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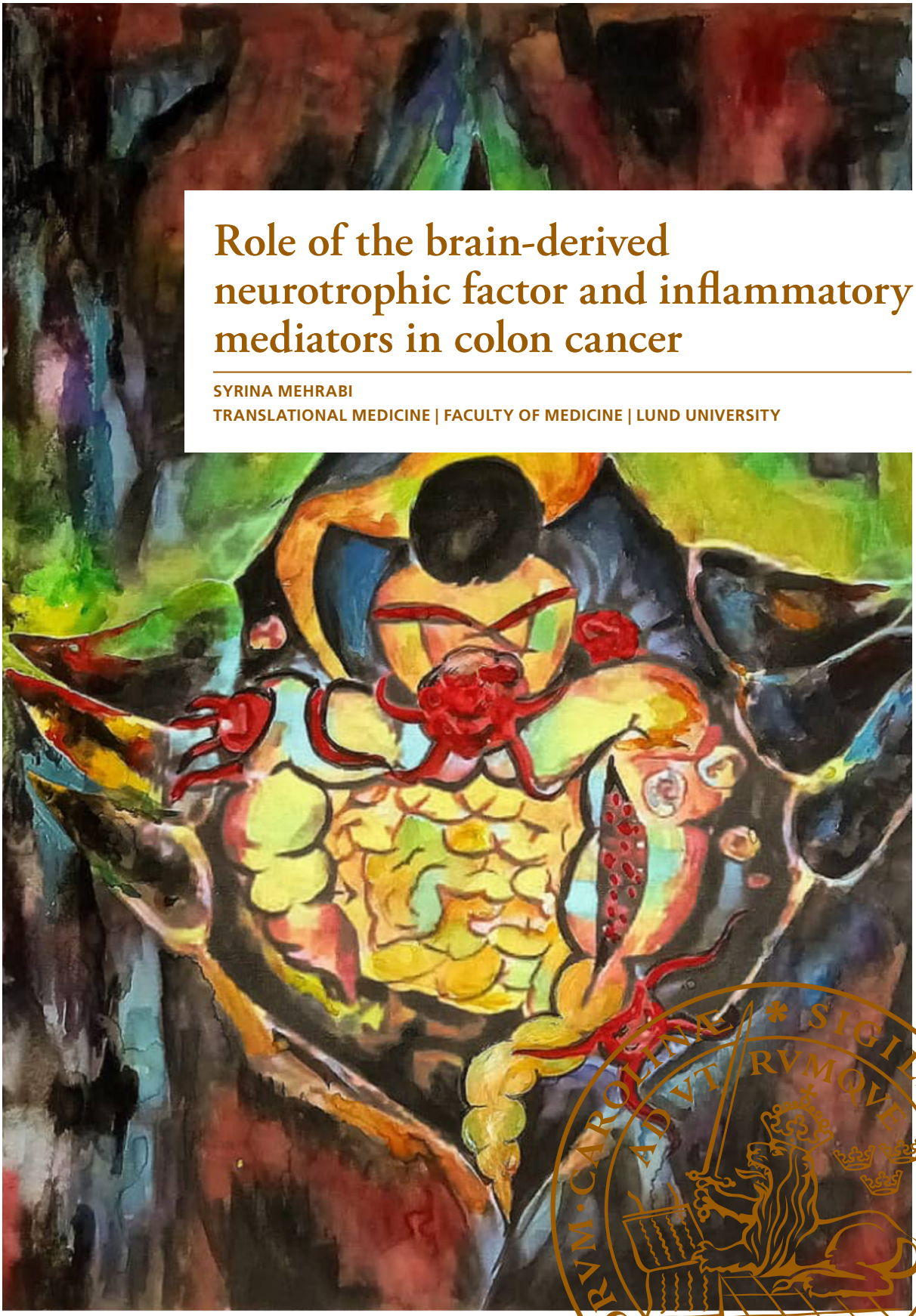

Role of the brain-derived neurotrophic factor and inflammatory mediators in colon cancer

SYRINA MEHRABI
TRANSLATIONAL MEDICINE | FACULTY OF MEDICINE | LUND UNIVERSITY

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About the author

Syrina Fred Mehrabi received a Master of Arts from Rome University of Fine Arts in 2003 and a Master of Pharmacy from Uppsala University in 2014. Syrina conducts her Ph.D. at the Department of Translational Medicine at Lund University. Her research focuses mainly on the tumor microenvironment, brain-derived neurotrophic factor, and inflammatory mediators, to find new prognostics and diagnostics biomarkers for early detection of colon cancer.

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UNIVERSITY

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Abstract

CRC is one of the most common causes of cancer death in the world. If CRC is diagnosed in the early stages, the patient's chance of survival is estimated at about 90%, with a very low probability of recurrence. The five-year DFS for CRC patients in stage III and IV drops to 63.3% and 18.9%, respectively. The current thesis aimed to identify potential new prognostic and predicting biomarkers for the detection of patients in the early stages of colon cancer.

In particular, this thesis:

1) studies the role of BDNF expression in stage II and III CC. By examining human biopsies, in vivo, and in vitro experiments, this study, to the best of our knowledge, for the first time shows a positive correlation between expression of CD66b, $CysLT_1R$, and BDNF. This study suggests BDNF, alone or in combination with other known molecular markers, as a good candidate to be prognostic and predictive biomarker for early detection of CC.

2) consider the problem of therapeutic resistance in CC patients and investigate the role of intermittent p-TrkB expression between the cytoplasm and nucleus. The results show that the cytoplasmic expression of TrkB and nuclear expression of $CysLT_1R$ are associated with poorer survival in CC patients. We also highlight the importance of further investigations of the role of TrkB expression in CC development

3) identifies a five-gene signature as a primary prognostic and diagnostic biomarker, in 5 independent published datasets for CRC patients in-silico. In all datasets, four tumorigenic genes (BDNF, PTGS2, GSK3B, and CTNNB1) are shown to be significantly upregulated and one tumor suppressor gene (HPGD) was significantly downregulated. The four suppressive tumorigenic genes were studied in the plasma of CRC patients to evaluate the diagnostic value in the disease. The results of this study showed that the suggested five-panel gene signature, with high accuracy, can be a promising indicator for predicting OS and RFS in patients with CRC. In addition, this study suggested the four highly sensitive tumor suppressor genes as potential diagnostic markers for CRC patients.

4) investigates altered expression of estrogen receptor beta (ERβ) in CRC. High expression of ERβ is shown to correlate with antitumorigenic activity, and a higher level of $CysLT_2R$, membrane β-catenin, and 15-hydroxy prostaglandin dehydrogenase (15-PGDH) expression, as well as lower levels of $CysLT_1R$, COX-2, and nuclear β-catenin. The current study confirmed the antitumor role of ERb-041 (an ERβ agonist), which corresponded to an inferior ability of the tumor cells to migrate, colonize and survive, including an increased apoptosis.

Key words: Colon cancer, neutrophils, $CysLT_1R$, BDNF, TrkB, tumor microenvironment, prognostic, predictive, diagnostic, gene signature

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To my sons Samuel Romeo and Benjamin Rafael

"The greatest obstacle to discovery is not ignorance, it is the illusion of knowledge."

Daniel J. Boorstin

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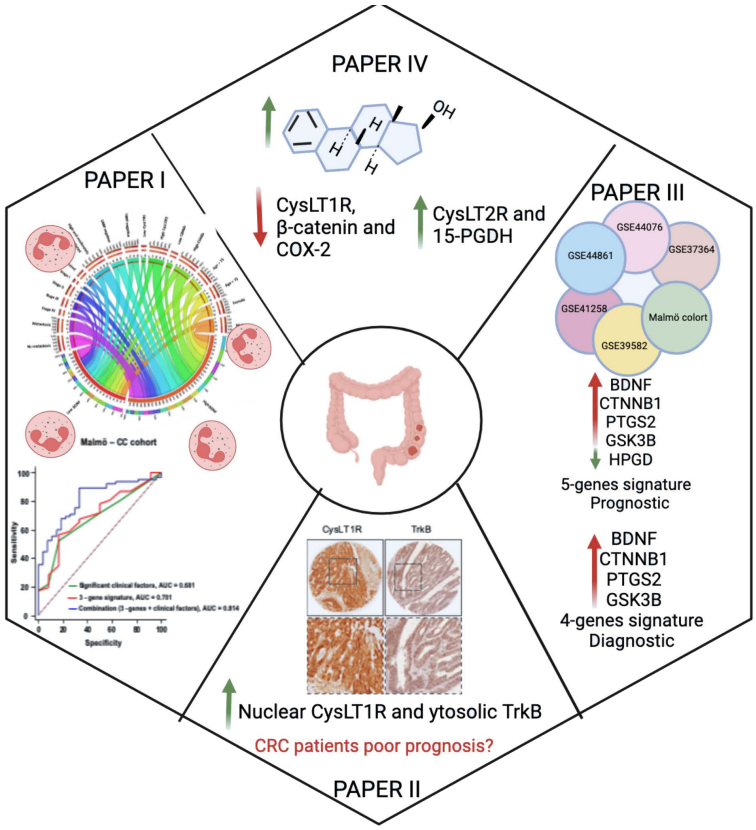
Brief Definition of Research topics



Colorectal cancer (CRC) is one of the most common cancer-related causes of death worldwide[1]. In the early stages of the disease, surgery and tumor resection are the primary and preferred methods of treatment. However, the success of these methods relies entirely on the time of diagnosis[2]. Colonoscopy programs are known to be the most efficient method for detecting this type of cancer, however, socioeconomic factors and the invasiveness of this method in nature are two setbacks for patients to use it[3]. At the time of diagnosis, around one-third of patients are in the stage of lymph node involvement (stage III) and one-quarter of them are in the stage of cancer cells invading adjacent tissues without lymph node involvement (stage II)[3]. Survival in CRC depends on the stage of the disease[4]. According to American Cancer Society (ACS), year 2020 the five-year disease-free survival (DFS) for CRC patients in stage I was about 90%, with a very low probability of recurrence. DFS was approximately 79.5% for stage II CRC patients and 63.3% and 18.9% for stage III and IV patients, respectively [5].

Treatment strategies for each of these stages differ from each other. For example, there is no evidence that chemotherapy can provide better treatment in stage II of the disease, while it is one of the most effective adjuvant therapies for patients with stage III colon cancer (CC)[6]. The overall aim of the current thesis is to identify potential new prognostic and predictable biomarkers for the detection of patients in the early stages of CC. More specifically, this thesis is going to explore whether the expression of brain-derived neurotrophic factor (BDNF) in stage II and III CC, alone or in combination with other known molecular marker, can be a good candidate as a prognostic/predictive biomarker for early detection of CRC. The secondary goal of the thesis is to investigate the role of estrogen receptors in CC and study its association with inflammatory features in order to assess the prognostic value of this ERß in women.

Paper I. Brain-Derived Neurotrophic Factor (BDNF) is highly expressed in some solid tumors including CRC[7]. In this study, neutrophil-derived BDNF expression was examined in a CC cohort with 72 patients in stage II and III of the disease. High BDNF and CD66b (a neutrophilic marker) expression were associated with shorter overall survival (OS). Mice lacking the expression of the Cysteinyl Leukotriene Receptor 1 ($CysLT_1R$- Leukotriene D4 (LTD4) receptor) had lower expression of BDNF. Furthermore, in a xenograft mouse model with human CC cells (SW480) with or without treatment with the $CysLT_1R$ antagonist showed a decrease in BDNF expression.

Brief summary: There was a positive correlation between CD66b, BDNF and $CysLT_1R$ expression and mice lacking the $CysLT_1R$ had a lower expression level compared to wild-type mice.

Paper II. (Manuscript), The clinical success of targeted therapy of CC patients is often limited by treatment resistance. BDNF and its receptor tropomyosin receptor kinase B (TrkB) have recently emerged as an anticancer target. In a CC cohort with 46 patients in stages II and III of the disease, the expression of TrkB are higher in the normal colon mucosa compared to its matched tumor tissues. However, the TrkB expression levels had no significant value in the overall survival of these patients. But the correlation between TrkB cytoplasmic expression and the nuclear $CysLT_1R$ expression was significant in the TCGA database with 259 patients. SW480 CC cells treated with recombinant BDNF showed accumulation of phosphorylated TrkB (p-TrkB) expression in the cell nucleus as opposite to SW480 cells treated with LTD4 where the p-TrkB expression was higher in the cytoplasm.

Brief summary: The expression of p-TrkB alternate between the cytoplasm or nucleus depending on the type of stimuli and its receptor.

Paper III. (Manuscript), The prognosis for CC is affected by various features found at the initial diagnosis. In this study, we identified a five-panel gene signature as a prognostic and early diagnostic biomarker in 5 independent in-silico CRCs cohorts, and the results were independently validated in one clinical cohort. In all the data sets, four tumorigenesis genes (BDNF, PTGS2, GSK3B, and CTNNB1) were significantly upregulated and one tumor suppressor gene (HPGD) was significantly down-regulated.

To evaluate the diagnostic value of the selected gene signatures, we used plasma samples from 19 CC patients and 9 matched healthy individuals as references.

Brief summary: This five-gene signature with good accuracy predicted overall survival (OS) and recurrent survival (RFS) in patients with CC. The four upregulated genes were shown, with high sensitivity, and proved to be promising diagnostic markers for CC patients.

Paper IV. Altered expression of estrogen receptor beta (ERβ) has been implicated in CRC. In this study, we showed that patients with high expression of ERβ had higher levels of the $CysLT_2R$, membrane β-catenin, and 15-Hydroxyprostaglandin dehydrogenase (15-PGDH), all of which have an antitumor effect and played a significant role in the survival for patients with CRC. In vitro data with CC cells treated with ERb-041 (an ERβ agonist) showed a lower ability to migrate, colonize, survive, and had higher apoptosis levels. In vivo, in a zebrafish xenograft model with human CC cells treated with ERb-041, tail metastases were lower compared to the control group.

Brief summary: ERβ was shown to have an antitumor effect on CRC, and the agonist ERb-041 may be useful in the treatment of CRC patients.

Keywords: CC, neutrophils, CysLT1R, BDNF, TrkB, tumor microenvironment, prognostic, predictive, diagnostic, gene signature

List of papers

Paper 1: Brain-Derived Neurotrophic Factor, Neutrophils and Cysteinyl Leukotriene Receptor 1 as Potential Prognostic Biomarkers for Patients with CC. Syrina F. Mehrabi, Souvik Ghatak, Lubna M. Mehdawi, Geriolda Topi, Shakti Ranjan Satapathy, Anita Sjölander. Cancers. DOI:10 3390/cancers13215520.

Paper 2: Cytosolic expression of the brain-derived neurotrophic factor receptor TrkB and nuclear cysteinyl leukotriene receptor 1 goes with poor prognosis for colon cancer patients. Syrina F Mehrabi, Souvik Ghatak, Lubna Mehdawi, Anita Sjölander, Shakti R Satapathy. (Manuscript)

Paper 3: Identification of a novel five-gene signature as a prognostic and diagnostic biomarker in colorectal cancers. Souvik Ghatak, Syrina F Mehrabi, Lubna M Mehdawi, Shakti Ranjan Satapathy, Anita Sjölander. (Manuscript)

Paper 4: Tumor-suppressive effect of oestrogen receptor β in colorectal cancer patients, CC cells, and a zebrafish model. Geriolda Topi, Shakti Ranjan Satapathy, Pujarini Dash, Syrina Fred Mehrabi, Roy Ehrnström, Roger Olsson, Marie-Louise Lydrup, Anita Sjölander. J Pathology DOI: 10.1002/path.5453.

List of Abbreviations

15-PGDH	15-Hydroxyprostaglandin Dehydrogenase
5-LOX	5-Lipooxygenase
AA	Arachidonic Acid
ACS	American Cancer Society
AJCC	American Joint Committee on Cancer
AOM	Azoxymethane
APC	Adenomatous Polyposis Coli
AUC	Area Under Curve
BDNF	Brain-Derived Neurotrophic Factor
CAC	Colitis-Associated Colon Cancer
cAMP	cyclic Adenosine Monophosphate
CC	Colon cancer
CD	Crohn's Disease
CEA	Carcinoembryonic Antigen
CIC	Cancer Initiating Cell
CMS	Consensus Molecular Subtypes
COX-2	Cyclooxygenase
CRC	Colorectal Cancer
CSC	Cancer Stem Cells
CTC	Circulating Tumor Cells
CTNNB1	Catenin Beta1
$CysLT_1R$	Cysteinyl Leukotriene Receptor 1
$CysLT_2R$	Cysteinyl Leukotriene Receptor 2
DSS	Dextran Sulfate Sodium
Erα	Estrogen Receptor alpha

ERβ	Estrogen Receptor beta
FAP	Familial Adenoma Polyposis
FLAP	Five Lipoxygenase Activating Protein
GDP	Guanosine Diphosphate,
GPCR	G-protein Couple Receptor
GSK3b	Glycogen Synthase Kinase 3
GTP	Guanosine Triphosphate
HNPCC	Lynch syndrome (Hereditary Nonpolyposis Colorectal Cancer
HPGD	15-Hydroxyprostaglandin Dehydrogenase
IBD	Inflammatory Bowel Diseases
IHC	Immunohistochemistry
IL	Interleukin
ISC	Intestinal multipotent progenitor or stem cells
LTD4	leukotriene D4
MAPK	Mitogen-Activated Protein Kinase
miRNAs	microRNAs
MK	Montelukast
MMR	Mismatch repair
MSH	DNA mismatch repair protein
MSI	microsatellite instability
MUC2	Mucin 2
NK	Natural Killer
NSAID	Nonsteroidal Anti-Inflammatory Drug
OS	Overall Survival
PBS	Phosphor Buffer Saline
PI3K	Phosphoinositide 3-Kinases
PTGS2	Prostaglandin-Endoperoxide Synthase 2
PTK	Protein Kinase C
pTrkB	phosphorylated Tropomyosin Kinase B
RFS	Recurrence Free Survival

TAM	Tumor Associated Macrophages
TAN	Tumor Associated Neutrophils
TCGA	The Cancer Genome Atlas
TGFß	Transforming Growth Factor ß
TMA	Tissue Microarray
TME	Tumor Microenvironment
TNFα	Tumor Necrosis Factor alpha
TNM	Tumor Node Metastasis
TP53	Tumor Protein P53
TrkB	Tropomyosin Receptor Kinase B
TSG	Tumor Suppressor Gene
UC	Ulcerative Colitis
WHO	World Health Organization
Wnt	Wingless-type mammary tumor virus integration site

My thesis discusses colorectal cancer in general; however, my research projects focus specifically on the subject of colon cancer.

Populärvetenskaplig Sammanfattning

Cancer är en icke-smittsam sjukdom som är den näst vanligaste dödsorsaken både i världen (17 %) och i Sverige (26 %). Med cirka 1,2 miljoner fall per år är tjocktarmscancer den fjärde vanligaste cancertypen i världen. Sjukdomen kan uppstå i alla åldrar men är vanligare hos personer över 50 år. Tillväxten av tjocktarmscancer är mycket långsam och den onormala vävnadstillväxten, polypen, blir gradvis malign. Det tar ungefär tio år från att en polyp debuterar till dess den har utvecklats till en malign tumör. Tidig upptäckt av denna sjukdom kan dramatiskt öka chanserna för att tillfriskna. Det största problemet med tjocktarmscancer är sen diagnos, ofta när sjukdomen har spridit sig till andra delar av kroppen och orsakat så kallade metastaser. Sjukdoms fri överlevnad är cirka 80 % för stadium II tjocktarm cancer (begränsad spridning av tumören till tarmväggen) och cirka 63 % för stadium III (tumörspridning till intilliggande lymfkörtlar) och 19% för stadium IV tjocktarmscancer (tumör som sträcker sig till omgivande lymfkörtlar). Risken för återfall beror ofta på hur tumören har spridit sig vid diagnos. Det övergripande målet med denna avhandling är att undersöka de prognostiska och potentiella riskindikatorer som kan förutsäga risken för återfall av tjocktarmscancer och svar på behandling. Studier har visat att förekomsten av inflammation i tumörens mikromiljö är förknippad med en dålig klinisk prognos. En neurotrofisk faktor, BDNF, är en nervtillväxtfaktor som finns i centrala nervsystemet, men även i många olika cancerformer, inklusive tjocktarmscancer.

I delarbete I undersöktes uttrycket av BDNF i tjocktarmscancer och resultaten visade att BDNF frisätts från neutrofiler i närvaro av cystinyl receptor 1 ($CyslT_1R$) och dess ligand LTD_4, ett ämne som frisätts vid inflammation. Med en kohortstudie av 72 patienter med tjocktarmscancer visades att höga nivåer av CD66b (en neutrofil markör), BDNF och LTD_4 är korrelerat med sämre överlevnad hos koloncancer patienter jämfört med de som har lägre uttryck av dessa proteiner. Detta fynd utvärderades i djurmodeller (in vivo), laboratoriestudier (in vitro), samt publicerade data från en tumörcancer genomisk atlas (TCGA), och alla resultat bekräftade våra fynd.

