The Human Microbiome and CRC

CRC and Microbiome Introduction

Colorectal cancer (CRC) is the second most fatal and the third most common cancer. In 2020, close to two million new colorectal cancer cases were estimated and short of one million deaths[1]. The known environmental risk factors for CRC include smoking, alcoholism, obesity, sedentary lifestyle, diabetes, and the Western diet, which explain the rising incidence of CRC in developing countries. Current therapies for CRC include surgical treatments, chemotherapy, and immunotherapy. Although the overall survival of patients has increased with the help of these therapies, CRC patients still face poor prognosis, late diagnosis, and low long-term survival [2,3]. The American Cancer Society lowered the age for recommended screening to 45 years of age in 2018 to increase the likelihood of early detection in individuals at risk; however, a mass screening effort is not recommended due to the high costs of colonoscopies and poor execution of diagnostic services [1]. Therefore, it is of great importance to find and/or refine less invasive and more economical methods to detect the disease, or even better, to act as a predictive biomarker, allowing possibilities to alleviate the growing burden of the disease.

The microbiome is involved in health and disease, affecting metabolism and immune functions. Together with environmental factors, changes in the gut microbiome are potentially supporting the initiation and development of CRC. Some studies have alluded to differences and patterns in overall composition and abundance of specific microbes between healthy individuals and CRC patients, yet there are still many inconsistencies between studies, warranting further research [4–7].

There are multiple stages of CRC, beginning with the growth of colorectal adenomas. In this early stage, there is evidence that the gut microbiome can be utilized to identify those at risk. Therefore, screening and early detection could be improved using gut microbiome changes as a prediction biomarker [4,6]. Interplay between the Microbiome and the Immune System contributes to CRC

A healthy gut epithelium can develop pre-cancerous lesions, polyps, due to multiple genetic pathways and the accumulation of various mutations leading to mechanisms enabling tumorigenesis and carcinogenesis, while also compromising the immune system [7]. The mechanisms involved between the microbiota and CRC are several and distinctive. The inflammation present in CRC is a result of both the microbiota and the immune system, whose intersection at the epithelial barrier of the colon can enable the development of bowel tumorigenesis [7,8]. The colon epithelium can undergo mutagenesis due to exposure to bacterial toxins damaging DNA. Epithelial proliferation can occur by mimicking of ligands, contributing to neoplasia [8]. The modulation of the immune system by the microbiota triggers immune responses and activating cells through immunomodulatory factors. These circumstances can affect the population, distribution and function of immune cell populations in the epithelium and underlying stroma, creating favorable microenvironments for tumor growth and promoting malignancy [8]. Important to note is that the communication between the innate immune system and the microbiome is extensive and bi-directional [9]; therefore, there could be domino effects due to changes in the homeostasis of either.

Another intriguing realization is that the induction of immunophenotypes and immunomodulatory functions do not correlate with microbial phylogeny [10–12]. Researching this discrepancy further by

analyzing gut microbes on the strain level could contribute to answering some of the questions relating to contradictions relating to composition and abundance of species in the gut microbiome that are often found. Especially since different strains of the same species can affect the host immune system in distinct and strikingly different ways. [10].

Changes throughout CRC development

Microbiome changes

Alterations in the microbiome in the presence of colorectal adenomas and CRC compared to healthy mucosa have been consistently found [13]. The question of whether these changes cause or are a result of the cancerous growth is still being debated [4]. Nevertheless, once defined decisively, such microbiome alterations are perfect candidates for early, non-invasive diagnosis [14,15] . The changes in the gut microbiome related to CRC are characterized by higher species richness, lower abundance of taxa known for their protective effects, increased abundance of taxa which have carcinogenic potential (**Error! Reference source not found.** below), and colonic biofilms [15,16].

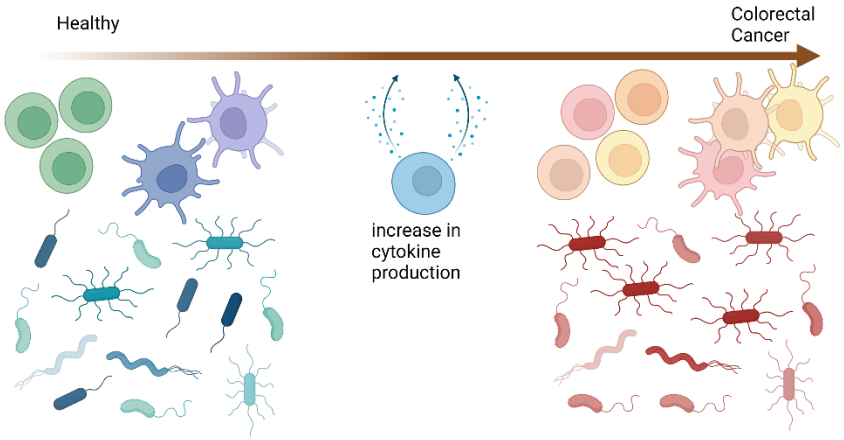

Figure 1: Immune environment changes, increases in pro-inflammatory cytokines, microbiome changes as CRC progresses in patients [4] Created with BioRender.com

The bacteria, *Fusobacterium nucleatum*, is related to CRC through multiple pathways and throughout the disease's progression, metastasis, and treatment resistance [13,17]. This bacteria can be measured in tumor tissue and fecal samples and has been suggested as a potential marker for CRC [15,18]. Intriguingly, *Fusobacterium nucleatum* is an oral bacteria species which translocates to the gut and colon in certain circumstances, making it an excellent example of patient physiological changes which lead to microbiome changes in composition and behavior and eventually affect colon cancer progression and aggressiveness. Another example is the strain *Escherichia coli* pks+ which synthesizes a genotoxin, colibactin, known for causing DNA damage that elevates the risk of CRC[18]. This *E. coli* was hence shown to deteriorate colorectal carcinoma.

