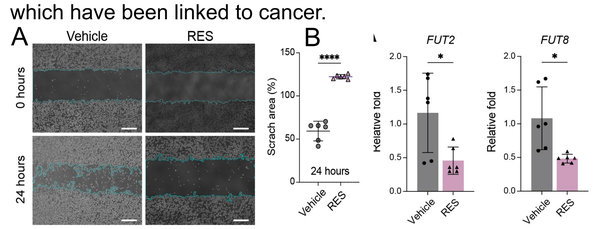

Fig 4. A. Anti-aging RES (100 mM) impairs the migration of DLD-1 cells. RB. T-qPCR analysis for *FUT2, FUT8,* in human epithelial colon carcinoma DLD-1 cells after treated with 100 mM resveratrol (RES) for 6 h.

Conclusions

- Our data supports that fucosylation increases with age and is linked to risk for colon cancer incidence.
- These findings are consistent with human data epithelium fucosylation data, suggesting the fucosylation process increased along with aging.
- Pathological role of IL22/IL22RA in aging conditions and that removing IL22RA could alleviate fucosylation.
- These findings promises a new immune-therapeutic approaches to reduce chronic gut inflammation

Methods

- **Aging colon cancer biopsies**. Histology for H&E and UEA staining comparing colon cancer in humans ranging from patients who are 26, 77 years of age, and those with adenocarcinoma.
- **Mouse colon staining**. An equal proportion of sex-match eight-week and 60-week-old mice (C57BL/6: M/F, n=8/group) colon excised and fix with 4% Paraformaldehyde and paraffin section for FITC labeled UEA and DAPI staining(6).
- **RNA isolation and qPCR.** Transcriptional analysis to detect inflammatory IL6, IL1b, TNFa, IL23, IL17, IL22, IL22RA, IL17RA, IL23R, p21, p16, FUT-2, and FUT-8 in colon RNA(6).
- **Flow cytometry analysis for UEA FITC.** Single suspension of the colon cell stained with UEA in FACS buffer at room temperature for 30 minutes. Cell acquire by BD FACS symphony, UND. Obtained data analysis Flowjo software(6).

Working Model

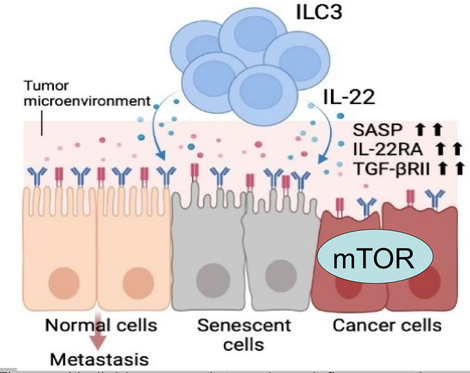

Fig5. The epithelial-immune interaction influences the tumor microenvironment. In aged epithelial cells, IL22RA and Fut2 are elevated, which indicates an increase in IL22/IL22RA-facilitated signaling and a worsening of the gut milieu favorable to benign-to-malignant cell transformation.

References

1. G. C. Leonardi, et al. Ageing: from inflammation to cancer. *Immun Ageing* ***15, 1 (2018).***
2. Terzic J, Karin M. Inflammation and colon cancer. Gastroenterology. 2010;138(6):2101-14.
3. Michaud M, et al. Proinflammatory cytokines, aging, and age-related diseases. J Am Med Dir Assoc. 2013;14(12):877-82.
4. K. Moriwaki, E. Miyoshi, Fucosylation and gastrointestinal cancer. *World J Hepatol* ***2, 151-161 (2010).***
5. Harrison, C. IL-22: linking inflammation and cancer. *Nat Rev Drug Discov* **12,** 505 (2013).
6. Mathur R, et al. Induction of autophagy in Cx3cr1(+) mononuclear cells limits IL-23/IL-22 axis-mediated intestinal fibrosis. Mucosal Immunol. 2019;12(3):612-23.

Acknowledgments

This research supported by UND DaCCOTA Feasibility award to RM