I delarbete II (pågående studie) undersöks effekten av BDNF receptorn TrkB på tjocktarmscancerprogression. Resultat från tumörvävnadsprover för 46 tjocktarmscancer patienter i stadier II och III visade högre uttryck av TrkB jämför med den normala tjocktarmsslemhinnan från samma patienter. TrkB-uttrycksnivåerna hade dock ingen betydelse för den totala överlevnaden hos de patienterna. Tidigare forskning från vår grupp indikerade på en sämre överlevnad för tjocktarmscancer patienter med högt uttrycket av $CysLT_1R$ i cellkärnan. mRNA analyser från 259 tjocktarmscancer patienter tillhörande ett TCGA data set visade en positiv korrelation mellan TrkB cytoplasmatiskt uttryck och det nukleära $CysLT_1R$-uttrycket. Resultaten från en in vitro

studie avslöjade ackumulering av aktiverad TrkB (fosfor-TrkB) receptor i cellkärnan när tjocktarmscancer celler behandlades med BDNF. Däremot ackumulerades aktiverad TrkB i cytoplasman när tjocktarmscancer celler behandlade med LTD_4. I nästa steg undersöks lokaliseringen av fosfor-TrkB uttryck i tjocktarmscancer vid olika stimuli och effekten det har på utvecklingen av tjocktarmscancer.

I delarbete III (manuskript) bestämdes en panel med en 5-gens signatur som prognostisk (kliniska eller biologiska faktorer som indikerar möjligheten till sjukdomsprogression och resultat) och diagnostisk (kliniska eller biologiska faktorer som möjliggör tidig upptäckt eller bekräftelse av cancern på ett icke-invasivt sätt eller identifiering av individer med en subtypen av sjukdom) biomarkör för patienter med tjocktarmscancer. Analyser av 5 oberoende publicerade tjocktarmscancer kohorter visade en signifikant uppreglering av fyra tumörstimulerande gener (*BDNF, PTGS2, GSK3B och CTNNB1*) och signifikant nedreglering av en tumörsuppresiv gen (*HPGD*). Den framtagna 5-gens signaturen bekräftades även som en prediktiv biomarkör för överlevnad och tillfrisknande utan återfall hos patienter med stadium I-III tjocktarmscancer. De fyra cancerstimulerande generna visade signifikant uppreglering i plasmaprover från 19 tjocktarmscancer patienter jämförd med 9 oberoende friska individer. Studien föreslår BDNF som en värdefull blodbaserad biomarkör, antingen ensam eller i kombination med andra cancermarkörgener, för screening för tjocktarmscancer.

I delarbete IV undersöks effekten av östrogenreceptor-beta (ERβ) i tjocktarmscancer. Denna studie visade att patienter med högt ERβ-uttryck hade högre nivåer av CysLT2R, membran β-catenin och 15-hydroxiprostaglandindehydrogenas (15-PGDH), som alla har tumör suppressiva effekter och förbättrar överlevnaden hos patienter med tjocktarmscancer. I in vitrostudier på tjocktarmscancerceller behandlades dessa med ERb-041 (en ERβ-agonist), som visade minskad förmåga hos tumören att migrera, kolonisera och överleva samt högre nivåer av tumörcellernas apoptos. In vivo experiment där djur behandlades med ERb-041 visade färre metastaser av tjocktarmstumörceller jämfört med kontrollgruppen. Arbetet föreslog ERβ och dess agonist ERb-041 som målterapi för behandling av CRC patienter

History of Cancer

Cancer is the second most popular cause of mortality in the world after cardiovascular disease[8] Today, having made earlier detection possible and access to better treatment technologies for the disease have enabled millions of people with cancer to live longer[9]. Cancer is a term that refers to a pathological disorder which is characterized by abnormal growth proliferation of cells in the body that can penetrate other organs and tissues of the body and change their structure and function[10]. But cancer is not a new disease. The Greek physician Hippocrates (470-370 BC) was the first who used the terms carcinos and carcinoma to describe non-ulcer forming and ulcer-forming tumors[11]. The name carcinoma was retrieved from the incision surface of a solid malignant tumor with branching veins around it that gave it a crab-like appearance[11]. The oldest documented case of cancer in ancient Egypt dates back to 1500 BC. In these descriptions written on papyrus, eight samples of breast ulcers have been mentioned as being treated by burning (cauterization) with a tool called a fire drill. "There is no cure," the author wrote of the disease[12].

Cancer refers to a group of diseases that have a common general phenotype which is defined as uncontrolled cell growth and proliferation[13]. Cancer is a genetic and multifactorial disorder that is usually caused by mutations in the DNA. All carcinogens alter DNA sequences, leading to mutations and the possibility of cell proliferation, preventing differentiation and escaping cell death[14]. As cancer cells proliferate, they ultimately form tumors and when the size of cancerous tumors increases, the process of angiogenesis is stimulated, during which the tumors acquire a new source of blood vessels and thus nutrition and oxygen. Finally, tumor cells target surrounding tissues as metastases[15].

Cancer-related gene mutations can be passed from parents to progenies, or a person can acquire them through somatic mutations. Approximately 85% of cancers occur in epithelial cells and are categorized as carcinomas that cover the internal organs and outer surfaces of the body. Cancers that develop in mesenchymal cells, such as bone or muscle, are called sarcomas[16]. Cancers of different origins have different characteristics, and the molecular mechanisms involved in the carcinogenesis of each cell type and the pattern of cell expansion from the original site are determined by the type of cancer[17]. In 2000, in a review by Hanahan and Weinberg, six hallmarks of most (but not all) cancers have been introduced (Figure 1). They suggested that for normal cells to become cancerous, they must acquire characteristics including: “1) the ability to divide in the absence of growth factor stimulation, 2) the ability to divide in the presence of anti-growth signals, 3) inability to apoptosis, 4) ability to maintain telomere length despite repeated cell division, 5) stimulation of angiogenesis, and 6) ability to attack

surrounding tissues and metastasize to other parts of the body[10]. Later, genomic instability and tumor-stimulating inflammation, which causes genetic diversity to accelerate their uptake and inflammation, were added to this list"[10]. In the background of all these characteristics, there is genomic instability and tumor-inducing inflammation, which leads to genetic diversity and distinct function in different types of cancer. Subsequently, two other important features of cancer were discovered and added to the list: reprogramming of energy metabolism and prevention of immune deficiency in the tumor. As tumors grow, they develop a complete cellular environment in and around themselves. This "tumor microenvironment" is composed of many different types of cells, including immune cells such as T lymphocytes and neutrophils[18]. Understanding the response of the tumor microenvironment to therapy is crucial to its treatment, especially when the tumor microenvironment inhibits the anti-tumor effects of the cellular immune system.

Despite the long history of cancer, no certain cure has yet been found for the disease. However, different treatments have been used over time and a few of them have been successful, but the effectiveness of these treatments depend a lot on the time of cancer diagnosis and are often successful when the cancer is in its early stages[19].

More insight into the nature of this disease along with considerations of personal genetic characteristics of patients and not only based on the stages of cancer they are at, will open new horizons in the field of cancer treatment.

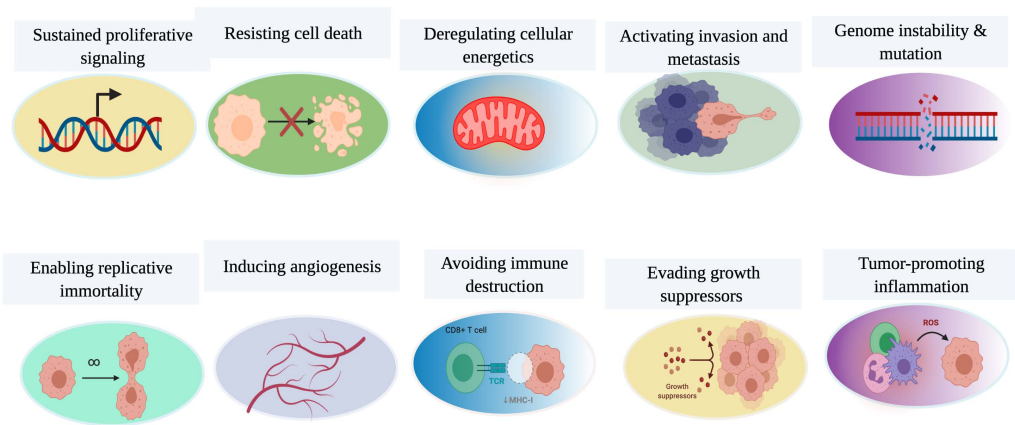


Figure 1. Hallmark of cancer, adapted from Hanahan and Weinberg, 2011[10]. Enabling characteristic (purple); Emerging hallmarks (blue).

Introduction

In 2019, the number of people diagnosed with CRC in Sweden was estimated at approximately 5000, and CRC caused over twenty-seven percent of all deaths in this country[20]. The five-year survival rate for CC, according to statistical research, is 68% in women and 64% percent in men, whereas this number for rectal cancer is 66% for both sexes[21]. In most cases, CRC starts as a small, noncancerous (benign) cell mass (called a polyp) on the inside of the colon or rectum. Polyps have different characteristics and are usually classified into adenomatous polyps (adenomas), sessile serrated polyps (SSPs), and traditional toothed adenomas (TSAs) or hyperplastic and inflammatory polyps. Over time, some adenomatous polyps can develop into CRC[22]. Inflammatory bowel disease (IBD) can cause CRC. Patients with ulcerative colitis and chronic Crohn's disease are at higher risk for CRC[23]. Inflammation may also be associated with other types of sporadic and hereditary bowel cancer[24]. However, little is known about the molecular mechanisms underlying the effect of inflammation on CRC.

CRC is a silent disease that takes years or even decades to develop, during which there are almost no specific symptoms, such as bleeding or pain, until diagnosis of metastasis. It usually takes approximately seven to ten years for an adenoma to develop into carcinoma[25]. Immune cells, cytokines, and other inflammatory mediators play an important role in the development of CRC, playing roles in growth, progression, and metastasis[26]. A thorough analysis of the interactions between tumor epithelial cells and their surrounding microenvironment is necessary to determine the prognosis of the tumor early. Therapeutic advances over the past decade have also been influential in increasing survival rates among CRC patients. However, most deaths from CRC occur in the metastatic stage[27]. Early diagnosis and early interventions can reduce mortality from the disease. Colonoscopy is currently considered the "gold standard for diagnosing CRC when combined with pathological examinations"[27]. However, cultural and socioeconomic problems associated with this test and its aggressiveness as a screening method are obstacles limiting its use[28]. Therefore, it would be advisable to find predictive biomarkers that can be used as non-invasive primary screening methods; colonoscopy can then be used as a secondary screening method for high-risk groups. Evaluating the possibility of finding specific biomarkers derived from the inflammatory microenvironment surrounding CRC cells was the main aim of the current thesis. On another level, this study aims to evaluate the relationship between estrogen receptors and inflammatory mediators in CRC and analyze their effect on CRC cell migration and wound healing.

Gastrointestinal (GI) tract

The gastrointestinal tract, also known as the digestive system or the alimentary canal, consists of hollow organs that are interconnected. The GI tract extends from the mouth to the anus and includes the pharynx, esophagus, stomach, small intestine, large intestine and rectum. In addition, associated secretory organs and glands, such as the pancreas, gallbladder, and liver, secrete substances into the digestive tract to facilitate digestion. Excess and unabsorbed material is stored in the form of feces in the rectum and anus. When the rectum is full, the nerves sense this fullness, and the pressure pushes the stool toward the anus; anal sphincter muscles relax, allowing the stool to enter the anal canal, pass through the pelvic muscles, and leave the body[29] (Figure 2).

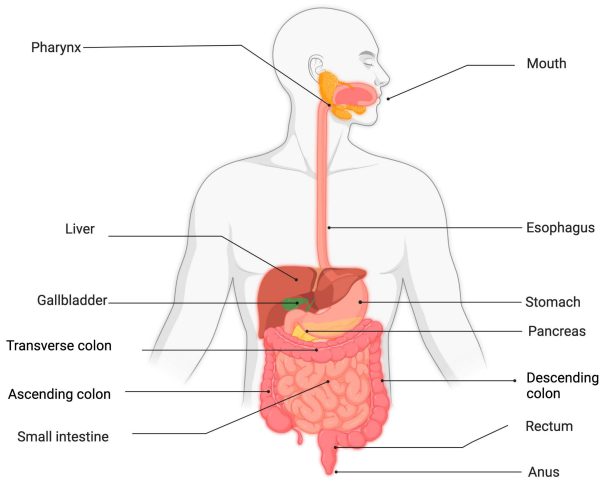


Figure 2. The human gastrointestinal tract.

Structure and function of the intestine

The lower part of the GI tract is called the intestine and is a twisted muscular tube that includes the small intestine and the large intestine[30]. The small intestine is the principal organ in the digestive system and is responsible for mixing and transporting intra-abdominal contents and producing enzymes and other compounds necessary for digestion and absorption of nutrients. The small intestine is located between the stomach and the large intestine and includes the duodenum, jejunum, and ileum[31]. The large intestine - also called the colon - consists of the cecum, ascending colon (right), transverse colon (horizontally), descending colon (left) and sigmoid colon, which connects to the rectum. The main function of the colon is the absorption of water and vitamins. Protein, unabsorbed fats, polysaccharides, bacterial biomass, undigested food debris, and water are excreted in the feces and eliminated from the body[32]. The structure of the small and large intestines is different for different reasons. There are no villi in the large intestine,

and the microvilli of epithelial cells are much less abundant in the large intestine than in the small intestine; goblet cells are more prominent in the large intestine, unlike endocrine cells, which are more prominent in the small intestine. In addition, crypt movement in the large intestine is slower than that in the small intestine. However, the general structures of the small intestine and large intestine are similar, and both consist of four distinct layers: the mucosa, submucosa, muscle, and serosa.

The inner layer of the intestine is called the mucosa, and its functions include secretion (of water, enzymes, mucus, antibodies, hormones, and acids) and absorption (of water, electrolytes, vitamins, bile, and nutrients), as well as serving as the first line of defense. This layer is designed for maximal absorption because it is covered with villi extruding into the lumen to increase the surface area. In the intestine, epithelial cell proliferation and regeneration take place regularly[33]. All the renewal processes are dependent on a limited number of intestinal multipotent progenitor or stem cells (ISCs). ISCs have two main properties: self-renewal and the ability to generate all intestinal differentiated cells[33]. Cells are transferred from the crypt to the villi and are classified into enterocytes, goblet cells, Paneth cells, or intrauterine cells[34]. Approximately one to seven intraepithelial lymphocytes are sparsely distributed per 100 epithelial cells, and similar to the case in the small intestine, these cells contain innate immune cells and T cells (CD8+)[35] (Figure 3). The submucosa contains blood vessels, nerves, and connective tissue. Throughout the GI tract, there is a neural network consisting of fibers and ganglion cells called the Meissner network. This network, along with the neural network in the outer muscle layer, forms the intestinal nervous system. This neural network is autonomous, although it also affects other parts of the body's nervous system. The muscularis is made up of two layers of smooth muscle: a narrow outer longitudinal layer that shortens and stretches the intestines and a thicker inner circular smooth muscle that causes contraction. The connective tissue between these two layers contains a neural network, the myenteric plexus or Auerbach’s plexus, and allows the muscle layers to transport waste products through peristalsis to the distal part of the colon. The outermost layer of the large intestine is the serosa. The serosa consists of mesothelial cells and protects the cecum and colon located in the abdominal cavity[36].

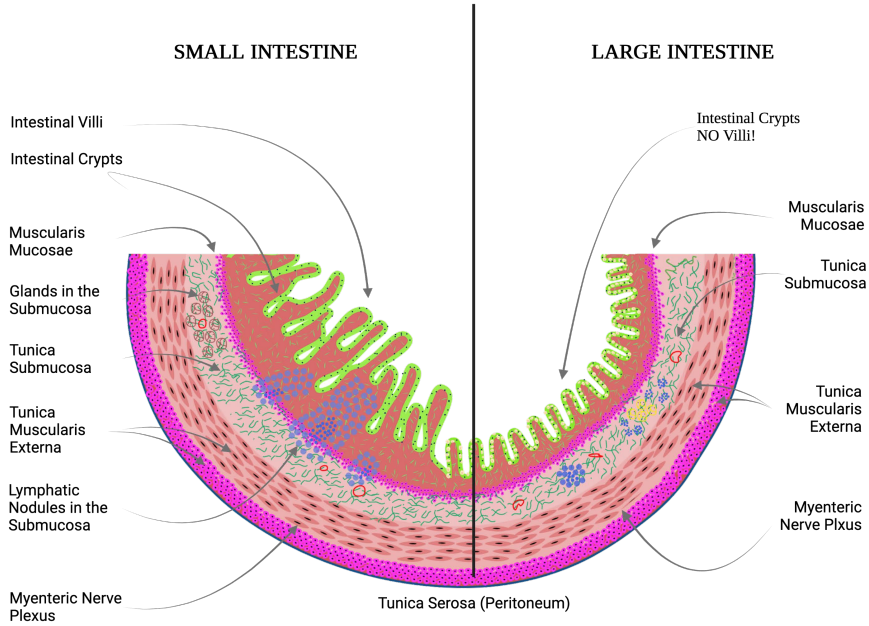


Figure 3. Difference between histology of the small and large intestine.

The Intestinal Microenvironment

Intestinal health largely depends on the balance between the intestinal microbiota and the host, where mucus, with its antimicrobial properties, limits the transmission of homogeneous and pathogenic microbes through the intestinal epithelial cell barrier[37]. Under steady-state conditions, microbial signaling and specific responses to each signal through complex immune system activation mechanisms contribute to intestinal homeostasis and keep the immune system ready for protective responses to pathogens[38]) (Figure 4).

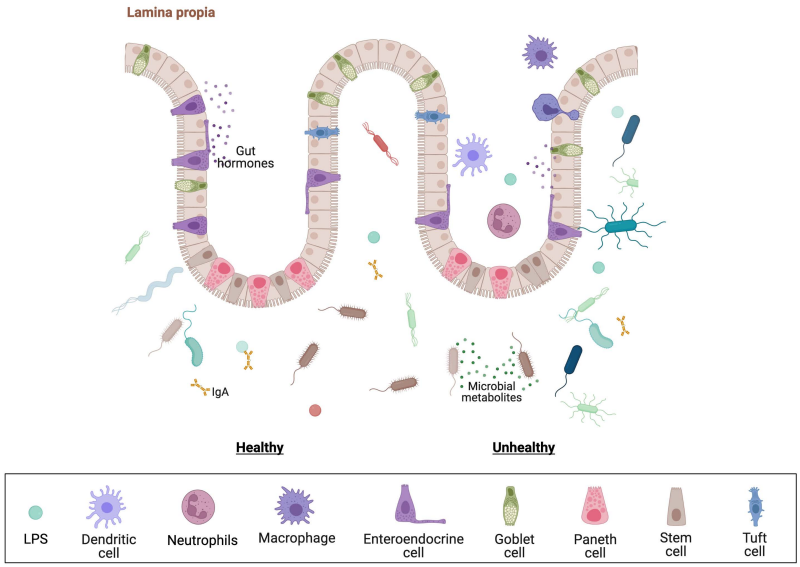


Figure 4. Intestinal microbiota in healthy (homeostasis), and unhealthy (dysbiosis) conditions. Intestinal dysbiosis has been described as a colorectal cancer risk factor.

The human colonic microbiota has a symbiotic relationship with the host and plays an essential role in maintaining immune and metabolic homeostasis and protection against pathogens[39]. Most GI bacteria are found in the large intestine, and the rest are found in the small intestine and stomach[40]. A healthy microbiome digests the elements in the chyme, which then moves along the colon, and bacterial fermentation converts the chyme into feces and produces vitamins such as vitamins K, B1, B2, B6, and B12 and biotin[41]. When disruption to microbiota homeostasis is caused by an imbalance in the microflora (dysbiosis), intestinal epithelial cells disintegrate, and the presence of germs in the intestinal lamina propria activates innate immune cells, leading to a series of diseases, including chronic inflammatory diseases called inflammatory bowel diseases (IBDs)[42].

Innate Immunity in the Intestine

The immune system adjusts its reactions under different conditions, depending on the type of pathogen and the organs affected, to cause minimal damage to the host cells. These basic immunological settings are called regional immunity[42]. The innate immune system is the first line of defense that protects the host against pathogens. Cells of the innate immune system primarily act as antigen-presenting cells and phagocytes. Their responses are thought to be immediate and nonspecific and include early detection of pathogens and prevention of them entering the host, removal of microbial residue, and activation of lymphocytes by expression and release of a large number of cytokines.

The innate immune system consists of physical and chemical barriers, antimicrobial peptides and pattern recognition receptors (PRRs) and various cell types, including neutrophils[43].

Neutrophils

Neutrophils are crucial regulators during microbial infection and are the first line of defense against pathogens and abnormal cells. Neutrophils enter the bloodstream from the bone marrow and migrate to the site of infection or inflammation, where they are eventually cleared by tissue-resident (Figure 5). They protect the body against bacteria by producing reactive oxygen species (ROS) and extracellular traps and by activating other immune cells[44].