Immune-tumor microenvironment alterations

The dissolution of the mucosal barrier by bacterial invasion gives bacteria and their metabolites direct access to the tissue resulting in the recruitment and functional changes of the innate and adaptive

immune cells (**Error! Reference source not found.**). The subsequent inflammation induces pro-inflammatory cytokines, reactive oxygen, and nitrogen species. Within the polyps the immune response is dampened with a continuous reduction of mature dendritic cells as the adenomas become cancerous [7]. The changes in cell-type distribution are still being researched, and clear answers are still not available for human polyps, but defining the immune microenvironment in early CRC development could be a crucial step [4].

Microbiome involvement in CRC development

CRC carcinogenesis is promoted through interactions of the gut microbiome and immune response [7]. An imbalance of the gut microbiome, dysbiosis, can disintegrate the mucus layer protecting the colonic epithelial barrier, creating an entry and direct pathway for pathogenic bacteria and their secretions to the underlying tissue. This invasion of bacteria stimulates immune cell-mediated responses increasing pro-inflammatory cytokines [7,19]. Altogether, within the tumor microenvironment, the bacteria are classified as drivers, directly carcinogenic, or passenger, opportunistic bacteria [15].

A factor connecting the gut microbiota and CRC risk is microbial metabolites. These small molecules result from the metabolism of dietary and host-derived compounds and can influence the population dynamics in the gut and host cells with a range of effects through various pathways, including carcinogenesis. Therefore, dysbiosis changes the metabolite homeostasis, which has been shown to cause the onset and progression of CRC [18]. The most influential metabolites are short-chain fatty acids (SCFAs), bile acids (BAs) and Tryptophan [3,18].

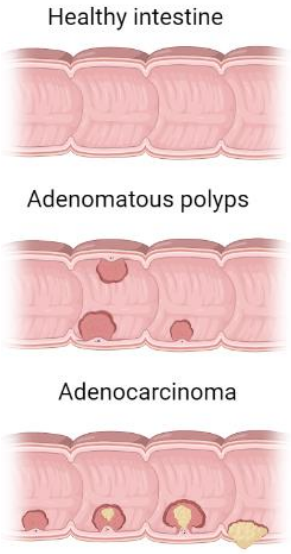

Figure 2: Stages of development from a healthy intestine to polyps to colorectal carcinogenesis.

Understanding the development of polyps and the importance of surveilling them and their microenvironment could increase the prospects of earlier diagnoses and treatment targets [7].

Solutions and challenges between Microbiome and CRC

There are exciting ideas and new research on harnessing the relationship of CRC with the microbiome, both before the carcinogenesis cascade fully commences and after CRC is well established (Figure 2).

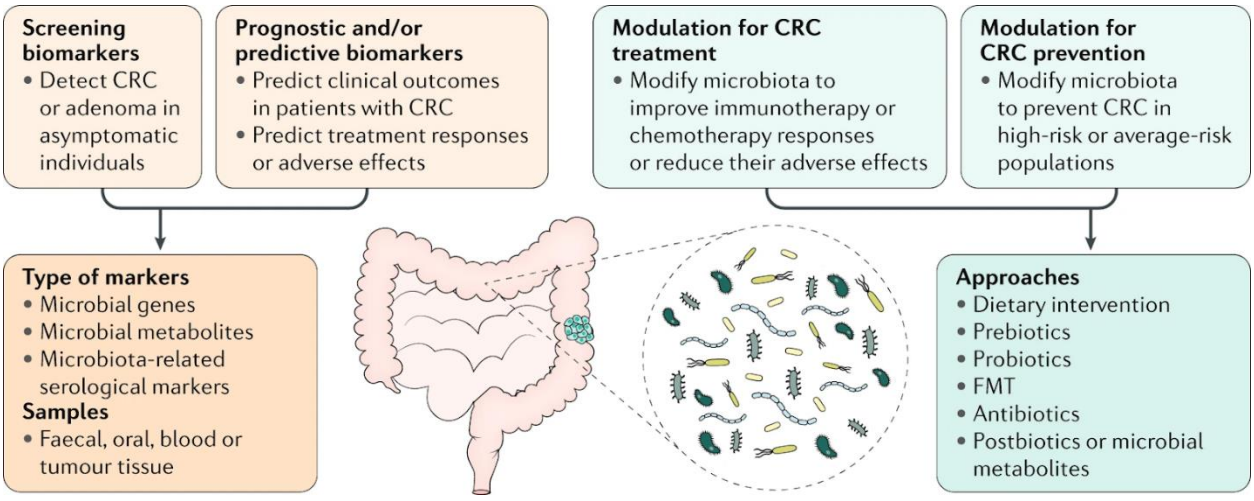

Figure 3: Potential clinical applications for the use of the gut microbiome in CRC, including screening and predictive biomarkers, as well as microbiota modulation for prevention and treatment [15].