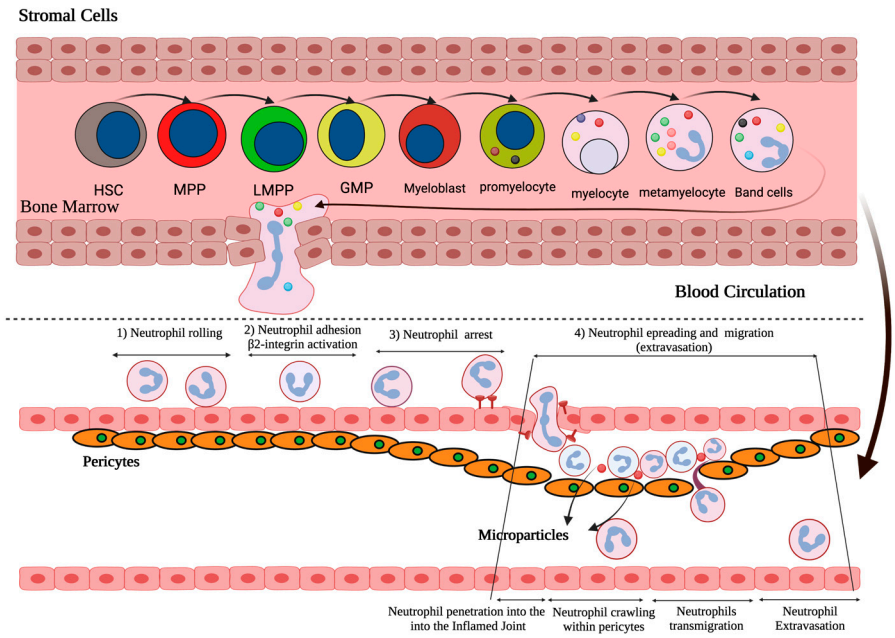


Figure 5. Neutrophil maturation and recruitment. Step 1, Maturation of neutrophils from hematopoietic stem cells: schematic process of transcription factor participation in myeloid cell development. Step 2, extravasation of neutrophils: The neutrophil migrates into the endothelial cell layer through the following steps: 1, rolling; 2, adhesion; 3, crawling, solid adhesion and patrolling; 4, extravasation into tissues and transmigration across the endothelial cell barrier, neutrophils are rolled along the endothelial basement membrane until they end up where there are narrow spaces between the pericytes. HSC, hematopoietic stem cell; MPP, multipotent progenitor; LMPP, lymphoid-primed multipotent progenitors; GMP, granulocyte monocyte progenitors.

Neutrophils make up fifty to seventy percent of leukocytes, and their presence in many cancers, including CRC, has been reported, but their role in cancer is not well defined[45]. Many studies have reported the tumor microenvironment and its secretions as important influencers of recruited neutrophils and tissue-resident immune cells. The TME itself is

constantly changing due to oncogenic signals from the growing tumor over time and affects other cells[46].

Tumor-associated neutrophils (TANs) are activated by a variety of cytokines, such as tumor growth factor β (TGF-β), and chemokines, such as CXCR2, present in the tumor microenvironment, and depending on the form of exposure, TANs can be categorized into proinflammatory and antitumorigenic (N1) or protumorigenic and anti-inflammatory (N2) TANs[47].

N2-TANs, with their genotoxic capacity are important etiological factors in carcinogenesis and can lead to oxidative DNA damage and activation of chemical carcinogenesis by releasing ROS[48]. They can also express nitric oxide synthase (iNOS) in the presence of TGFβ or arginase 1 (ARG1) and inhibit the antitumor response of CD8+ T lymphocytes. N1-TANs play a cytotoxic role in tumors and are characterized by the secretion of proinflammatory cytokines (IL-12, TNF-α, GM-CSF, and VEGF). They can also absorb chemokines such as CCL3, CXCL9, and CXCL10 or activate DCs by secreting TNF-α[49](Figure 6).

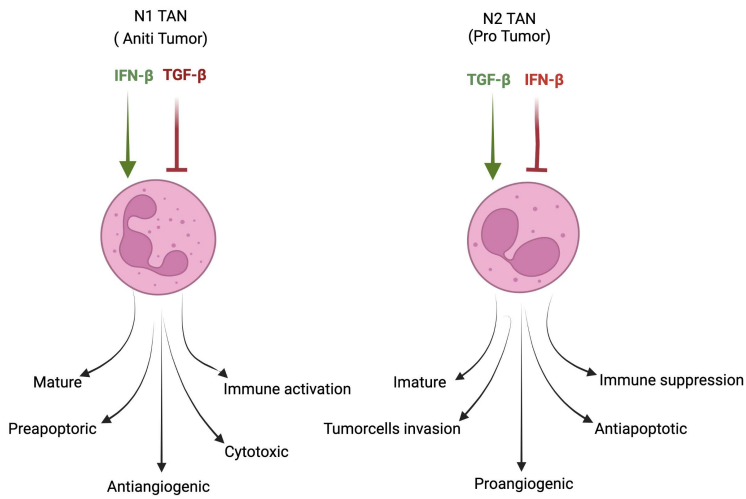


Figure 6. Polarization and activation of N1 and N2 TAN

The presence of neutrophils is observed in chronic inflammatory bowel disease [49]. Research has shown that in intestinal inflammation, neutrophils cause induced cell arrest and replication failure in colon epithelial cells, which can lead to the production of the carcinogenic compound N-nitroso and facilitate CC[50]. New research has shown neutrophils in colitis-associated CRC (CAC) lesions, where they produce the proinflammatory cytokine IL-1β, which may be a factor promoting CAC formation[51].

Inflammation

Inflammation is part of the body's natural healing system and helps fight injuries and infections[52]. However, inflammation does not only occur in response to injury. Sometimes the immune system is activated for unknown reasons without damage or infection, and because there is nothing to fight, it begins to destroy healthy cells.

Inflammation can be acute or chronic. Symptoms of acute inflammation include swelling, dilation of blood vessels, heat, redness, and pain. When the danger that threatens the body is eliminated, there are factors in the blood that restore the condition to normal. Chronic inflammation may not be severe or acute, but it is dangerous because it can lead to a wide range of diseases, including autoimmune diseases such as inflammatory bowel disease[53].

Inflammatory Bowel Diseases (IBDs) and Colonic carcinogenesis

IBD is an irreversible, lifelong autoimmune disease that covers a wide range of clinical phenotypes and has various ages of onset, but clinical manifestations usually vary depending on the patient's age at diagnosis[53]. IBD is thought to be due to the complex interaction of environmental, microbial, and host factors, including genetic factors, although the mechanism of onset of the disease remains unclear[54]. Crohn's disease (CD) and ulcerative colitis (UC) are two well-known clinical forms of IBD; CD can affect any part of the gastrointestinal tract from the mouth to the anus, while UC is limited to the large intestine. CAC that develops in the context of chronic inflammation differs from sporadic CRC[55] (Figure 7). CAC is an inflammation-dysplasia-carcinoma sequence and has greater malignant potential, while CRC arises from an adenoma-carcinoma sequence[24]. Survival in patients diagnosed with CAC is lower than that in patients with CRC[56]. In patients with a recent diagnosis of UC, the risk of developing CRC is increased 20-30 times compared to that in the healthy population[57]. In addition, eight to ten years after the diagnosis of UC, the risk of developing CAC increases[57]. The pathophysiological mechanisms behind intestinal carcinogenesis in IBD, especially UC, are unclear.

Cancer stem cells (CSCs), also known as tumor-initiating cells, are a small subset of cells in a tumor. These cells have the ability to self-renew and differentiate into diverse specialized cell types and have high tumorigenic and metastatic potential [57]. The most important function of the inflammatory response is to eradicate foreign agents, which destroy tissue homeostasis[58]. Under normal circumstances, after tissue repair or removal of the pathogen, inflammation is relieved, and the homeostatic state is recovered[58]. In chronic inflammation, a variety of cytokines and chemokines present in the inflammatory microenvironment, such as tumor necrosis factor α (TNFα) and interleukins (ILs) such as IL1b and IL6, and secreted by immune cells trigger CSC-related pathway, such as the wingless integration site (WNT)-β-catenin and TNFα-NFκB pathways. In this way, patients who are exposed to IBD are at greater risk of developing CRC[59].

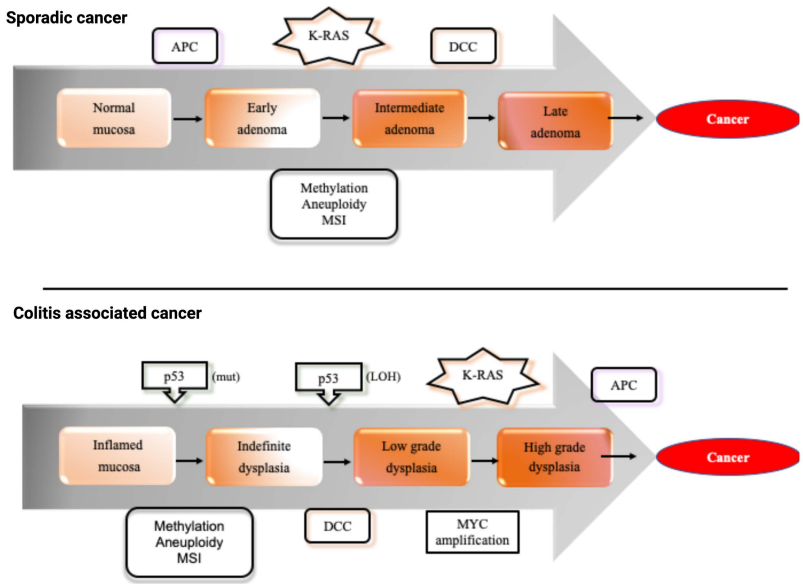


Figure 7. Comparison of molecular changes in sporadic colon cancer and colitis-associated colon cancer. APC mutations occur earlier in early sporadic (upper) than colitis-associated colon carcinogenesis (lower). Conversely, p53 appears to follow an opposite pattern of mutational order in the two types of cancer and stage earlier in the onset of colon carcinogenesis (lower), then that of sporadic carcinogenesis (upper).

Tumor Microenvironment

The tumor microenvironment (TME) plays a key role in the formation, onset, and progression of tumors. Characterization of the TME by cell and molecular profiles shows that there are different cell types in this environment that stimulate neoplastic changes and metastasis, protect the tumor from the host immune system and lead to resistance to treatment[60]. The TME consists of proliferating tumor cells, the tumor stroma, blood vessels, associated tissue cells, and a variety of infiltrating inflammatory cells. Tumor cells produce signals that cause disruption in the function of immune cells. In addition, immune cells can be the source of signals that stimulate tumor growth[61]. Infiltration of inflammatory cells found in tumors is chronic and enriched by myeloid suppressor cells (MSCs) and regulatory T cells (Tregs). Macrophages and neutrophils are important myeloid cells in the innate immune system and the most important stimuli for inflammatory responses and have a major impact on tumor progression[62]. TAMs and TANs may show opposite behaviors that are affected by the composition of the tumor microenvironment[62]. In general, TANs are protomorphic factors in different types of tumors and have the least effect of all leukocytes on the survival rate among patients with solid tumors[62]. Understanding the interactions between immune components and tumor cells at the molecular level can open up potential therapeutic pathways (Figure 8).

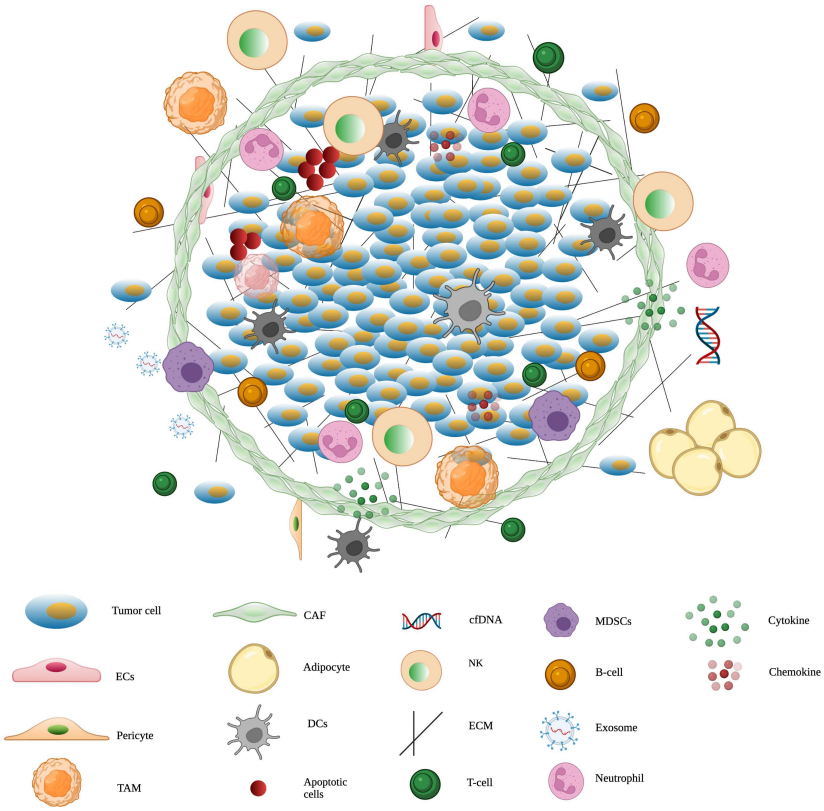


Figure 8. The tumor microenvironment (TME). Tumor cells affect all different cellular and non-cellular components in non-malignant tumors, altering and accelerating them, and advantageously transforming them into more aggressive growth of themselves.Many factors such as chemokines, cytokines, growth factors, cfDNA, etc. stimulate the activity of tumor cells. CAFs, cancer-associated fibroblasts; DC, dendritic cells; ECs, endothelial cells; ECM, extracellular matrix; MDSCs, myeloid-derived suppressor cells; NKs, natural killer cells; TAM, tumor-associated macrophages.

Colorectal Cancer

Colon cancer is usually divided into two categories: disease that extends from the proximal colon to the spleen bend (the cecum, ascending colon and transverse colon) and disease that extends to the left side of the colon (the descending colon and sigmoid colon). When CC occurs within 15 cm of the anal sphincter, it is classified as rectal cancer. Rectal cancer has a higher relapse rate and is more prone to lung metastases, whereas CC has a better prognosis and is more inclined to liver metastases[63]. CC usually begins with gland-like growths called adenomatous polyps (precancerous) inside the colon. Colonic polyps are benign and superfluous tissues in the colon that occur after a series of mutations (abnormalities) in cellular DNA[64]. Intestinal polyps are so common that approximately one-third of adults over the age of 50 develop them[65].

Epidemiology

CRC is one of the most common and lethal cancers worldwide[1]. According to the International Agency for Research on Cancer (IARC) in 2020, CRC was "the most common cause of death after breast cancer in women and the third most common cause of death after lung and prostate cancer in men" [66]. The incidence and mortality of CRC are not the same worldwide. Globally, the highest numbers are in Australia, New Zealand, Europe and North America, and the lowest are in India, parts of Africa, and South America[67]. The prevalence and mortality of CRC seem to be related to geographical elements, socioeconomic status, and a variety of modifiable behaviors, such as diet, physical activity, smoking, and obesity[68]. Although the prevalence of CRC in Western societies is higher than that in developing countries, due to the greater potential for CRC screening and early detection of the disease, CRC mortality is lower in these countries[68]. In 2019, the Swedish Cancer Foundation reported approximately 4,800 new cases of CC and 2,200 cases of rectal cancer. In total, "almost 75% of CRC patients were over 65 years old and approximately 5% of patients diagnosed with CRC were under 50 years old". Among the patients diagnosed with CC, approximately 2,500 were female, and 2,300 were male. For rectal cancer patients, approximately 800 of the patients were female, and approximately 1400 of the patients were male. CRC was the fourth most common form of cancer in Sweden in 2019[20]. (Table 1)

Table 1. Colorectal cancer in Sweden 2019 (Cancerfonden)

Tumor Site	Female	Male	Total
Colon	2507	2391	4898
Rectum	831	1360	2191
Total	3338	3751	7089

Risk Factors and Etiology

CRC is a clear example that reflects the complex interaction between the environment and genetic background in the pathogenesis of a common tumor. "The lifetime risk of CRC and CRC death is approximately 5-6% and 2.5%, respectively"[69]. This risk is exacerbated by hereditary factors, lifestyle, and environment[70]. Most CRCs are sporadic, meaning that the pathogenesis is affected by the patient's point mutations that appear throughout life, and most cases are not related to inherited syndromes but "affect individual cells and their offspring". Seventy percent of all CRCs are diffuse[71]. In fact, approximately 20% of all CRC cases have a known potential inherited cause, and approximately 5% of familial syndromes occur with a high genetic risk of the disease[72].

Environmental features, including a wide range of often adverse cultural, social, and lifestyle-related habits, are considered to be very important factors in the development of CRC. Age and Western world lifestyle factors, including low physical activity, obesity, a high-fat diet, smoking, alcohol consumption, IBD and family history, carry a

high risk for the development of CRC[73]. However, some of the risk factors for developing CRC, including overweight and smoking, are modifiable, while others, such as age or hereditary factors, are not[23].

Hereditary CRC syndromes are autosomal dominant and can be caused by changes in several sensitivity sites that have additive effects[74]. In approximately 70% of CRC cases, we can observe a specific mutation that translates into a morphological change in gene sequence, leading to adenoma and subsequent carcinoma formation[71]. "Familial adenomatous polyposis (FAP) and Lynch syndrome, also known as hereditary nonpolyposis CRC (HNPCC), are the most known hereditary CRC syndromes"[71].

FAP is observed in approximately 1% of CRC cases and is a result of mutation of the adenomatous polyposis coli (APC) gene on chromosome 5. APC, which is also known as deleted in polyposis 2.5 (DP2.5), is a multidomain tumor suppressor protein that controls many cellular activities, such as mitosis and migration[75], and together with AXIN1 and GSK3β regulates wnt signaling by managing the subcellular localization and stability of CTNNB1 (β-catenin)[76]. The three most well-known phenotypes in FAP are classic FAP, attenuated FAP (AFAP), and Gardner syndrome[74].

Lynch syndrome, also termed hereditary nonpolyposis CRC (HNPCC), is observed in approximately two-three percent of all CRC cases and is caused by mutations in the DNA mismatch repair genes MLH1, MSH2, MSH6, and PMS2[77].

Other CRC syndromes include Peutz-Jegher syndrome, caused by hereditary mutations in the tumor suppressor gene STK11 (LKB1), and MUTYH-associated polyposis (MAP), caused by modifications in the MUTYH gene, which affect the monitoring and correction of DNA and cellular defects during cell division[78]. (Figure 9)

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There are two major differences between FAP and HNPCC:

> ***The number of mutated genes:*** In FAP, only the APC gene has abnormalities, while in HNPCC, there are several gene mutations that cause the spread of precancerous conditions.
>
> ***The presence of polyps or cellular glands that can become cancerous:*** FAP is known to have more than 100 benign polyps, but patients with a defective gene with HNPCC have fewer polyps. However, these polyps can become cancerous faster than usual.

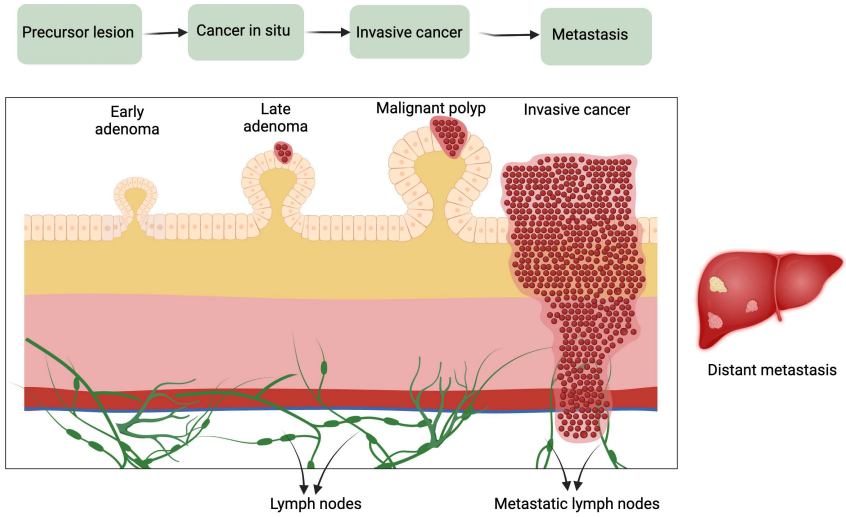

Figure 9. The progression of tumor-initiating cells from aberrant colon crypt until their subsequent transition and transformation from primary adenoma to malignant polyps and finally to invasive metastatic cancer.

Unfortunately, most CCs are "silent tumors". They grow slowly and often induce no symptoms until they reach large sizes. However, CC can be prevented and treated if it is diagnosed early[74].

Genomic Classification

In CRC, molecular alterations based on genetic instability are classified by two general mechanisms: chromosomal instability (CIN); and microsatellite instability (MSI)[79].

CIN, also known as the adenoma-carcinoma sequence, occurs in approximately 60 to 70% of cases and is characterized by extensive imbalances in chromosome number (aneuploidy) and high differentiation grade; in addition, cases with CIN are infrequently mucinous, seldom show lymphocytic infiltration, and often have poor prognosis[80]. The genomic mutations caused by CIN, often found on chromosomes 1, 5, 8, 17 or 18, lead to “activation of the proto-oncogene KRAS; inactivation of tumor suppressor genes such as APC, TP53, SMAD2, SMAD4, DCC, PIK3CA; and loss of heterozygosity (LOH)”[71]. The Wnt/β-catenin signaling pathway is required for intestinal homeostasis[81]. In sporadic CRC, inactivation of APC causes stable, nonphosphorylated β-catenin in the cytoplasm to accumulate and translocate into the nucleus by inducing activation of the Wnt/β-catenin signaling pathway, resulting in transcription of downstream target genes[82]. Wnt hyperactivation is the major oncogenic driving force in CRC[83].

The second type of genomic instability in CRC, MSI, occurs in approximately 15% of all CRC cases and approximately 3% of all Lynch syndrome cases[84]. MSI is a

hypermutable phenotype that usually occurs as a result of loss of DNA mismatch repair (MMR) activity, often related to wild-type TP53 and a diploid pattern of chromosome instability[85]. The progression of neoplasms requires inherited defects in the DNA-MMR system in neoplastic cells. Lynch syndrome is the predominant inherited syndrome associated with mutation of MMR genes (often MSH2 or MLH1), and there is often one mutated and one wild-type variant of the MMR allele in all somatic cells[86]. The rest of the cases of MSI are due to sporadic mutations and recurrent hypermethylation of gene promoters, such as hypermethylation of the MLH1 promoter, the V300E mutation in the BRAF gene, and mutations causing loss of TP53 and p16 functions[87], (Figure 10).

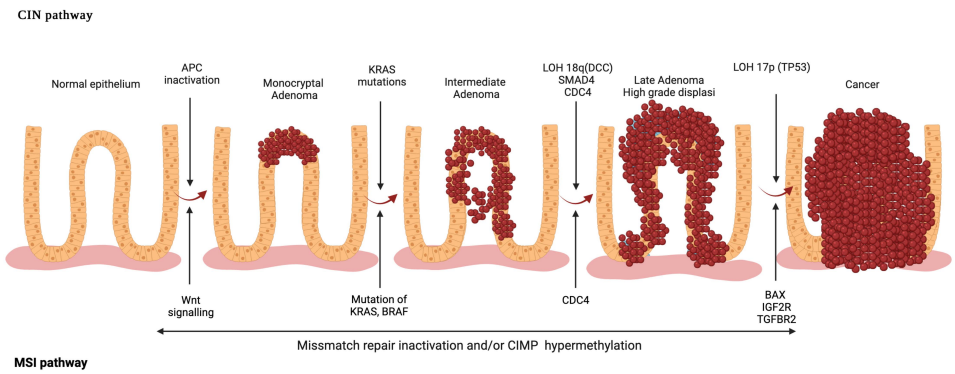

Figure 10. Molecular modification in colon cancer. Mutational changes in colon tumor-forming genes promote the histopathological malignancy of benign tumor cells to metastatic cancer. APC adenomatous polyposis coli; Bax, a major proapoptotic member of the Bcl2 family; CDC4, cell division control protein 4; CIN, chromosomal instability; IGF2R, insulin-like growth factor 2 receptor; DCC, deleted in colorectal cancer; LOH, heterozygosity; MSI, Microsatellite instability; SMAD4, SMAD Family Member 4; TGFBR2, Transforming growth factor; TP53, tumor protein p53. Adapted from (Walther A, et al., 2009).

Due to the heterogeneity of CRC, classification of disease type is pivotally important in predicting patient prognosis and determining treatment strategies. The consensus CRC subtyping, established in 2015, categorizes CRC into four molecular subspecies (CMSs) with distinct molecular and biological characteristics: CMS1 (immune, 14%), in which immune gene hyperactivity is highly associated with microsatellite instability (MSI-h); CMS2 (canonical, 37%), in which activation of epithelial/canonical wnt/β-catenin signaling indicates over activity of the epithelial growth factor pathway and higher expression of epidermal growth factor receptor (EGFR); CMS3 (metabolic, 13%), a metabolic disorder caused by increased activity in glutaminolysis and lipogenesis; and CMS4 (mesenchymal, 23%), which is considered a pro-inflammatory type with activation of TGFβ and epithelial-mesenchymal transition (EMT) genes, stromal invasion and angiogenesis, more advanced stage and chemoresistance[88].

Symptoms

Many patients with CRC do not experience symptoms in the early stages of the disease. The symptoms vary for different patients depending on, e.g., the tumor size and location. Two important symptoms of CRC are rectal bleeding and changed bowel habits (diarrhea and constipation). The feeling that the colon is not completely empty from feces, bloating, gastric pain and mucus in the feces are also symptoms exhibited in CRC. High fatigue, weakness, and weight loss can occur in advanced stages of the disease[89].

Diagnosis

Early diagnosis of CC is critical to improving patient outcomes and survival[90]. According to the American Cancer Society (ACS), people with a history of CC or colonic adenomatous polyps, or IBD, strong hereditary background of CRC, or a history of radiation therapy of the bowel and pelvis are at higher risk of developing CC at some point in their lives[91].

Noninvasive methods include various tests based on stool samples. In the stool sample, blood, immunochemical and DNA markers are assessed. This method should be repeated annually. If a stool sample shows a positive result, a colonoscopy should be performed. In invasive screening methods (sigmoidoscopy, colonoscopy, and colonography), the structure of the colon and rectum is observed. This type of screening is performed with a scope (an instrument with a light and small camera at the end) that is inserted through the rectum or performed with special imaging tests (X-rays). The number of recommended colonoscopies is less than screening based on stool tests and usually needs to be repeated every five or ten years, depending on the type of method. Colonoscopy is now considered the gold standard of screening methods[92].

After a preliminary diagnosis of CRC, the patient is usually tested to determine the extent (stage) and location of the tumor, and selection of the best CRC treatment strategy hinges on correct disease staging.

Pathology and Staging

The cancer stage describes how widely the cancer has developed in the body and helps to determine how serious and dangerous the cancer is and what the best treatment is. Cancer stage is also used for statistics on cancer survival. The most common classification system for CRC malignancy is the tumor-lymph node-metastasis (TNM) staging system promulgated by the American Joint Committee on Cancer[93]. TNM staging is often used in solid tumors to provide a broad prognosis based on cancer stage aimed at predicting survival and providing more effective therapeutic recommendations for patients[94].

The first stage of cancer is called stage 0, followed by stages 1 to 4. In general, the lower the cancer stage is, the less widespread the cancer, and higher stages are associated with more spread. Early stages of cancer have a lower stage and less spread. Although each person's experience is different, cancer patients who have disease of the same stage are viewed similarly and are often treated in the same way.

The TNM staging system

Table 2. TNM classification according to the 8th edition of AJCC Cancer Staging Manual

(Tumor) size	TX	Primary tumor can not be assessed	
	T0	No sign of the primary tumor	
	T1:	Tumor is found only in submucosa	
	T2	Tumor has grown into muscularis propria	
	T3	Tumor invades through muscularis propria into the subserosa	
	T4	T4a	Tumor perforates visceral peritoneum
		T4b	Tumor has grown into or has attached to other structures
(Node) involvement	NX	No regional lymph node can be assessed	
	N0	No sign of the regional lymph node metastasis	
	N1	A small number of the nearest regional lymph nodes (less than four) have been invaded by tumor cells	
	N2	Tumor cells have invaded more lymph nodes in the nearby areas	
	N3	Tumor cells are now found in many lymph nodes as well as in more distant lymph nodes	
	MX	No distant metastasis can be assessed	
(Metastasis) appearance	M0	No distant metastasis	
	M1	Distant metastasis	

T: The letter T (tumor) and numbers 0 to 4 are used to describe the rate of growth of the primary tumor and invasion into the adjacent tissues. T0 shows no signs of tumor, while T1-T4 are assigned based on increasing tumor size, progression and invasion.

N: The letter N (node) characterizes the lymph nodes in the area near the large intestine that are involved in the tumor. Lymph nodes are immune organs that contain lymph fluid. These nodules are widely spread throughout the body, including the armpits and intestine, and are associated with lymphatic vessels. Lymph nodes contain many immune cells, such as B and T cells. In the TNM system, N0 indicates no tumor spread in regional nodes, while N1-N3 indicates some degree of nodal expansion, with gradual distal expansion from N1 to N3 (Sapin MR, 2007).

M: The letter M (metastasis) describes cancer that has spread beyond the regional lymph nodes to other parts of the body, including the liver or lungs, which is called distant metastasis. In the TNM system, M0 is classified as a tumor without distant metastasis, while M1a, M1b, and M1cc indicate spread to 1 area, more than 2 areas, and spread to the peritoneal surface, respectively.

Recurrence

Return of cancer after treatment usually happens sometime after the initial treatment in the same place it starts or in another part of the body. Recurrent cancer anywhere in the body retains the original name. For example, CC may recur in the colon or rectum or may have spread to the lungs and liver, but in all cases, it is called CC.

Recurrent cancer is usually divided into three subgroups: local recurrence is when the tumor has recurred where it first appeared, regional recurrence is when the tumor returns in the lymph nodes near its original location, and distant recurrence is when the cancer reappears in another part of the body and at a considerable distance from the original site (often the lung or liver in the case of CC)[19].

To date, no reliable method has been found to predict the recurrence of CC after removal of the primary tumor. This shortcoming may be offset by the identification and clinical use of molecular and biological markers to identify patients with early-stage CC and improve the prognosis of patients at high risk for recurrence.

Treatment

The specific treatment strategy for CRC largely depends on the exact site of cancer and the stage at which the tumor was identified. Most colon polyps can be removed by colonoscopy. In the early stages of CRC, large benign tumors are removed by surgery. Advanced CRC can be treated in different ways depending on its location. "Treatment includes surgery, radiation therapy, chemotherapy, and targeted therapy"[95].

Colorectal treatment may include any of the following or a combination of these:

-Surgery

-Radiation therapy

-Therapy using medication (chemotherapy, targeted therapy, or immunotherapy)

In stage 0 CRC, polyps can only be observed in the inner lining of the colon, and surgery is generally the first treatment choice. In stage I CRC, when the tumor has penetrated into the colon wall but not outside the walls or to the adjacent lymph nodes, if the tumor is totally removed by colonoscopy, with no cancer cells left around the site of the removed part of the colon, no other treatment is needed. In stage II CRC, the breadth of the tumor is large, and it has grown through the colon wall and probably adjacent tissues but not lymph nodes. This is one of the most challenging stages of CRC to treat. Surgery and tumor removal are the first treatment options. To date, no effective adjuvant therapy has been proposed for stage II CRC. Adjuvant therapy is used only if indicated by the physician based on the patient's risk scores for recurrence. For stage II rectal cancer, radiation therapy is normally combined with chemotherapy before or after tumor resection. In stage III CRC, the tumor has invaded lymph nodes. Treatment for this stage includes removal of the tumor and all lymph nodes involved, followed by adjuvant

therapy. In cases where it is difficult to remove the entire tumor, neoadjuvant chemotherapy in combination with radiation therapy (called chemoradiation) may be an option. In stage IV CRC, the cancer has metastasized and migrated to another part of the body, such as the liver or lungs, via the bloodstream. Removal of the colon and metastatic organs is the first treatment option. Metastatic stage 4b in CRC differs from stage 4a in that it represents metastases to more than one site or peritoneal metastasis. Often, patients with stage 4b disease will not benefit from resection treatment, and their treatment is usually palliative[91].

Treatment of recurrence of CRC includes removing the recurrent cancer. If all the cancer tissue cannot be removed by surgery, chemotherapy becomes the main treatment[93,96].

Survival and Prognosis

Patient, treatment, and tumor-related characteristics are the three main prognostic factors in CRC. The survival of CRC is strongly dependent on the stage of the disease at the time of diagnosis and the biology of the tumor. The approximate 5-year survival at different stages of CRC is as follows: stage I, 92%; IIA, 87%; IIB, 65%; IIIA, 90%; IIIB, 72%; and stage IIIC, 53%. CRC is more difficult to treat and has a worse prognosis when it spreads to other parts of the body. The relative expected survival after 5 years of stage IV or metastatic CRC is 12%[1]. The survival rate of CRC patients of all stages has been steadily improving in recent years. Nevertheless, the improvement in patient survival is not evident in all geographical zones and is better in countries with high life expectancy and good access to modern professional health care[68]. In many cases, survival estimation may not be accurate. Although the disease stage is relatively predictable, due to the differences in subgroups and the exceptions of each classification as well as differences in patient overall health, the stage of CRC may not correctly predict the prognosis for the disease. Approximately 85% of relapses occur during the first two years after initial resection in both local and limited liver metastasis cases. Liver metastases are more frequently found two years after primary tumor resection. Metastases found less than 12 months from diagnosis of the primary resection indicate a worse prognosis[97].

Early detection of liver metastases in patients with treated locoregional disease and in patients with treated metastases increases the number of patients who benefit from metastatic resection and chemotherapy. If recurrent disease is not identified in time, it may no longer be removable and therefore can only be treated with palliative chemotherapy[98]. However, in many cases, survival statistics do not include tumor heterogeneity, tumor markers, or proteins that affect treatment, and therefore, such statistics may not be reliable for determining prognosis[99,100]. A biomarker-based prognostication tool may improve the evaluation of CRC risk and may also provide insight into actionable therapeutic targets[101].

Biomarkers

In medicine, "biomarker" is a portmanteau of "biological marker" that refers to an indicator that is measurable, specific, accurate, and consistent[102]. A biomarker is a factor in the blood, tissues, or body fluids that indicates a patient's clinical condition in the presence of disease, infection, or other abnormal conditions. Biomarkers have a variety of uses, including being used in monitoring patient response to treatment and disease progression[103]. In cancer, biomarkers are often produced by tumors or chemicals secreted in the body in response to cancer. Biomarkers can be observed in several forms. They may be found in feces or secretions such as urine and sputum that do not require invasive methods to isolate. The presence of biomarkers in the blood can also be detected through minimally invasive tests, such as a simple blood sample (e.g., carcinoembryonic antigen (CEA), a CRC marker). Some biomarkers are present in tissues and organs, and tissue biopsy or organ imaging is required to identify them[104].

Types of Biomarkers

Cancer cells contain a large number of genetic changes, including gene rearrangements, point mutations, and gene amplifications. These genetic changes disrupt the molecular pathways that regulate cell proliferation, survival, and metastasis. This change can be in the structure of the marker (qualitative changes) or in the value of the marker (quantitative changes)[105]. In situations in which these changes are common or in most patients with a particular type of tumor, they can be used as biomarkers to predict prognosis and develop therapies[106].

Deoxyribonucleic acid (DNA) is a commonly used biomarker. Mutations in proto-oncogenes that bring about the formation of oncogenes are commonly used to assess cancer. Tumor inhibitory genes that help prevent cancer can also be considered biomarkers. "Other abnormal genetic changes, such as the number of copies of a particular gene and the fusion of genes not normally seen (by translocation) with each other, are also used as biomarkers"[106]. The sources of DNA can be plasma, serum, sputum, saliva, stool, cerebrospinal fluid, or circulating tumor cells (CTCs)[107].

Ribonucleic acid (RNA) is very similar to DNA, and changes in it can indicate disease. One of the key factors that has led to the growth of cancer research and biomarkers is small noncoding RNA molecules called microRNAs (miRNAs). miRNAs are known as markers for various types of cancer, including CRC, breast cancer, leukemia, liver, lung, and pancreatic cancer[108].

Proteins are probably the most prominent type of biomarkers. Their responsibility is to control most cellular processes. Proteins can be a very reliable indicator of certain diseases because proteins, unlike DNA molecules and certain RNAs, are rapidly formed and destroyed. Proteins can indicate the current state of illness in the body[109].

Viruses can also be considered a biomarker. Viral infections are involved in 15 to 20% of cancer cases[110].

Bacteria can also act as biomarkers. Bacteria that induce mild chronic inflammation are known to cause cancer. Helicobacter pylori, for example, is a bacterium found in the stomach wall that has been linked to the formation of ulcers and stomach cancer[110].

Exosomes are small bubble-shaped (vesicular) structures that are secreted by cells. These structures may contain miRNA, RNA, DNA, or specific proteins in cancer cells[111]. Exosomes are found in body fluids, including blood and urine. In many cancers, exosomes have been observed to prepare sites far from the primary cancer site for metastasis. Many ongoing efforts have been made to use exosomes as a tool to diagnose cancer and predict the likelihood of cancer occurring[112].

Cancer cells can be used as biomarkers. Cancer cells migrating through the bloodstream, called circulating tumor cells (CTCs), can be used as a marker for disease progression. Cancer stem cells (CSCs) can also be used to monitor disease[113].

Application of Biomarkers in CRC

Biomarkers can be divided into several categories based on the information they give in the evaluation of patient status in different clinical situations:

> ***Patient status (diagnosis):*** The purpose of using biomarkers as a diagnostic tool is to identify cancer in its early stages. These tests can be used to determine the specific biology of the cancer, and can also be used to guide treatment.
>
> ***Determining the probable outcome for the patient (prognosis):*** Prognostic biomarkers are used to determine the rate of cancer progression.
>
> ***Predicting disease progression:*** A predictive biomarker is used to assess how a patient responds to a particular treatment. A predictive biomarker can also be used to determine the maximum effect of drugs or chemotherapeutic agents in specific patients.
>
> ***Monitoring disease progression****:* Biomarkers can be used to measure treatment effects and thus guide decisions on subsequent treatment(s). [114]

Biomarker Requirements

Although many molecules have the potential to act as biomarkers, only a small number have been approved[115]. A biomarker is considered effective when it can detect primary tumors and is very specific for the disease. This molecule must be present in the body in sufficient quantities to be detectable. Preferably, the biomarker should be easily identifiable in the infected person's blood, serum, or other body fluids. It should also have other features, such as low-test cost and simple test performance[116].

Limitations of Biomarkers

Even though biomarkers may seem perfect in theory, there are several practical limitations to their use. “Although biomarkers have been used successfully to detect and monitor disease, there is a conflict between sensitivity (ability of a test to identify people with the disease), specificity (ability of a test to exclude people who do not have the disease), and cost”[117]. “Another caveat for non-tissue-specific biomarkers is when the level of a biomarker is affected by a noncancer disease, and utility for cancer detection may also be compromised” [118]. An example of such a biomarker is the prostate-specific antigen (PSA) test, as the PSA level is increased in prostate cancer but also in benign prostatic hyperplasia (enlarged prostate) and prostatitis (inflammation of the prostate)[119].

To date, biomarkers in CRC include DNA biomarkers (such as mutation of DNA (MMR)[120]; RNA biomarkers (especially miRNAs as diagnostic, treatment-related and prognostic biomarkers)[121]; protein biomarkers, which are considered the most accurate biomarkers (e.g., KRAS, CEA, and TP53)[122]; small molecule metabolite biomarkers (including volatile compounds that can be detected by metabolic techniques)[123]; and biomarkers related to changes in the composition of the intestinal microbiota[124]. Ongoing studies are underway to identify biomarker panels with high sensitivity and specificity for the early detection of CRC.

Other biomarkers in CRC may include inflammatory responses and associated microenvironmental changes[125]. Recently, a number of neutrophil proteins in the feces, including calprotectin, fecal lactoferrin, lysozyme, myeloperoxidase, and elastase, have been studied as specific markers of intestinal inflammation[126]. On the other hand, the structure of the gastrointestinal tract creates permanent interactions between its epithelial tissue and the intestinal microbiota, so the inflammatory effect in CRC is more significant than that in any other neoplasm[127].

Cancer cells have a high number of genetic alterations, including gene rearrangements, point mutations, and gene modifications. These genetic modifications cause disturbances in the molecular pathways regulating cell proliferation, survival, and metastasis. If these high-sensitivity modifications can be measured and are specific to a particular type of cancer, they can be viable candidates as biomarkers to predict survival and for the development of a variety of therapies[14].

Leukotrienes

Chronic inflammation and an impaired immune system are two important and significant components in the microenvironment of the tumor, and extensive studies are necessary to evaluate tumor growth and response to immune therapies. The biological mechanism in patients with chronic inflammation induces arachidonic acid (AA) production and leads to the generation of anti-inflammatory eicosanoids leukotrienes

and prostaglandins via the isoenzymes 5-lipoxygenase (5-LOX) and cyclooxygenase (COX), respectively. Leukotrienes and prostaglandins are inflammatory lipid mediators found in higher concentrations in the lining of the colon[128] (Figure 11).