The most evident challenges with these solutions are the lack of optimal testing and implementation regimens and the uncertainty regarding sensitivity and specificity [15,20]. Novel approaches to microbiome based computational analysis are necessary to decipher the microbiota-related diagnosis factors (whether microbial strains or functional attributes) and their role in the various phases of CRC. Important potential sources of innovation in this area are the fields of Artificial Intelligence (AI), Deep Learning (DL) and metagenomics analyses. Such approaches must be validated comprehensively using prospective clinical trials, like the ones performed by BiotaX.

References

1. Sung H, Ferlay J, Siegel RL, *et al.* Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. CA Cancer J Clin 2021;**71**(3):209–49.
2. Li S, Liu J, Zheng X, *et al.* Tumorigenic bacteria in colorectal cancer: mechanisms and treatments. Cancer Biol Med 2022;**19**(2):147.
3. Stott KJ, Phillips B, Parry L, May S. Recent advancements in the exploitation of the gut microbiome in the diagnosis and treatment of colorectal cancer. Biosci Rep 2021;**41**(7):20204113.
4. Song M, Chan AT, Sun J. Influence of the Gut Microbiome, Diet, and Environment on Risk of Colorectal Cancer. Gastroenterology 2020;**158**(2):322–40.
5. Feng Q, Liang S, Jia H, *et al.* Gut microbiome development along the colorectal adenoma–carcinoma sequence. Nat Commun 2015 61 2015;**6**(1):1–13.
6. Dai Z, Coker OO, Nakatsu G, *et al.* Multi-cohort analysis of colorectal cancer metagenome identified altered bacteria across populations and universal bacterial markers. Microbiome 2018;**6**(1):70.
7. Tse BCY, Welham Z, Engel AF, Molloy MP. Genomic, Microbial and Immunological Microenvironment of Colorectal Polyps. Cancers 2021, Vol 13, Page 3382 2021;**13**(14):3382.
8. Hanahan D. Hallmarks of Cancer: New Dimensions. Cancer Discov 2022;**12**(1):31–46.
9. Zheng D, Liwinski T, Elinav E. Interaction between microbiota and immunity in health and disease. Cell Res 2020;**30**(6):492–506.
10. Hajjo H, Geva-Zatorsky N. Strain-level immunomodulatory variation of gut bacteria. FEBS Lett 2021;**595**(9):1322–7.
11. Teh JJ, Berendsen EM, Hoedt EC, *et al.* Novel strain-level resolution of Crohn's disease mucosa-associated microbiota via an ex vivo combination of microbe culture and metagenomic sequencing. ISME J 2021 1511 2021;**15**(11):3326–38.
12. Yang C, Mogno I, Contijoch EJ, *et al.* Fecal IgA Levels Are Determined by Strain-Level Differences in Bacteroides ovatus and Are Modifiable by Gut Microbiota Manipulation. Cell Host Microbe 2020;**27**(3):467-475.e6.

13. de Vos WM, Tilg H, Van Hul M, Cani PD. Gut microbiome and health: mechanistic insights. Gut 2022;**71**(5):1020–32.

14. Xie YH, Gao QY, Cai GX, *et al.* Fecal Clostridium symbiosum for Noninvasive Detection of Early and Advanced Colorectal Cancer: Test and Validation Studies. EBioMedicine 2017;**25**:32–40.

15. Wong SH, Yu J. Gut microbiota in colorectal cancer: mechanisms of action and clinical applications. Nat Rev Gastroenterol Hepatol 2019 1611 2019;**16**(11):690–704.

16. Drewes JL, White JR, Dejea CM, *et al.* High-resolution bacterial 16S rRNA gene profile meta-analysis and biofilm status reveal common colorectal cancer consortia. Npj Biofilms Microbiomes 2017 31 2017;**3**(1):1–12.

17. Engevik MA, Danhof HA, Ruan W, *et al.* Fusobacterium nucleatum secretes outer membrane vesicles and promotes intestinal inflammation. MBio 2021;**12**(2):1–17.

18. Li J, Zhang A, Wu F, Wang X. Alterations in the Gut Microbiota and Their Metabolites in Colorectal Cancer: Recent Progress and Future Prospects. Front Oncol 2022;**12**:285.

19. Candela M, Turroni S, Biagi E, *et al.* Inflammation and colorectal cancer, when microbiota-host mutualism breaks. World J Gastroenterol 2014;**20**(4):908.

20. DeDecker L, Coppedge B, Avelar-Barragan J, Karnes W, Whiteson K. Microbiome distinctions between the CRC carcinogenic pathways. Https://DoiOrg/101080/1949097620201854641 2021;**13**(1).