Leukotrienes (LTs) regulate the function of immune cells in a paracrine and cell type-dependent manner. All leukocytes produce leukotrienes, and leukotriene production is also associated with the production of histamine and prostaglandins in mast cells, which act as inflammatory mediators. Leukotrienes are divided into two types: dihydroxy fatty acid leukotriene B4 (LTB_4), including LTC_4 and its metabolites LTD_4 and LTE_4, and cysteinyl leukotrienes (CysLTs). LTB_4 is a secondary chemoattractant secreted by neutrophils and is the most prominent leukotriene in acute inflammatory responses. LTB4 is responsible for activating other leukocytes and increasing their survival[129]. LTC_4, LTD_4, and LTE_4 are three structurally similar, but functionally distinct, eicosanoid lipids known for mediating inflammation, bronchoconstriction, and vascular leakage. The fatty acid-peptide conjugate LTC4 is mainly produced by myeloid cells, such as macrophages/monocytes, neutrophils, eosinophils, and mast cells. It can be converted into its metabolites, the potent constrictor LTD_4 and the stable degradation metabolite LTE4[130]. The presence of LTD_4 leads to smooth muscle contraction, mucus production, and asthma and allergic rhinitis pathogenesis. Inhibition of LTD_4 production or activity by antagonists such as montelukast is used to treat these disorders[131].

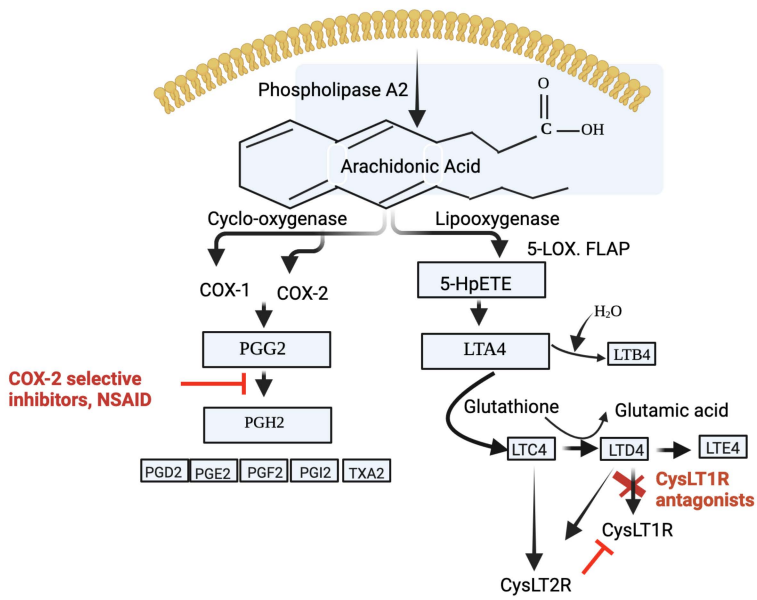

Figure 11. Schematic drawing of arachidonic acid metabolism pathway. Alox-5, arachidonate 5-lipoxygenase; COX, cyclooxygenase; FLAP, 5-lipoxygenase activating protein; 5-HpETE, 5-hydroperoxyacosatetraenoic acid; LTC_4, LTD_4, LTE4 LTs, cysteinyl leukotrienes LTC_4, LTD_4 and LTE_4; LOX, lipoxygenase; LTs, leukotrienes; PG, Prostaglandin.

Cysteinyl Leukotriene Receptor 1 ($CysLT_1R$)

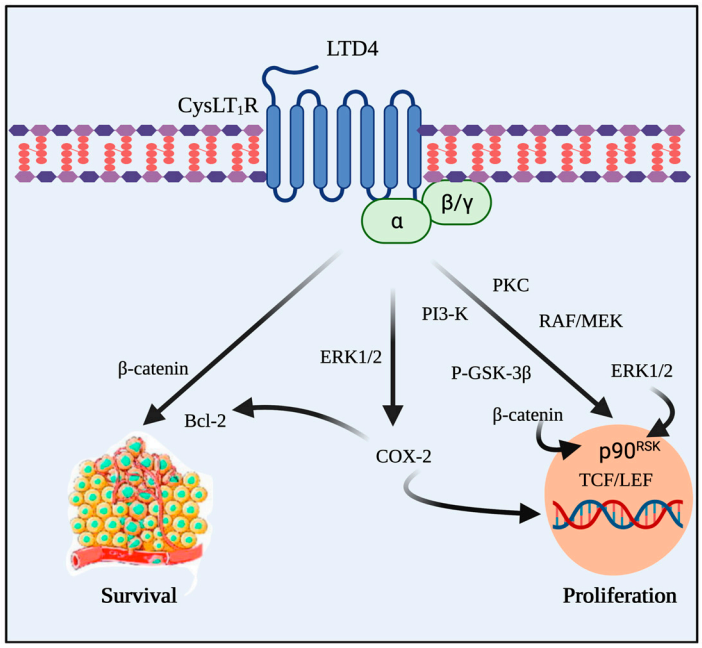

Figure 12. LTD_4/$CysLT_1R$ signaling cascade induces cell survival and cell proliferation in colon cancer. Bcl2, B-cell lymphoma 2; GSK3β, Glycogen synthase kinase-3β; MEK, Mitogen-activated protein kinase kinase; PI3K, Phosphoinositide 3-kinases; PLC, Phospholipase C; TCF/LEF, T cell factor/lymphoid enhancer factor family.

Leukotrienes mediate their functions by binding to G protein-coupled receptors (GPCRs): high-affinity BLTL and low-affinity BLT2 binding receptors for LTB_4 and high-affinity $CysLT_1R$ and $CysLT_2R$ for LTD_4 and LTC_4, respectively[132]. LTE_4, as a relatively stable cysteinyl leukotriene, has the lowest affinity for both CysLTRs (Laidlaw T, et al., 2012). LTE_4 is partly responsible for a range of inflammatory effects, such as bronchoconstriction, vasoconstriction and smooth muscle contraction[133].

$CysLT_1R$ and $CysLT_2R$ are both G protein-coupled receptors located on the plasma membrane of several cell types[134]. Initially, these two receptors were thought to be structurally similar, but after being cloned and characterized, they were shown to be derived from different chromosomes and were homologous in only 38% of their sequences. $CysLT_1R$ is a high-affinity receptor for LTD_4, while $CysLT_2R$ is a high-affinity receptor for LTC_4. Both receptors are expressed in immune cells (such as macrophages, neutrophils, mast cells, and B lymphocytes), as well as smooth muscle cells and heart, brain, and spinal cord tissues[135]. Research has shown that low expression of $CysLT_1R$ and high expression of $CysLT_2R$ are associated with better prognosis and longer survival for CRC patients (Figure 12). $_{LTD4}$ signaling via the G protein-coupled receptor $CysLT_1R$ leads to a series of downstream signaling pathways. LTD_4-induced

$CysLT_1R$ signaling regulates several carcinogenic proteins, such as COX-2, β-catenin and Bcl-2, in intestinal epithelial cells. Upregulation of $CysLT_1R$ in CC correlates with a poor prognosis. When $CysLT_1R$ is downregulated via gene knockdown or antagonists such as montelukast, the effect of carcinogenesis decreases[136]. Expression of $CysLT_1Rs$ is a primary event in CC progression and seems to mediate the invasion and metastasis of tumor cells to other parts of the body (Savari, et al., 2013). For this reason, retrospective clinical follow-up research on LTD_4 and $CysLT_1R$ could be valuable for the detection of new potential biomarkers in CC.

G Protein-Coupled Receptors

GPCRs, with seven membrane-spanning domains (7TM), are a large group of protein receptors located on the outer surface of cell membranes. GPCRs bind to an intracellular heterotrimeric protein called a G-protein, which consists of three distinct subunits (α, β, and γ). Prior to ligand binding to the receptor, the inactive G protein α subunit releases GDP and binds GTP to take on its active state. The subunits of the G-protein are then split into the α subunit and the βγ subunit, and then either or both α or β are able to activate downstream signals. To turn off the signaling pathway, GTP in its active state is hydrolyzed to GDP, and the α subunit is inactivated. The α/β subunits rejoin to form the inactive G protein[137]. GPCRs exhibit an important role in the body's physiological interactions and have been implicated in many steps of tumor progression, including tumor cell proliferation, angiogenesis, invasion, and metastasis. Understanding the mechanisms of responsiveness of a given GPCR can shed light on the functional impact of the receptor in physiological and disease states but also identify regulatory molecules as potential and remarkable biomarkers for the early detection of cancer.

Brain-Derived Neurotrophic Factor

Neurotrophins (NTs) are a family of growth factors that play important roles in the development, maintenance, survival, and death of cells in the nervous system[138]. Neurotrophins are made up of at least 4 family members: nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), neurotrophin-3 (NT-3), and neurotrophin (NT-4/5)[139]. The brain is considered the main regulator of neuronal differentiation and synaptic plasticity, and the process of cell death also plays an important role in learning and memory. Brain-derived neurotrophic factor is the most important trophic factor known in the nervous system and is expressed throughout the CNS. BDNF, despite its name, is not only found in the brain but is also expressed in a variety of tissues and cells, including the retina, kidneys, and colon[140]. Various studies have shown that in the hippocampus and cortex of mice exposed to proinflammatory mediators, BDNF expression is significantly reduced[141]. BDNF is recognized as an essential modulator in central physiological and pathological pain. High levels of this protein in the colon epithelium and lamina propria in patients with IBS are significantly associated with

symptoms of abdominal pain and increases in neurotransmitters such as substance P and serotonin[142] BDNF essentially mediates cellular biological effects via its high-affinity tyrosine kinase receptor, called tropomyosin-related kinase B (TrkB) receptor. The TrkB receptor binds to BDNF via the extracellular N-terminus and, after autophosphorylation, activates intracellular signaling pathways[143].

Receptor Tyrosine Kinases (RTKs)

The process by which molecular signaling is transmitted from the extracellular surface to the cytosol is known as signal transduction and typically amplifies and produces multiple intracellular signals for every receptor that is activated. Protein kinase activation is often part of a larger signal transduction pathway, such as the platelet activation signal pathway[144]. Protein kinases reversibly catalyze the phosphorylation of substrate proteins using adenosine triphosphate (ATP). The ATP binding site is well conserved in all protein kinases and enables their phosphorylation, which is one of the most critical steps in cell signal transduction[145].

RTKs are a group of membrane proteins that participate in the regulation of intracellular processes, such as growth, survival, differentiation and motility, by transmitting signals from the extracellular environment to the cytoplasm or cell nucleus. Ligand binding to RTK causes activation of different molecular and cellular responses, and each receptor monomer is coupled to another monomer (dimerization) and phosphorylated[146] (Figure13). Activation of RTKs leads to phosphorylation of downstream intracellular signaling pathways and subsequent changes in cell physiology and behavior. Internal domains of RTKs mediate intracellular pathways such as the PI3K, JAK, STAT, AKT, ERK, and MAPK pathways[147]. In cancer cells, due to mutations in oncogenes and tumor-inhibiting genes, several intracellular transmission pathways are strongly activated, ultimately resulting in differentiation, increased cell proliferation and inhibition of apoptosis[148]. In recent years, the understanding of the molecular processes involved in intracellular pathways in cancer has led to the identification of chemical biomarkers that target these pathways and either inhibit or stimulate signaling in cancer cells.

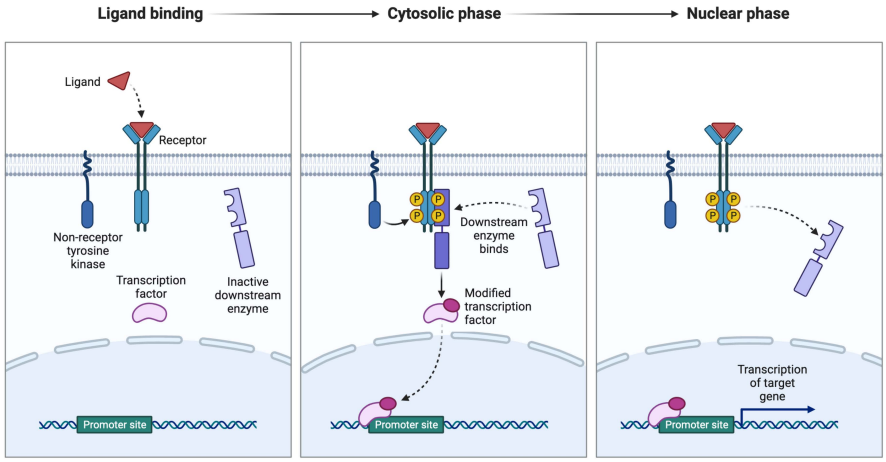

Figure 13. Canonical RTK signaling cascade. When the tyrosine kinase receptor (RTKs) binds to its ligand, the receptor is dimerized and activated. The intracellular kinase domains of the receptors are in the correct orientation so that oligomerization and trans autophosphorylation of tyrosine occur in the activation ring (ring A) and subsequent kinase activation. Phospho-tyrosine residues, in turn, cause secondary transphosphorylation in the kinase insert, which induces RTK phosphorylation. Secondary messenger proteins activate downstream proteins, which in turn send signals to regulate gene transcription to the nucleus. RTK, Receptor tyrosine kinase

BDNF/TrkB Signaling

BDNF is a homodimer protein formed of a signal peptide sequence and an N-glycosylation site located at nerve terminals and transmitted anterogradely throughout neurons[149]. Released BDNF binds to the tropomyosin B (TrkB) receptor, a member of the tyrosine kinase family, resulting in its dimerization and subsequent autophosphorylation[150,151]. BDNF activation of TrkB induces chemoresistance through activation of phosphatidylinositol-3-kinase (PI3K)/Akt signaling and mitogen-activated protein kinases (MAPKs), which in turn affect gene expression by activating transcription factors, such as cAMP response element-binding (CREB) protein. Activated AKT causes activation of mammalian target of rapamycin (mTOR) and suppresses autophagy. Induction of MAPK and extracellular signal-regulated kinases (ERK1/2) increases apoptotic markers that induce cell survival, as well as phosphorylation of synapsin-1, which mediates the release of synaptic vesicles, causing activation of CREB[152] (figure 14). BDNF/TrkB activity can even modulate proinflammatory mediators such as NF-κB and lead to limitation or enhancement of the inflammatory response[153]. In most tumors, BDNF/TrkB signaling leads to several important biological processes in tumor cells, including proliferation[154], migration, and epithelial-mesenchymal transmission[155], as well as resistance to apoptosis and anoikis (a form of detached apoptosis) and suppression of antitumor immunity[156]. High expression of BDNF in neoplastic tissue compared to non-neoplastic adjacent tissue from the same person and the presence of both BDNF mRNA and protein in serum in CRC patients make BDNF a possible candidate biomarker in CRC[157].

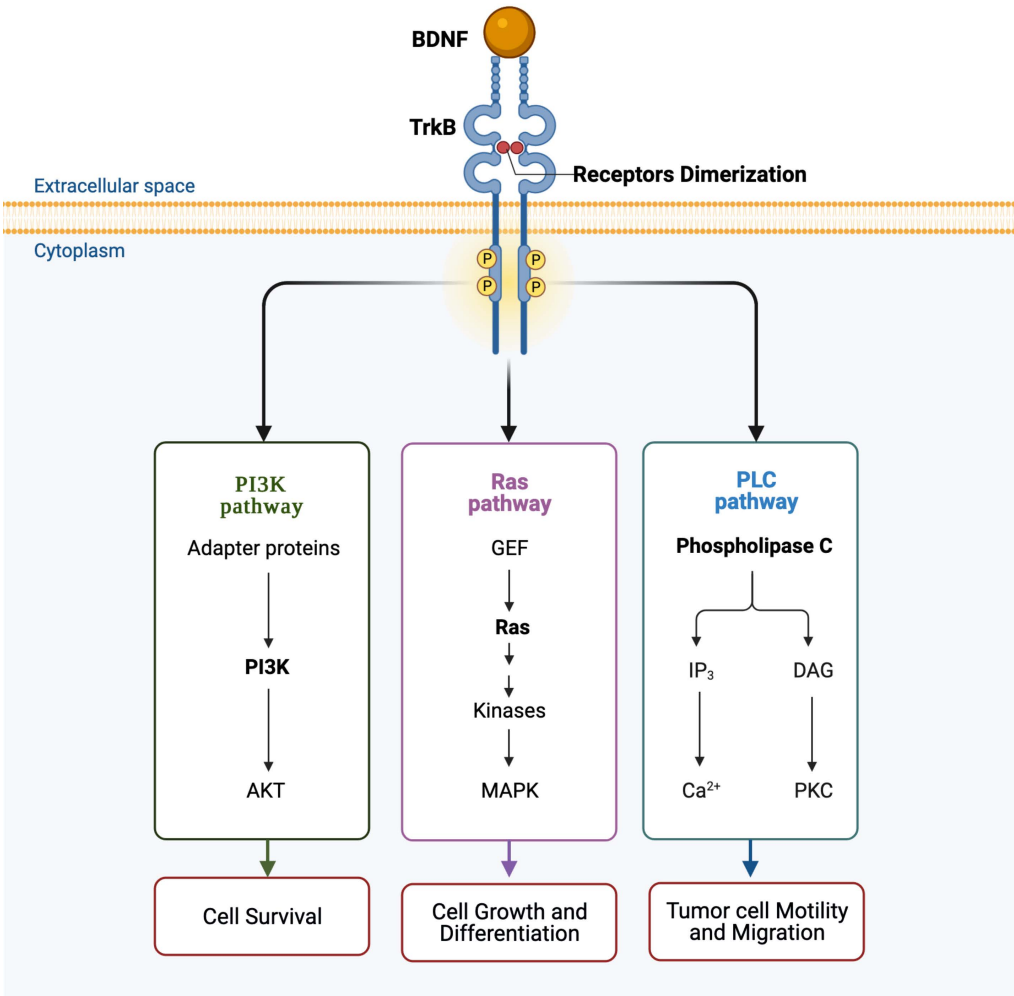

Figure 14. Schematic drawing of the BDNF / TrkB signaling cascade. BDNF / TrkB activation generally activates the signal paths PI3K, Ras, and PLC γ. The activity summary of this pathway is as follows: Activation of the PI3K pathway leads to cell survival. Activation of the Ras pathway leads to cell growth and differentiation. Activation of the PLCγ pathway leads to tumor cells motility and migration. All three pathways link to the CREB transcription factor, which can regulate gene expression. diacylglycerol. AKT, Protein Kinase B (PKB); CREB, cAMP response element-binding protein, DAG, Diacylglycerol; IP3, Inositol Trisphosphate; MAPK, mitogen-activated protein kinase; PKC, Protein Kinase C.

Hormones in Cancer

Hormones are chemicals secreted by endocrine glands, including the ovaries and testicles. Hormones are responsible for making cancer cells grow in certain cancers, including breast and prostate cancer. In other cases, hormones can kill cancer cells, shorten the growth cycle of these cells, or prevent them from growing. Hormone therapy, which is used as a treatment for cancer, means using medicine that enhances the activity of these specific hormones or stops the body from producing them[158], but using them optimally to achieve the best health benefits and prevent side effects is still a major challenge[159].

Estrogen and estrogen receptor beta (ERβ) in CRC

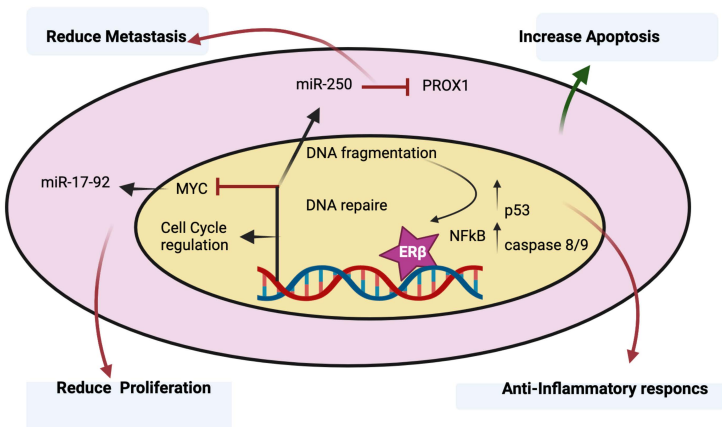

Figure 15. Molecular mechanism for ERβ-mediated anti-tumorigenic activity. Adapted from Williams C, et al., 2016[160].

Young women with CRC exhibit better survival than young men[161]. Previous research has shown the role of female hormones in preventing CC. Sex, age and the amount of estrogen in a woman's body are three important factors that increase the likelihood of recovery and life expectancy[162]. Research has shown that women with CC who are less than 45 years old live on average two years longer than men with the disease[163], and respond better to anticancer drugs. Hormone replacement therapy and estrogen injections will increase the chances of disease improvement in postmenopausal women[164]. Estrogens are steroid hormones that are important for the sexual and reproductive organs in women and men. Estrogens regulate various physiological characteristics in the female body, such as bone integrity, muscle mass, subcutaneous visceral fat, and homeostasis[165]. Abnormal estrogen activity can lead to a wide range of diseases, including CRC. Estrogen signals through two nuclear receptors, ERα and ERβ.

These receptors are encoded by two distinct genes (ESR1 and ESR2) on two different chromosomes[165]. ERα and ERβ show major differences in their biological functions dependent on both nuclear and extranuclear signaling. ERα is amplified by differential expression of proapoptotic and anti-apoptotic proteins as well as cyclin D1 to promote cell cycle transfer due to promoted proliferative signals in cells, while ERβ can activate the proapoptotic signal cascade in the absence of ERα and via the p38/MAPK pathway to induce antitumor and antiproliferative effects[166]. ERβ is the predominant estrogen receptor in both normal and malignant colonic epithelium, while little is known about the level of ERα protein in the colon epithelium or in CRC. ERα and ERβ interact with many receptors and signaling substances, including tyrosine kinases, scaffold proteins, and guanine (G) nucleotide exchange proteins, and are involved in activating cytoplasmic signaling pathways. Previous studies have shown that ERβ expression decreases during colon tumorigenesis and that the level of expression is inversely related to the prognosis of disease[167] (figure 15). Further knowledge of ERβ activity in CRC may enable prevention of the disease and provide more treatment options for tumors with high ERβ expression.

Aims

Specific Aim

The general aim of this thesis was to investigate the role of BDNF in CC and its microenvironment by focusing on inflammatory mediators, as well as the estrogen hormone and its receptor.

Paper I: The main goal of this project was to investigate better strategies for diagnosing CC. This study focused on the neutrophil/BDNF/$CysLT_1R$ axis as an independent prognostic marker for the early detection of CC.

Paper II: The main goal of this project was to investigate the correlation between BDNF/TrkB and LTD_4/$CysLT_1R$ for the early detection of CC. Here, we focused on the molecular interactions between TrkB and $CysLT_1R$.

Paper III: The main goal of this project was to identify a new and robust gene signature based on the link between BDNF and cancer diagnosis that could predict tumor recurrence in patients with stage I, II, and III CC.

Paper IV: The main goal of this project was to investigate the correlation between ERβ and inflammatory mediators and the antitumor effect of ERβ and its selective agonist ERB-041 in CRC.

Patient data and Methodology

Patient Material

In Papers I, II, and III, the Malmö Hospital cohort including 72 formalin-fixed and paraffin-embedded samples and matched normal mucosa samples from primary CC was used. Tumor microarrays were stained with hematoxylin and eosin according to the manufacturer's instructions for further study with immunohistochemistry.

In Paper I, 197 CC patients with stage I, II or III disease from the public TCGA-COAD dataset were included.

In Paper II, 259 CC patients in stages I, II and III from the public TCGA-COAD dataset were included.

In Paper III, a total of 1758 CRC samples from public TCGA-COAD cohorts including five discovery cohorts, two training validation cohorts and one RNA-seq-based clinical cohort were included. Additionally, 19 plasma samples from CC patients and 9 plasma samples from healthy donors were used in the study.

In Paper IV, 314 female CRC patients were studied.

Methodology

All experiments were repeated at least three times.

In Paper I, using IHC, we analyzed the protein markers CD66b, $CysLT_1R$, and BDNF. To determine the transcriptional correlation between BDNF and $CYSLT_1R$, total RNA from colon tumor tissues and matched normal tissues from patients with CC was isolated according to the manufacturer's protocol. Real-time PCR was performed, the cDNA samples were normalized to HPRT1, and the results were prepared using the comparative threshold cycle (Ct) method. To study and validate the expression of related genes, we also used a published TCGA dataset. For in vivo experiments, two mouse models were used: colitis-associated mice lacking the cyslt1 gene (and reference mice) and a xenograft mouse model in which mice were administered the CC cell line SW480. We determined relevant gene and protein expression in vivo using qPCR, IHC, and Western blot.

In Paper II, using IHC, we stained colon tissue samples from 46 patients with CC and assessed TrkB expression based on the intensity of staining in the tumor tissue as well as adjacent normal tissue. The SW-480 CC cell line was cultured using RPMI-1640 medium with 10% fetal bovine serum (FBS). At 80% confluence, the cell culture media was changed to 0.5% FBS, and the cells were then treated with either recombinant BDNF or LTD_4 at different times. Treated cells were used to either analyze gene expression using qPCR or investigate the localization of receptors using immunofluorescence. A published TCGA dataset was used to evaluate the correlation of TrkB with $CysLT_1R$ mRNA levels. All the results were analyzed by suitable statistical methods.

In Paper III, a systematic gene expression assay was performed in 5 independents in-silico CRC cohorts, and 5 differentially expressed genes were detected in CC. mRNA analyses of the 5 significant differentially regulated genes (*BDNF*, *PTGS2*, *GSK3β*, *CTNNB1* and *HPGD*) were performed between the normal colon and tumor tissues to analyze patient 5-year survival. The results of the mRNA assays were normalized using RMA algorithms (multiarray average). Using Cox regression analysis, the risk scores were calculated based on survival analyses, and the prognostic utility of the selected genes was estimated in each dataset (using the Youden index value as a cutoff). Using IHC, primary tumor tissues and the adjacent mucosa were stained with antibodies against the proteins of interest, and the correlations between the mRNA expression of *BDNF* and 4 other genes *PTGS2*, *GSK3B, CTNNB1* (COX-2, GSK3β and β-catenin) and *HPGD* (15-PGDH) were analyzed. To evaluate our suggested five-gene signature as a diagnostic marker, plasma was collected from 19 CC patients and 9 healthy independent controls. According to the manufacturer's protocol, cell-free plasma was provided and stored at -80 °C in 1 ml of QIAzol reagent (Qiagen). In addition, 6 tissue samples and adjacent normal mucosa from patients with CC from Malmö Hospital were used. Total RNA isolated from tissue samples and plasma samples along with controls was sent to the Lund University sequencing facility for sequencing for transcriptome analysis. A sequencing library was prepared, and mRNA expression levels were measured by reads per million mRNAs mapped (RPM). To measure mRNA for the four-gene signature, total RNA in CC patient plasma and relevant controls was isolated with a RNeasy mini kit; the levels were normalized to β-actin and analyzed using qRT–PCR. The results were analyzed by statistical methods.

In Paper IV for in vitro experiments, four CC cell lines, SW-480, HCT-116, Caco-2, and HT-29, were treated with or without the ERβ agonist ER-041 and subjected to further tests to study CRC progression (migration, wound healing, clonogenic cell survival, cell survival, and colonosphere formation tests). *T*o analyze the expression of genes of interest in CC cell lines treated with or without ER-041, qRT–PCR was used. For in vivo studies, 3 different mouse models were used to confirm the correlation between ERβ and antitumor membrane-associated β-catenin, cysteinyl leukotriene receptor 2 ($CysLT_2R$), and 15-hydroxyprostaglandin dehydrogenase (15-PGDH), and a negative correlation of ERβ with nuclear β-catenin, cysteinyl leukotriene receptor 1 ($CysLT_1R$), and cyclooxygenase-2 (COX-2) was identified. A zebrafish study was conducted to study the antimetastatic effect of ERβ.

Results and Discussion

Paper I: Brain-Derived Neurotrophic Factor, Neutrophils and Cysteinyl Leukotriene Receptor 1 as Potential Prognostic Biomarkers for Patients with CC168.

Main findings:

A significant positive correlation between the levels of neutrophils and BDNF and between the levels of $CysLT_1R$ and BDNF was found (P = 0.02 and P = 0.03, respectively), as were correlations with CC patient prognosis.

There was significant downregulation of BDNF expression in mice lacking the cysltr1 gene and in a mouse xenograft model with human SW480 CC cells treated with the $CysLT_1R$ antagonist montelukast compared to that in wt mice and untreated mice, respectively (P<0.001).

Potential predictive value of CD66b, BDNF and $CysLT_1R$ expression as an independent prognostic predictor for CC progression with and without regard to clinicopathological factors (sex, TNM stage and LNM) was identified in CC patients (P= 0.01).

Previous studies have shown that high neutrophil infiltration in the tumor is associated with a poor prognosis for CC patients.

To study the role of tumor-associated neutrophils, we used the neutrophil marker CD66b in CC tissues and matched normal mucosa. Our results showed that tumor infiltration of neutrophils is higher than that in normal colon mucosa and associated with poor prognosis for CC patients.

We differentiated human leukemia cell lines (HL-60) into neutrophil-like cells (NLCs). NLCs were treated with LTD_4 or TNFα for 24 hours, and then the medium was collected and used to investigate cytokine secretion. Analyses of the data showed significant increases in both BDNF and $CysLT_1R$ secretion in NLC media (data not shown). Next, we examined the expression levels of CD66b, $CysLT_1R$, and BDNF in 56 tumor tissue samples from CC patients with stage I, II, and III disease from the Malmö CC cohort and observed a positive correlation between the expression of all three proteins. mRNA data from 197 CC patients from the TCGA database confirmed our finding.

Previously, our group showed that low levels of $CysLT_1R$ and high levels of $CysLT_2R$ are associated with a better prognosis for CC patients. In addition, BDNF, which is a nerve growth factor for the central nervous system, contributes to the differentiation, maturation, and survival of nerve cells. In cancer, BDNF binds to its high-affinity receptor TrkB, which phosphorylates and increases tumor cell proliferation and contributes to metastasis via epithelial-mesenchymal transition (EMT).

Interestingly, our results showed a low level of BDNF expression in the intestinal tissue of mice lacking the *cysltr1* gene. A mouse xenograft model with human cancer cells treated with montelukast, a leukotriene receptor antagonist, also showed lower levels of BDNF expression than the control group.

We also confirmed these results by measuring BDNF in mouse tissues using Western blotting analysis.

The results from the Malmö cohort were further validated with the TCGA cohort, in which high BDNF expression independently correlated with poorer OS in CC patients.

Sex, TNM stage, and LNM have been shown to be significantly associated with OS in patients with CC and are often used to estimate the prognosis of CC patients. We calculated the risk score for CD66b, $CysLT_1R$ and BDNF and clinical factors (sex, TNM stage and LNM) in stage I-III CC patients from the Malmö cohort. We found a strong correlation between the clinical factors and CD66b, $CysLT_1R$ and BDNF, which makes them possible candidate predictors for high-risk CC patients. We examined the three CD66b, $CysLT_1R$ and BDNF signature markers in both the Malmö cohort and TCGA (mRNA) datasets, and the results indicated that the signature containing CD66b, $CysLT_1R$ and BDNF had significant positive value and high accuracy as a predictor for high-risk CC patients. This signature in combination with clinical factors obtained a high AUC (AUC = 0.81), implying high accuracy, sensitivity and specificity of the three indicators that therefore may be able to be used in combination as a marker for high-risk CC patients.

What is new:

To the best of our knowledge, the study showed, for the first time, a significant positive correlation between CD66b, BDNF, and $CysLT_1R$ expression in CC.

Our data revealed the CD66b/BDNF/$CysLT_1R$ signature as an independent prognostic biomarker for CC patient survival.

We reported that the *CD66b/BDNF/CYSLTR1* genes signature along with clinical features (TNM stage and LNM) might be valuable as a prognostic marker to identify patients with poor prognosis.

Paper II: Cytosolic expression of the brain-derived neurotrophic factor receptor TrkB and nuclear cysteinyl leukotriene receptor 1 goes with poor prognosis for CC patients.

Main findings:

Significantly higher expression of TrkB in the normal colon mucosa compared to colon tumors was found in CC patients (P= 0.0002).

No correlation between TrkB expression and CC patient overall survival was found (P= 0.3).

LTD_4 induced increased *NTRK2* (TrkB) gene expression in human SW480 CC cells.

We found a significant correlation between *NTRK2* and *CYSLTR1* gene expression in TCGA-COAD (P= 0.001).
There was nuclear accumulation of phosphorylated TrkB in SW480 CC cells (P= 0.006).

This study is part of an ongoing project aimed at investigating the expression and subcellular localization of TrkB in CC in the presence or absence of its ligand, BDNF. In addition, the biological effects of the activated isoform of TrkB in human CC were assessed.

Research has indicated that targeted therapy against TrkB/BDNF greatly reduces tumor growth in patients with CC. The colon is known as a site for the expression of neurotrophins and their receptors. In the study, TrkB expression was analyzed in both colon tumor cells and normal matched colon tissues from patients with CC, as well as in the SW480 CC cell line. High TrkB expression intensity was observed in only 6% of tumor tissues, while 24% of normal colon tissues from the same patient showed high TrkB expression. The expression level of the tyrosine kinase receptor did not show any effect on CC patient OS. Our preliminary data showed that the effect of TrkB expression in CC patients may be mostly due to the localization of the receptor on tumor cells rather than the total amount of TrkB expression. We showed that TrkB is abundantly expressed in CC cells, and the expression of TrkB when CC cells were stimulated with BDNF or LTD_4, a high-affinity ligand for $CysLT_1R$, increased in these cells.

Extensive research has been performed on TrkB's extracellular domain, which has a high affinity for BDNF. However, TrkB can also be activated by other ligands. Our data revealed that stimulation of cancer cells with LTD_4 might cause TrkB phosphorylation.

Studies have shown that TrkB can possibly be activated by GPCRs. Activated TrkB can stimulate an intracellular mitogenic cascade, followed by a downstream signaling pathway through the well-characterized PI3K/Akt pathway, causing uncontrolled growth of cancer cells.

In the current study, using a published available TCGA-COAD cohort, we showed a positive correlation between *NTRK2* and *CYSLTR1* gene expression in CC patients. The same correlation was observed between TrkB and $CysLT_1R$ protein expression in colon tumor tissues from CC patients belonging to the Malmö cohort. However, whether LTD_4 activates TrkB is still unclear. TrkB has several different isoforms, including a full-length (TrkB-FL), a truncated membrane receptor (TrkB-T), and an intracellular fragment (TrkB-ICD) isoform. In CRC, two different domains of TrkB have been found mutated, whereas in lung cancer, the extracellular domain of the TrkB receptor seems to be mutated. Using IHC, we showed a similar pattern with high expression of phosphorylated TrkB in healthy tissues from the skin, breast, prostate colon, kidney, and uterus.

To investigate the localization of TrkB expression in CC cells, we treated SW480 CC cells with recombinant BDNF (rBDNF) at different time points. We found the appearance of phosphorylated TrkB receptors after 10 minutes of treatment, with accumulation in the cytoplasm. Our results indicated translocation of the pTrkB receptor to the nucleus after stimulation of SW-480 CC cells with rBDNF for 20 minutes.

Research questions and future plans

Is the localization of TrkB in the nucleus in CC cells induced by soluble factors from other cell types? If it is, which of the TrkB domains are activated?

How does blocking BDNF and LTD_4 affect CRC progression?

Paper III: Identification of a novel five-gene signature as a prognostic and diagnostic biomarker in colorectal cancers.

Main findings:

A promising role of a gene signature containing *BDNF, PTGS2, CTNNB1, HPGD* and *GSK3B* as independent biomarker of OS and RFS in patients with CRC was identified.

The five-gene signature alone was superior to the tumor stage for prognostic evaluation, and the results differed slightly from those obtained using TNM stage and lymph node metastasis.

A significant AUC value (>0.75) indicated a good prognostic ability of the five-gene signature.

Significant upregulation of mRNA expression of the four genes, *BDNF, PTGS2, CTNNB1*, and *GSK3B*, was found in CRC patient plasma samples.

To investigate whether our four-gene signature is a suitable blood-based diagnostic predictive marker, assessment of OS and RFS of patients and an extensive study of blood samples from CRC patients are required.

The five-year survival drops from 80% for patients in the early stages to 63% for patients with CRC in the later stages of the disease. Colonoscopy screening is currently the most accurate diagnostic tool for CRC patients, but given its invasiveness and associated socioeconomic issues, the need to find non-invasive blood-based methods to reach more individuals for CRC screening is substantial.

A reproducible and robust gene signature can be very helpful for a more precise clinical diagnosis of the disease. In this study, we performed a comprehensive assessment of transcriptome profiling data and found a novel signature for the early diagnosis and prognosis of CRC patients. We used five in-silico CRC cohorts as discovery, training, and validation cohorts to identify gene signatures based on changes in *BDNF* mRNA expression in CRC. All data were filtered, and differential gene expression analyses were performed in five independent in-silico CRC cohorts to create the associated gene signature for CRC, with genes considered significant if their expression was more than log2(fold change) upregulated or downregulated in tumor tissues compared with adjacent normal mucosa.

Using the Gene Ontology database and the discovery cohort, 32 cancer-related and dysregulated genes, including five genes (*BDNF*, *PTGS2, CTNNB1*, *HPGD*, and *GSK3B*), were initially identified. All the genes were associated with the overall survival of CRC patients ($P<0.05$).

For prognostic assessment and to identify the expression level of the five selected genes in adjacent normal and tumor samples from CRC patients, we used in-silico detection cohorts. Four genes (*BDNF*, *PTGS2*, *CTNNB1*, and *GSK3B*) were significantly upregulated and one gene (*HPGD*) was significantly downregulated in primary tumor tissues compared to adjacent normal tissues in all five in-silico datasets. Correlation matrices and heatmaps were generated for the selected markers, and we found a significant association between *BDNF* mRNA expression and all four other genes in primary tumor tissues compared with adjacent colon mucosa and colon mucosa from healthy donors.

We determined the risk scores for the five selected genes, compared mRNA expression in high-risk versus low-risk CRC patients using the Youden index cutoff from the training TCGA dataset (N = 324), and built a prognostic model to calculate the risk scores for selected genes for CRC patients with stage I, II and III disease. We found that

patients who belonged to the high-risk group had a worse OS than those who belonged to the low-risk group. Using data from the in-silico cohort, we compared the prognostic performance of the risk scores for the five selected genes and found a significant improvement in the risk scores for each individual marker. Our data indicated that relapse-free survival for patients in the high-risk group was significantly worse than that for patients in the low-risk group. Nonsurvivors and recurrent CRC patients had significantly high-risk scores for the five selected genes in this training TCGA-COAD patient cohort.

To validate the prognostic utility of the five-gene risk score, we employed a validation cohort and assigned patients to high and low risk score groups with the same cutoff used for the in-silico training cohort. High-risk patients had worse OS than low-risk patients (HR = 3.36, P = 0.01). We obtained the same results in the in-silico validation cohort. Time-dependent ROC curves revealed a high AUC value of 0.82, which confirmed the predictive accuracy of our suggested model. The ROC analysis showed that the five-gene panel with the clinical risk stratification system was significantly correlated with OS (AUC = 0.89, P<0.001).

To validate our biomarker panel, we used a training cohort with plasma sample and clinical data. The level of *HPGD* gene expression was considered insufficient to determine the validity of the biomarker panel in plasma, and therefore, the gene was removed from the five investigated genes.

Furthermore, to evaluate our selected genes as an mRNA-based signature, we used 6 colon tumors and 6 matched normal tissues for RNA-seq-based mRNA expression profiling. All four genes showed significant upregulation in tumor tissues.

Next, based on our mRNA-seq data, we measured the levels of the 4 selected genes (*BDNF*, *PTGS2*, *CTNNB1*, and *GSK3B*) in a plasma-based microarray in an in-silico training cohort, and the results showed significant upregulation of the 4 genes in the cohort.

To estimate the robustness of our four-gene panel, we selected a clinical validation cohort with 19 CRC cases and 9 healthy independent controls. Upregulation of all 4 genes was significant in the CRC patient plasma. The results revealed a robust AUC value of 0.83 (P<0.0001) in CRC patients.

What is new:

The study proposed a five-gene signature (*BDNF, PTGS2, CTNNB1, HPGD, and GSK3B*) that was found to be an improved potent prognostic biomarker compared to currently available clinical pathological risk factors.

Based on the results obtained, the gene signature is a potential independent predictor of tumor recurrence, which, in combination with TNM stage and LNM, can offer a more personalized risk assessment in patients with stage II/III CRC.

Upregulation of 4 of the 5 selected mRNAs in plasma from CRC patients was correlated with BDNF, and these factors could be used either alone or in combination with *PTGS2, CTNNB1, and GSK3B* as diagnostic markers for CRC.

Research questions and future plans

Large-scale cohort validation of the diagnostic utility of our suggested 4-gene signature as an affordable, noninvasive CRC screening marker is warranted.

Paper IV: Tumor-suppressive effect of estrogen receptor β in colorectal cancer patients, CC cells, and a zebrafish model[169].

Main findings:

There was a negative correlation between Erβ and the tumorigenesis markers $CysLT_1R$, β-catenin and COX-2.

There was a positive correlation between Erβ and the antitumor mediators $CysLT_2R$ and 15-PGDH.

Erβ and $CysLT_2R$ levels are positively associated with CRC OS and DFS.
The Erβ agonist ErB-041 reduced CC cell migration and colony formation and cell survival but induced apoptosis of human CC cells.

In vivo models showed a suppressive effect of Erβ on CC metastasis.

Our group, like others, has previously reported the antitumor effects of ERβ in CRC, showing that high expression of ERβ is correlated with better OS and DFS in women suffering from the disease.

In this study, we investigated the antitumor effect of ERβ and its selective agonist ERB-041. Using in-silico, in vitro and in vivo experiments, we showed that the presence of ERβ reduced cell proliferation, migration and invasion. IHC analysis revealed that women with CRC who had higher expression levels of ERβ had significantly higher levels of membrane β-catenin, $CysLT_2R$ and 15-PGDH, all of which have antitumor effects, and lower levels of nuclear β-catenin, $CysLT_1R$ and cyclooxygenase-2 (COX-2), which have tumor-promoting effects.

In vitro experiments with three different CC cell lines showed that ERβ was significantly positively correlated with $CysLT_2R$, membrane β-catenin and 15-PGDH and negatively correlated with $CysLT_1R$, nuclear β-catenin and COX-2. In addition, CC cells treated with the ERβ agonist ERB-041 showed a decrease in $CysLT_1R$, active β-catenin and COX-2 levels but increased levels of $CysLT_2R$ and 15-PGDH, as well as

increased apoptosis, compared to untreated control groups. IHC staining of the intestine of the CAC mouse model lacking the cysltr1 gene showed higher expression of ERβ, while the CAC mouse model lacking the cysltr2 gene showed lower expression of ERβ. The APCmin/+ spontaneous mouse model, with activated Wnt-β-catenin signaling, showed prevented phosphorylation and degradation of β-catenin, which caused its translocation into the cell nucleus. Using a zebrafish xenograft model, we found less distant metastases in fish treated with ERB-041 than in vehicle-treated fish.

What is new:

Induction of ERβ expression is significantly correlated with antitumorigenic activity and results in a reduced metastatic burden in CRC. Our results support the hypothesis that the ERβ agonist ERB-041 has a suppressive effect on tumor cells and is beneficial for CRC patients

Conclusions

Paper I. High expression of CD66b, $CysLT_1R$, and BDNF in human tumor tissues indicated worse overall survival for CC patients. There was a positive correlation between $CysLT_1R$ and BDNF in CC, where BDNF expression was affected by the presence or absence of $CysLT_1R$ in the TME. The data suggest that *CD66b, CYSLTR1*, and *BDNF* gene signatures may have prognostic value as predictive biomarkers for CC and may be an appropriate option for identifying high-risk CC patients in the early stages of the disease.

Paper II. We found no association between TrkB expression levels and the overall survival of CC patients. In in vitro experiments, our results showed an increase in TRKB mRNA expression levels in SW480 CC cells during rBDNF or LTD_4 stimulation. In addition, we found different degrees of phosphorylated TrkB translocation into the cytosol and nucleus in CC cells at different times after exposure of SW cells to rBDNF. The duration of stimulation of CC cells with rBDNF may play a role in the cytosolic or nuclear localization of the TrkB receptor. Further studies are needed to investigate the localization of TrkB receptors in CC cells and their effects on tumor cell progression.

Paper III. The main findings of this project confirm that the five-gene signature (*BDNF, PTGS2, CTNNB1, HPGD, and GSK3B*) is an effective stratification marker for patients with stage I, II, and III CRC who are at risk of relapse based on their clinical results. This tool could be a new prognostic biomarker with much higher accuracy than the currently used biomarkers for prognostication. Considering the five-gene signature in combination with clinically validated progression-related risk factors such as TNM stage and LNM can provide an accurate and noninvasive screening strategy to assess personal risk. The presence of four out of five gene markers in CRC tissues in the plasma of CRC patients, which can be easily assessed in clinical practice, was correlated with BDNF, and these factors with significant plasma expression levels could be an effective, robust tools for CRC screening.

Paper IV. The survival of female CRC patients with high ERβ expression was longer than that of patients with lower ERβ expression. ERβ and its agonist ERB-041 reduced cell proliferation, migration and invasion in both in vitro and in vivo experiments on CC cells. Women with CRC who had higher expression levels of ERβ also had higher levels of antitumor factors such as membrane β-catenin, $CysLT_2R$ and 15-PGDH, and tumor-enhancing effects were shown to be lower due to lower expression levels of nuclear β-catenin, $CysLT_1R$ and COX-2. In addition, xenograft zebrafish treated with ERB-041 showed fewer distant metastases. In summary, we believe that ERβ has an

antitumor effect in CRC and that its agonist ERb-041 may be a treatment for CRC patients.

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References

1. Rawla P, Sunkara T, Barsouk A. Epidemiology of colorectal cancer: incidence, mortality, survival, and risk factors. doi:10.5114/pg.2018.81072
2. American Cancer Society. Colorectal Cancer Facts and Figures 2020-2022. *Am cancer Soc*. 2020;66(11).
3. Lo SH, Halloran S, Snowball J, Seaman H, Wardle J, Von Wagner C. Colorectal cancer screening uptake over three biennial invitation rounds in the English bowel cancer screening programme. *Gut*. 2015;64(2). doi:10.1136/gutjnl-2013-306144
4. Jemal A, Siegel R, Ward E, Murray T, Xu J, Thun MJ. Cancer Statistics, 2007. *CA Cancer J Clin*. 2007;57(1):43-66. doi:10.3322/canjclin.57.1.43
5. Hong Y, Kim J, Choi YJ, Kang JG. Clinical study of colorectal cancer operation: Survival analysis. *Korean J Clin Oncol*. 2020;16(1). doi:10.14216/kjco.20002
6. Varghese A. Chemotherapy for Stage II Colon Cancer. *Clin Colon Rectal Surg*. 2015;28(4). doi:10.1055/s-0035-1564430
7. Fujikawa H, Tanaka K, Toiyama Y, et al. High TrkB expression levels are associated with poor prognosis and EMT induction in colorectal cancer cells. *J Gastroenterol*. 2012;47(7). doi:10.1007/s00535-012-0532-0
8. Nagai H, Kim YH. Cancer prevention from the perspective of global cancer burden patterns. *J Thorac Dis*. 2017;9(3). doi:10.21037/jtd.2017.02.75
9. Chen X, Gole J, Gore A, et al. Non-invasive early detection of cancer four years before conventional diagnosis using a blood test. *Nat Commun*. 2020;11(1). doi:10.1038/s41467-020-17316-z
10. Hanahan D, Weinberg RA. Hallmarks of cancer: The next generation. *Cell*. 2011;144(5):646-674. doi:10.1016/j.cell.2011.02.013
11. Papavramidou N, Papavramidis T, Demetriou T. Ancient greek and greco-Roman methods in modern surgical treatment of cancer. *Ann Surg Oncol*. 2010;17(3). doi:10.1245/s10434-009-0886-6
12. Chintamani. The paradigm shifts in the management of breast cancer—have we finally arrived? *Indian J Surg*. 2013;75(6). doi:10.1007/s12262-013-1022-1
13. Balmain A. Cancer genetics: From Boveri and Mendel to microarrays. *Nat Rev Cancer*. 2001;1(1). doi:10.1038/35094086
14. Baylin SB, Jones PA. Epigenetic determinants of cancer. *Cold Spring Harb Perspect Biol*. 2016;8(9). doi:10.1101/cshperspect.a019505
15. Farnsworth RH, Lackmann M, Achen MG, Stacker SA. Vascular remodeling in cancer. *Oncogene*. 2014;33(27). doi:10.1038/onc.2013.304

16. Taylor BS, Barretina J, Maki RG, Antonescu CR, Singer S, Ladanyi M. Advances in sarcoma genomics and new therapeutic targets. *Nat Rev Cancer*. 2011;11(8). doi:10.1038/nrc3087
17. Luzzi KJ, MacDonald IC, Schmidt EE, et al. Multistep nature of metastatic inefficiency: Dormancy of solitary cells after successful extravasation and limited survival of early micrometastases. *Am J Pathol*. 1998;153(3). doi:10.1016/S0002-9440(10)65628-3
18. Binnewies M, Roberts EW, Kersten K, et al. Understanding the tumor immune microenvironment (TIME) for effective therapy. *Nat Med*. 2018;24(5). doi:10.1038/s41591-018-0014-x
19. Society AC. Colorectal Cancer Facts and Figures 2020-2022. *Am cancer Soc*. 2020;66(11).
20. Cancerfonden. Cancerfonden. Published 2020. https://www.cancerfonden.se/om-cancer/statistik/tjocktarmscancer
21. Koo JH, Leong RWL. Sex differences in epidemiological, clinical and pathological characteristics of colorectal cancer. *J Gastroenterol Hepatol*. 2010;25(1). doi:10.1111/j.1440-1746.2009.05992.x
22. Tanaka T. Colorectal carcinogenesis: Review of human and experimental animal studies. *J Carcinog*. 2009;8. doi:10.4103/1477-3163.49014
23. Kim ER, Chang DK. Colorectal cancer in inflammatory bowel disease: The risk, pathogenesis, prevention and diagnosis. *World J Gastroenterol*. 2014;(29). doi:10.3748/wjg.v20.i29.9872
24. Itzkowitz SH, Yio X. Inflammation and cancer - IV. Colorectal cancer in inflammatory bowel disease: The role of inflammation. *Am J Physiol - Gastrointest Liver Physiol*. 2004;287(1 50-1). doi:10.1152/ajpgi.00079.2004
25. Stigliano V, Sanchez-Mete L, Martayan A, Anti M. Early-onset colorectal cancer: A sporadic or inherited disease? *World J Gastroenterol*. 2014;20(35). doi:10.3748/wjg.v20.i35.12420
26. Qu X, Tang Y, Hua S. Immunological Approaches Towards Cancer and Inflammation: A Cross Talk. *Front Immunol*. 2018;9:563. doi:10.3389/fimmu.2018.00563
27. Siegel RL, Miller KD, Sauer AG, et al. Colorectal cancer statistics, 2020. *CA Cancer J Clin*. 2020;70(3). doi:10.3322/caac.21601
28. Issa IA, NouredDine M. Colorectal cancer screening: An updated review of the available options. *World J Gastroenterol*. 2017;23(28). doi:10.3748/wjg.v23.i28.5086
29. Cheng LK, O'Grady G, Du P, Egbuji JU, Windsor JA, Pullan AJ. Gastrointestinal system. *Wiley Interdiscip Rev Syst Biol Med*. 2010;2(1). doi:10.1002/wsbm.19
30. Leng-Peschlow E. Acceleration of large intestine transit time in rats by sennosides and related compounds. *J Pharm Pharmacol*. 1986;38(5). doi:10.1111/j.2042-7158.1986.tb04589.x
31. Kimchi ET, Gusani NJ, Kaifi JT. Anatomy and physiology of the small intestine. *Greenfield's Surg Sci Princ Pract Fifth Ed*. Published online 2012. doi:10.1016/b978-0-323-40232-3.00071-6
32. Rose C, Parker A, Jefferson B, Cartmell E. The characterization of feces and urine: A review of the literature to inform advanced treatment technology. *Crit Rev Environ Sci Technol*. 2015;45(17). doi:10.1080/10643389.2014.1000761

33. Barker N, Wetering M Van De, Clevers H. The intestinal stem cell. *Genes Dev*. 2008;22(14). doi:10.1101/gad.1674008
34. Umar S. Intestinal stem cells. *Curr Gastroenterol Rep*. 2010;12(5). doi:10.1007/s11894-010-0130-3
35. Sheridan BS, Lefrançois L. Intraepithelial lymphocytes: To serve and protect. *Curr Gastroenterol Rep*. 2010;12(6). doi:10.1007/s11894-010-0148-6
36. Jaladanki RN, Wang J-Y. Regulation of Gastrointestinal Mucosal Growth. *Colloq Ser Integr Syst Physiol From Mol to Funct*. 2011;3(2). doi:10.4199/c00028ed1v01y201103isp015
37. Okumura R, Takeda K. Maintenance of intestinal homeostasis by mucosal barriers. *Inflamm Regen*. 2018;38(1). doi:10.1186/s41232-018-0063-z
38. Cheng Y, Ling Z, Li L. The Intestinal Microbiota and Colorectal Cancer. *Front Immunol*. 2020;11. doi:10.3389/fimmu.2020.615056
39. Thursby E, Juge N. Introduction to the human gut microbiota. *Biochem J*. 2017;474(11). doi:10.1042/BCJ20160510
40. Browne HP, Neville BA, Forster SC, Lawley TD. Transmission of the gut microbiota: Spreading of health. *Nat Rev Microbiol*. 2017;15(9). doi:10.1038/nrmicro.2017.50
41. Rowland I, Gibson G, Heinken A, et al. Gut microbiota functions: metabolism of nutrients and other food components. *Eur J Nutr*. 2018;57(1). doi:10.1007/s00394-017-1445-8
42. Kho ZY, Lal SK. The Human Gut Microbiome – A Potential Controller of Wellness and Disease. *Front Microbiol*. 2018;9:1835. doi:10.3389/fmicb.2018.01835
43. Alberts B, A J, J L. Analyzing Protein Structure and Function. *Mol Biol Cell*. Published online 2002.
44. Brinkmann V, Reichard U, Goosmann C, et al. Neutrophil Extracellular Traps Kill Bacteria. *Science (80-)*. 2004;303(5663). doi:10.1126/science.1092385
45. Rosales C. Neutrophil: A cell with many roles in inflammation or several cell types? *Front Physiol*. 2018;9(FEB). doi:10.3389/fphys.2018.00113
46. Quail DF, Joyce JA. Microenvironmental regulation of tumor progression and metastasis. *Nat Med*. 2013;19(11). doi:10.1038/nm.3394
47. Uribe-Querol E, Rosales C. Neutrophils in cancer: Two sides of the same coin. *J Immunol Res*. 2015;2015. doi:10.1155/2015/983698
48. Fridlender ZG, Sun J, Kim S, et al. Polarization of Tumor-Associated Neutrophil Phenotype by TGF-β: "N1" versus "N2" TAN. *Cancer Cell*. 2009;16(3). doi:10.1016/j.ccr.2009.06.017
49. Masucci MT, Minopoli M, Carriero MV. Tumor Associated Neutrophils. Their Role in Tumorigenesis, Metastasis, Prognosis and Therapy. *Front Oncol*. 2019;9. doi:10.3389/fonc.2019.01146
50. Campregher C, Luciani MG, Gasche C. Activated neutrophils induce an hMSH2-dependent G2/M checkpoint arrest and replication errors at a (CA)13-repeat in colon epithelial cells. *Gut*. 2008;57(6). doi:10.1136/gut.2007.141556
51. Shang K, Bai YP, Wang C, et al. Crucial Involvement of Tumor-Associated Neutrophils in the Regulation of Chronic Colitis-Associated Carcinogenesis in Mice. *PLoS One*. 2012;7(12). doi:10.1371/journal.pone.0051848

52. Chen L, Deng H, Cui H, et al. Inflammatory responses and inflammation-associated diseases in organs. *Oncotarget*. 2018;9(6). doi:10.18632/oncotarget.23208
53. Oeckinghaus A, Hayden MS, Ghosh S. Crosstalk in NF-κB signaling pathways. *Nat Immunol*. 2011;12(8). doi:10.1038/ni.2065
54. Maloy KJ, Powrie F. Intestinal homeostasis and its breakdown in inflammatory bowel disease. *Nature*. 2011;474(7351). doi:10.1038/nature10208
55. Hendrickson BA, Gokhale R, Cho JH. Clinical aspects and pathophysiology of inflammatory bowel disease. *Clin Microbiol Rev*. 2002;15(1). doi:10.1128/CMR.15.1.79-94.2002
56. Watanabe T, Konishi T, Kishimoto J, Kotake K, Muto T, Sugihara K. Ulcerative colitis-associated colorectal cancer shows a poorer survival than sporadic colorectal cancer: A nationwide Japanese study. *Inflamm Bowel Dis*. 2011;17(3). doi:10.1002/ibd.21365
57. Castaño-Milla C, Chaparro M, Gisbert JP. Systematic review with meta-Analysis: The declining risk of colorectal cancer in ulcerative colitis. *Aliment Pharmacol Ther*. 2014;39(7). doi:10.1111/apt.12651
58. Jurjus A, Eid A, Kattar S Al, et al. Inflammatory bowel disease, colorectal cancer and type 2 diabetes mellitus: The links. *BBA Clin*. 2016;5. doi:10.1016/j.bbacli.2015.11.002
59. Romano M, Francesco F De, Gringeri E, et al. Tumor Microenvironment Versus Cancer Stem Cells in Cholangiocarcinoma: Synergistic Effects? *J Cell Physiol*. 2016;231(4). doi:10.1002/jcp.25190
60. Whiteside TL. Immune suppression in cancer: Effects on immune cells, mechanisms and future therapeutic intervention. *Semin Cancer Biol*. 2006;16(1). doi:10.1016/j.semcancer.2005.07.008
61. Dvorak HF. Tumors: wounds that do not heal. Similarities between tumor stroma generation and wound healing. *N Engl J Med*. 1986;315(26). doi:10.1056/NEJM198612253152606
62. Lança T, Silva-Santos B. The split nature of tumor-infiltrating leukocytes: Implications for cancer surveillance and immunotherapy. *Oncoimmunology*. 2012;1(5). doi:10.4161/onci.20068
63. Li J, Yuan Y, Yang F, et al. Expert consensus on multidisciplinary therapy of colorectal cancer with lung metastases (2019 edition). *J Hematol Oncol*. 2019;12(1). doi:10.1186/s13045-019-0702-0
64. Testa U, Pelosi E, Castelli G. Colorectal Cancer: Genetic Abnormalities, Tumor Progression, Tumor Heterogeneity, Clonal Evolution and Tumor-Initiating Cells. *Med Sci*. 2018;6(2). doi:10.3390/medsci6020031
65. Song M, Emilsson L, Bozorg SR, et al. Risk of colorectal cancer incidence and mortality after polypectomy: a Swedish record-linkage study. *Lancet Gastroenterol Hepatol*. 2020;5(6). doi:10.1016/S2468-1253(20)30009-1
66. 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):209-249. doi:10.3322/caac.21660
67. Hull R, Francies FZ, Oyomno M, Dlamini Z. Colorectal cancer genetics, incidence and risk factors: In search for targeted therapies. *Cancer Manag Res*. 2020;12. doi:10.2147/CMAR.S251223

68. Haggar FA, Boushey RP. Colorectal Cancer Epidemiology: Incidence, Mortality, Survival, and Risk Factors. doi:10.1055/s-0029-1242458

69. Terdiman JP, Conrad PG, Sleisenger MH. Genetic testing in hereditary colorectal cancer: Indications and procedures. *Am J Gastroenterol*. 1999;94(9). doi:10.1111/j.1572-0241.1999.01356.x

70. Chen X, Jansen L, Guo F, Hoffmeister M, Chang-Claude J, Brenner H. Smoking, Genetic Predisposition, and Colorectal Cancer Risk. *Clin Transl Gastroenterol*. 2021;12(3). doi:10.14309/ctg.0000000000000317

71. Fearon ER, Vogelstein B. A genetic model for colorectal tumorigenesis. *Cell*. 1990;61(5). doi:10.1016/0092-8674(90)90186-I

72. Johns LE, Houlston RS. A systematic review and meta-analysis of familial colorectal cancer risk. *Am J Gastroenterol*. 2001;96(10). doi:10.1111/j.1572-0241.2001.04677.x

73. Bishehsari F, Mahdavinia M, Vacca M, Malekzadeh R, Mariani-Costantini R. Epidemiological transition of colorectal cancer in developing countries: Environmental factors, molecular pathways, and opportunities for prevention. *World J Gastroenterol*. 2014;20(20). doi:10.3748/wjg.v20.i20.6055

74. Jasperson KW, Tuohy TM, Neklason DW, Burt RW. Hereditary and Familial Colon Cancer. *Gastroenterology*. 2010;138(6). doi:10.1053/j.gastro.2010.01.054

75. Nelson S, Näthke IS. Interactions and functions of the adenomatous polyposis coli (APC) protein at a glance. *J Cell Sci*. 2013;126(Pt 4):873-877. doi:10.1242/JCS.100479

76. Shang S, Hua F, Hu ZW. The regulation of β-catenin activity and function in cancer: therapeutic opportunities. *Oncotarget*. 2017;8(20):33972. doi:10.18632/ONCOTARGET.15687

77. Lynch HT, de la Chapelle A. Hereditary colorectal cancer. Guttmacher AE, Collins FS, eds. *N Engl J Med*. 2003;348(10):919-932. doi:10.1056/NEJMRA012242

78. Duan FX, Gu GL, Yang HR, Yu PF, Zhang Z. Must Peutz-Jeghers syndrome patients have the LKB1/STK11 gene mutation? A case report and review of the literature. *World J Clin Cases*. 2018;6(8). doi:10.12998/wjcc.v6.i8.224

79. Armaghany T, Wilson JD, Chu Q, Mills G. Genetic alterations in colorectal cancer. *Gastrointest Cancer Res*. 2012;5(1):19-27. Accessed December 17, 2021. https://pubmed.ncbi.nlm.nih.gov/22574233/

80. Lengauer C, Kinzler KW, Vogelstein B. Genetic instability in colorectal cancers. *Nature*. 1997;386(6625). doi:10.1038/386623a0

81. Clevers H. Wnt/beta-catenin signaling in development and disease. *Cell*. 2006;127(3):469-480. doi:10.1016/J.CELL.2006.10.018

82. Behrens J, Von Kries JP, Kühl M, et al. Functional interaction of beta-catenin with the transcription factor LEF-1. *Nature*. 1996;382(6592):638-642. doi:10.1038/382638A0

83. Filippo C De, Luceri C, Caderni G, et al. Mutations of the APC gene in human sporadic colorectal cancers. *Scand J Gastroenterol*. 2002;37(9). doi:10.1080/003655202320378248

84. Hampel H, Frankel WL, Martin E, et al. Screening for the Lynch Syndrome (Hereditary Nonpolyposis Colorectal Cancer). *N Engl J Med*. 2005;352(18). doi:10.1056/nejmoa043146

85. Poulogiannis G, Frayling IM, Arends MJ. DNA mismatch repair deficiency in sporadic colorectal cancer and Lynch syndrome. *Histopathology*. 2010;56(2). doi:10.1111/j.1365-2559.2009.03392.x

86. Umar A, Boland CR, Terdiman JP, et al. Revised Bethesda Guidelines for hereditary nonpolyposis colorectal cancer (Lynch syndrome) and microsatellite instability. *J Natl Cancer Inst*. 2004;96(4). doi:10.1093/jnci/djh034

87. Mundade R, Imperiale TF, Prabhu L, Loehrer PJ, Lu T. Genetic pathways, prevention, and treatment of sporadic colorectal cancer. *Oncoscience*. 2014;1(6). doi:10.18632/oncoscience.59

88. Guinney J, Dienstmann R, Wang X, et al. The consensus molecular subtypes of colorectal cancer. *Nat Med*. 2015;21(11). doi:10.1038/nm.3967

89. Hamilton W, Round A, Sharp D, Peters TJ. Clinical features of colorectal cancer before diagnosis: A population-based case-control study. *Br J Cancer*. 2005;93(4). doi:10.1038/sj.bjc.6602714

90. Safaee A, Moghimi-Dehkordi B, Fatemi SR, Ghiasi S, Nemati-Malek F, Zali MR. Characteristics of colorectal mucinous adenocarcinoma in Iran. *Asian Pac J Cancer Prev*. 2010;11(5):1373-1375. Accessed December 17, 2021. https://pubmed.ncbi.nlm.nih.gov/21198295/

91. American Cancer Society. Colorectal Cancer Risk Factors. Published 2021. https://www.cancer.org/cancer/colon-rectal-cancer/causes-risks-prevention/risk-factors.html

92. American Cancer Society. Colorectal Cancer Screening Tests. Published 2021. https://www.cancer.org/cancer/colon-rectal-cancer/detection-diagnosis-staging/screening-tests-used.html

93. Weiser MR. AJCC 8th Edition: Colorectal Cancer. *Ann Surg Oncol*. 2018;25(6):1454-1455. doi:10.1245/s10434-018-6462-1

94. Terán MD, Brock M V. Staging lymph node metastases from lung cancer in the mediastinum. *J Thorac Dis*. 2014;6(3):230-236. doi:10.3978/J.ISSN.2072-1439.2013.12.18

95. Xie YH, Chen YX, Fang JY. Comprehensive review of targeted therapy for colorectal cancer. *Signal Transduct Target Ther*. 2020;5(1). doi:10.1038/s41392-020-0116-z

96. Liu Q, Luo D, Cai S, Li Q, Li X. P–TNM staging system for colon cancer: Combination of P-stage and AJCC TNM staging system for improving prognostic prediction and clinical management. *Cancer Manag Res*. 2018;10. doi:10.2147/CMAR.S165188

97. Leung U, Gönen M, Allen PJ, et al. Colorectal cancer liver metastases and concurrent extrahepatic disease treated with resection. *Ann Surg*. 2017;265(1). doi:10.1097/SLA.0000000000001624

98. Xing M, Kooby DA, El-Rayes BF, Kokabi N, Camacho JC, Kim HS. Locoregional therapies for metastatic colorectal carcinoma to the liver - An evidence-based review. *J Surg Oncol*. 2014;110(2). doi:10.1002/jso.23619

99. Sharma S V, Lee DY, Li B, et al. A Chromatin-Mediated Reversible Drug-Tolerant State in Cancer Cell Subpopulations. *Cell*. 2010;141(1). doi:10.1016/j.cell.2010.02.027

100. Nowell PC. The clonal evolution of tumor cell populations. *Science (80-)*. 1976;194(4260). doi:10.1126/science.959840

101. Nikolouzakis TK, Vassilopoulou L, Fragkiadaki P, et al. Improving diagnosis, prognosis and prediction by using biomarkers in CRC patients (Review). *Oncol Rep*. 2018;39(6). doi:10.3892/or.2018.6330
102. Lippi G, Somma S Di, Plebani M. Biomarkers in the emergency department. Handle with care. *Clin Chem Lab Med*. 2014;52(10). doi:10.1515/cclm-2014-0726
103. Mueller WH. Biological markers in epidemiology. Edited by B. S. Hulka, T. C. Wilcosky, and J. D. Griffith. xi + 236 pp. New York: Oxford University Press, 1990, $40.00 (cloth). *Am J Hum Biol*. 1991;3(2). doi:10.1002/ajhb.1310030225
104. Hauptman N, Glavač D. Colorectal Cancer Blood-Based Biomarkers. *Gastroenterol Res Pract*. 2017;2017. doi:10.1155/2017/2195361
105. Hamon S, Dussert S, Deu M, et al. Effects of quantitative and qualitative principal component score strategies on the structure of coffee, rubber tree, rice and sorghum core collections. In: *Genetics Selection Evolution*. Vol 30. ; 1998. doi:10.1186/1297-9686-30-s1-s237
106. Sidransky D. Emerging molecular markers of cancer. *Nat Rev Cancer*. 2002;2(3). doi:10.1038/nrc755
107. Alix-Panabières C, Pantel K. Clinical applications of circulating tumor cells and circulating tumor DNA as liquid biopsy. *Cancer Discov*. 2016;6(5). doi:10.1158/2159-8290.CD-15-1483
108. Huang Z, Huang D, Ni S, Peng Z, Sheng W, Du X. Plasma microRNAs are promising novel biomarkers for early detection of colorectal cancer. *Int J Cancer*. 2010;127(1). doi:10.1002/ijc.25007
109. Shastri YM, Loitsch S, Hoepffner N, et al. Comparison of an established simple office-based immunological FOBT with fecal tumor pyruvate kinase type M2 (M2-PK) for colorectal cancer screening: Prospective multicenter study. *Am J Gastroenterol*. 2008;103(6). doi:10.1111/j.1572-0241.2008.01824.x
110. Mishra A, Verma M. Cancer biomarkers: Are we ready for the prime time? *Cancers (Basel)*. 2010;2(1). doi:10.3390/cancers2010190
111. Bhome R, Vecchio F Del, Lee GH, et al. Exosomal microRNAs (exomiRs): Small molecules with a big role in cancer. *Cancer Lett*. 2018;420. doi:10.1016/j.canlet.2018.02.002
112. Tickner JA, Urquhart AJ, Stephenson SA, Richard DJ, O'Byrne KJ. Functions and therapeutic roles of exosomes in cancer. *Front Oncol*. 2014;4 MAY. doi:10.3389/fonc.2014.00127
113. Kreso A, Dick JE. Evolution of the cancer stem cell model. *Cell Stem Cell*. 2014;14(3). doi:10.1016/j.stem.2014.02.006
114. Henry DFHNL. Cancer biomarkers. *Mol Oncol*. Published online 2012:140-146.
115. Amur S, Lavange L, Zineh I, Buckman-Garner S, Woodcock J. Biomarker qualification: Toward a multiple stakeholder framework for biomarker development, regulatory acceptance, and utilization. *Clin Pharmacol Ther*. 2015;98(1). doi:10.1002/cpt.136
116. Rhea JM, Molinaro RJ. Cancer biomarkers: surviving the journey from bench to bedside. *MLO Med Lab Obs*. 2011;43(3).
117. Hartwell L, Mankoff D, Paulovich A, Ramsey S, Swisher E. Cancer biomarkers: A systems approach. *Nat Biotechnol*. 2006;24(8). doi:10.1038/nbt0806-905

118. Trevethan R. Sensitivity, Specificity, and Predictive Values: Foundations, Pliabilities, and Pitfalls in Research and Practice. *Front Public Heal*. 2017;5. doi:10.3389/fpubh.2017.00307

119. Diamandis EP. Cancer biomarkers: Can we turn recent failures into success? *J Natl Cancer Inst*. 2010;102(19). doi:10.1093/jnci/djq306

120. Sinicrope FA, Okamoto K, Kasi PM, Kawakami H. Molecular Biomarkers in the Personalized Treatment of Colorectal Cancer. *Clin Gastroenterol Hepatol*. 2016;14(5). doi:10.1016/j.cgh.2016.02.008

121. Chen B, Xia Z, Deng YN, et al. Emerging microRNA biomarkers for colorectal cancer diagnosis and prognosis. *Open Biol*. 2019;9(1). doi:10.1098/rsob.180212

122. Goetz LH, Schork NJ. Personalized medicine: motivation, challenges, and progress. *Fertil Steril*. 2018;109(6). doi:10.1016/j.fertnstert.2018.05.006

123. Aboud OA, Weiss RH. New opportunities from the cancer metabolome. *Clin Chem*. 2013;59(1). doi:10.1373/clinchem.2012.184598

124. Weir TL, Manter DK, Sheflin AM, Barnett BA, Heuberger AL, Ryan EP. Stool Microbiome and Metabolome Differences between Colorectal Cancer Patients and Healthy Adults. *PLoS One*. 2013;8(8). doi:10.1371/journal.pone.0070803

125. Liu Z, Zhang Y, Niu Y, et al. A systematic review and meta-analysis of diagnostic and prognostic serum biomarkers of colorectal cancer. *PLoS One*. 2014;9(8). doi:10.1371/journal.pone.0103910

126. Langhorst J, Elsenbruch S, Koelzer J, Rueffer A, Michalsen A, Dobos GJ. Noninvasive markers in the assessment of intestinal inflammation in inflammatory bowel diseases: Performance of fecal lactoferrin, calprotectin, and PMN-elastase, CRP, and clinical indices. *Am J Gastroenterol*. 2008;103(1). doi:10.1111/j.1572-0241.2007.01556.x

127. Chen J, Pitmon E, Wang K. Microbiome, inflammation and colorectal cancer. *Semin Immunol*. 2017;32. doi:10.1016/j.smim.2017.09.006

128. Cathcart MC, Lysaght J, Pidgeon GP. Eicosanoid signalling pathways in the development and progression of colorectal cancer: Novel approaches for prevention/intervention. *Cancer Metastasis Rev*. 2011;30(3-4). doi:10.1007/s10555-011-9324-x

129. Chou RC, Kim ND, Sadik CD, et al. Lipid-Cytokine-Chemokine Cascade Drives Neutrophil Recruitment in a Murine Model of Inflammatory Arthritis. *Immunity*. 2010;33(2). doi:10.1016/j.immuni.2010.07.018

130. Peters-Golden M, Henderson WR. Mechanisms of disease: Leukotrienes. *New Engl J Med [NEJM]*. 2007;357(18). doi:10.1056/NEJMra071371

131. Osman J, Savari S, Chandrashekar NK, Bellamkonda K, Douglas D, Sjölander A. Cysteinyl leukotriene receptor 1 facilitates tumorigenesis in a mouse model of colitis-associated colon cancer. *Oncotarget*. 2017;8(21). doi:10.18632/oncotarget.16718

132. Nakamura M, Shimizu T. Leukotriene receptors. *Chem Rev*. 2011;111(10). doi:10.1021/cr100392s

133. Minigh J. Leukotriene E4. *xPharm Compr Pharmacol Ref*. Published online 2007. doi:10.1016/B978-008055232-3.62025-2

134. Kanaoka Y, Boyce JA. Cysteinyl leukotrienes and their receptors; emerging concepts. *Allergy, Asthma Immunol Res*. 2014;6(4). doi:10.4168/aair.2014.6.4.288

135. Laidlaw TM, Boyce JA. Cysteinyl leukotriene receptors, old and new; implications for asthma. *Clin Exp Allergy*. 2012;42(9). doi:10.1111/j.1365-2222.2012.03982.x

136. Savari S, Vinnakota K, Zhang Y, Sjölander A. Cysteinyl leukotrienes and their receptors: Bridging inflammation and colorectal cancer. *World J Gastroenterol*. 2014;20(4). doi:10.3748/wjg.v20.i4.968

137. Kobilka BK. G protein coupled receptor structure and activation. *Biochim Biophys Acta - Biomembr*. 2007;1768(4). doi:10.1016/j.bbamem.2006.10.021

138. Lu B, Nagappan G, Lu Y. BDNF and synaptic plasticity, cognitive function, and dysfunction. *Handb Exp Pharmacol*. 2015;220. doi:10.1007/978-3-642-45106-5_9

139. Hallböök F. Evolution of the vertebrate neurotrophin and Trk receptor gene families. *Curr Opin Neurobiol*. 1999;9(5). doi:10.1016/S0959-4388(99)00011-2

140. Yamamoto M, Sobue G, Yamamoto K, Terao S, Mitsuma T. Expression of mRNAs for neurotrophic factors (NGF, BDNF, NT-3, and GDNF) and their receptors (p75NGFR, TrkA, TrkB, and TrkC) in the adult human peripheral nervous system and nonneural tissues. *Neurochem Res*. 1996;21(8). doi:10.1007/BF02532343

141. Miranda M, Morici JF, Zanoni MB, Bekinschtein P. Brain-Derived Neurotrophic Factor: A Key Molecule for Memory in the Healthy and the Pathological Brain. *Front Cell Neurosci*. 2019;13. doi:10.3389/fncel.2019.00363

142. Yu YB, Zuo XL, Zhao QJ, et al. Brain-derived neurotrophic factor contributes to abdominal pain in irritable bowel syndrome. *Gut*. 2012;61(5). doi:10.1136/gutjnl-2011-300265

143. Haniu M, Montestruque S, Bures EJ, et al. Interactions between brain-derived neurotrophic factor and the TRKB receptor. Identification of two ligand binding domains in soluble TRKB by affinity separation and chemical cross-lingking. *J Biol Chem*. 1997;272(40). doi:10.1074/jbc.272.40.25296

144. Li Z, Zhang G, Feil R, Han J, Du X. Sequential activation of p38 and ERK pathways by cGMP-dependent protein kinase leading to activation of the platelet integrin α IIbβ3. *Blood*. 2006;107(3). doi:10.1182/blood-2005-03-1308

145. Fukami Y, Lipmann F. Reversal of Rous sarcoma-specific immunoglobulin phosphorylation on tyrosine (ADP as phosphate acceptor) catalyzed by the src gene kinase. *Proc Natl Acad Sci U S A*. 1983;80(7 I). doi:10.1073/pnas.80.7.1872

146. Hubbard SR. Structural analysis of receptor tyrosine kinases. *Prog Biophys Mol Biol*. 1999;71(3-4). doi:10.1016/S0079-6107(98)00047-9

147. Steelman LS, Pohnert SC, Shelton JG, Franklin RA, Bertrand FE, McCubrey JA. JAK/STAT, Raf/MEK/ERK, PI3K/Akt and BCR-ABL in cell cycle progression and leukemogenesis. *Leukemia*. 2004;18(2). doi:10.1038/sj.leu.2403241

148. Aubrey BJ, Kelly GL, Janic A, Herold MJ, Strasser A. How does p53 induce apoptosis and how does this relate to p53-mediated tumour suppression? *Cell Death Differ*. 2018;25(1). doi:10.1038/cdd.2017.169

149. Altar CA, Cai N, Bliven T, et al. Anterograde transport of brain-derived neurotrophic factor and its role in the brain. *Nature*. 1997;389(6653). doi:10.1038/39885

150. Thiele CJ, Li Z, McKee AE. On Trk - the TrkB signal transduction pathway is an increasingly important target in cancer biology. *Clin Cancer Res*. 2009;15(19). doi:10.1158/1078-0432.CCR-08-0651

151. Dong Q, Ji YS, Cai C, Chen ZY. LIM Kinase 1 (LIMK1) interacts with Tropomyosin-related Kinase B (TrkB) and mediates Brain-derived Neurotrophic Factor (BDNF)-induced axonal elongation. *J Biol Chem*. 2012;287(50). doi:10.1074/jbc.M112.405415
152. Panja D, Bramham CR. BDNF mechanisms in late LTP formation: A synthesis and breakdown. *Neuropharmacology*. 2014;76(PART C). doi:10.1016/j.neuropharm.2013.06.024
153. Carter BD, Kaltschmidt C, Kaltschmidt B, et al. Selective activation of NF-κB by nerve growth factor through the neurotrophin receptor p75. *Science (80-)*. 1996;272(5261). doi:10.1126/science.272.5261.542
154. Pearse RN, Swendeman SL, Li Y, Rafii D, Hempstead BL. A neurotrophin axis in myeloma: TrkB and BDNF promote tumor-cell survival. *Blood*. 2005;105(11):4429-4436. doi:10.1182/blood-2004-08-3096
155. Kupferman ME, Jiffar T, El-Naggar A, et al. TrkB induces EMT and has a key role in invasion of head and neck squamous cell carcinoma. *Oncogene*. 2010;29(14):2047-2059. doi:10.1038/onc.2009.486
156. Okugawa Y, Tanaka K, Inoue Y, et al. Brain-derived neurotrophic factor/tropomyosin-related kinase B pathway in gastric cancer. *Br J Cancer*. 2013;108(1). doi:10.1038/bjc.2012.499
157. Tanaka K, Okugawa Y, Toiyama Y, et al. Brain-Derived Neurotrophic Factor (BDNF)-induced Tropomyosin-related kinase B (Trk B) signaling is a potential therapeutic target for peritoneal carcinomatosis arising from colorectal cancer. *PLoS One*. 2014;9(5). doi:10.1371/journal.pone.0096410
158. Diamanti-Kandarakis E, Bourguignon JP, Giudice LC, et al. Endocrine-disrupting chemicals: An Endocrine Society scientific statement. *Endocr Rev*. 2009;30(4). doi:10.1210/er.2009-0002
159. Daly E, Vessey MP, Barlow D, Gray A, McPherson K, Roche M. Hormone replacement therapy in a risk-benefit perspective. In: *Maturitas*. Vol 23. ; 1996. doi:10.1016/0378-5122(95)00978-7
160. Nie X, Xie R, Tuo B. Effects of Estrogen on the Gastrointestinal Tract. *Dig Dis Sci*. 2018;63(3). doi:10.1007/s10620-018-4939-1
161. Koo JH, Jalaludin B, Wong SKC, Kneebone A, Connor SJ, Leong RWL. Improved survival in young women with colorectal cancer. *Am J Gastroenterol*. 2008;103(6). doi:10.1111/j.1572-0241.2007.01779.x
162. Chidi-Ogbolu N, Baar K. Effect of estrogen on musculoskeletal performance and injury risk. *Front Physiol*. 2019;10(JAN). doi:10.3389/fphys.2018.01834
163. Soerjomataram I, Thong MSY, Ezzati M, Lamont EB, Nusselder WJ, Poll-Franse LV Van De. Most colorectal cancer survivors live a large proportion of their remaining life in good health. *Cancer Causes Control*. 2012;23(9). doi:10.1007/s10552-012-0010-2
164. Hirokazu Uemura Masaya Takikawa MITY. Hormone replacement therapy in postmenopausal women. *J Med Invest*. Published online 2003:136-145.
165. Ohlsson C, Engdahl C, Börjesson AE, et al. Estrogen receptor-α expression in neuronal cells affects bone mass. *Proc Natl Acad Sci U S A*. 2012;109(3). doi:10.1073/pnas.1111436109

166. Galluzzo P, Caiazza F, Moreno S, Marino M. Role of ERβ palmitoylation in the inhibition of human colon cancer cell proliferation. *Endocr Relat Cancer*. 2007;14(1). doi:10.1677/ERC-06-0020
167. Caiazza F, Ryan EJ, Doherty G, Winter DC, Sheahan K. Estrogen receptors and their implications in colorectal carcinogenesis. *Front Oncol*. 2015;5(FEB). doi:10.3389/fonc.2015.00019
168. Mehrabi SF, Ghatak S, Mehdawi LM, Topi G, Satapathy SR, Sjölander A. Brain-Derived Neurotrophic Factor, Neutrophils and Cysteinyl Leukotriene Receptor 1 as Potential Prognostic Biomarkers for Patients with Colon Cancer. *Cancers (Basel)*. 2021;13(21):5520. doi:10.3390/cancers13215520
169. Topi G, Satapathy SR, Dash P, et al. Tumour-suppressive effect of oestrogen receptor β in colorectal cancer patients, colon cancer cells, and a zebrafish model. *J Pathol*. 2020;251(3). doi:10.1002/path.5453