Role of novel tumor suppressors in colon cancer : Mechanisms and therapeutic opportunities

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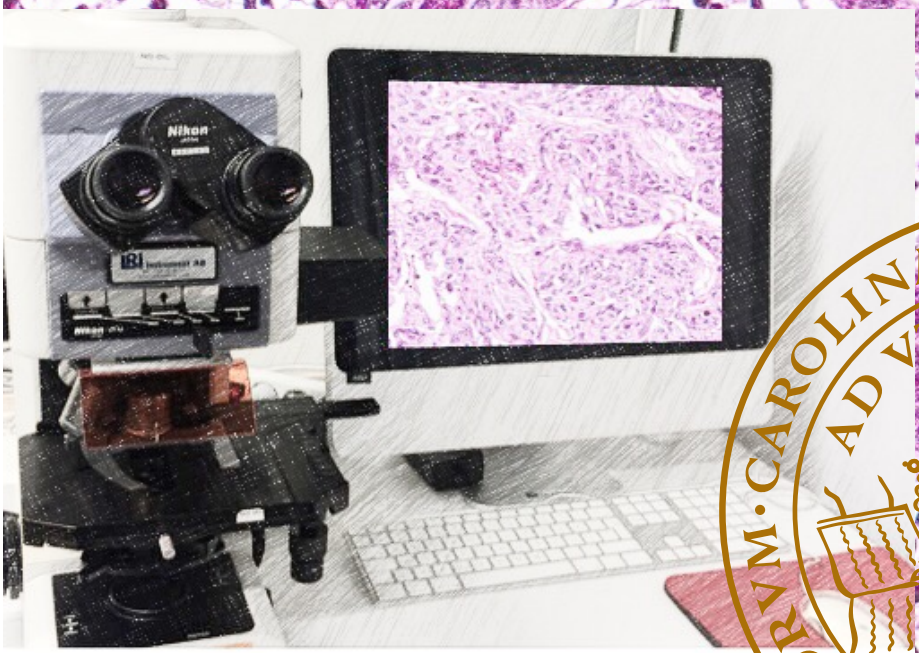

Role of novel tumor suppressors in colon cancer: Mechanisms and therapeutic opportunities

Lubna Mohammed Mehdawi

LUND
UNIVERSITY

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Abstract

Colon cancer is the third most common cancer in the world and the fourth most common cause of cancer related deaths. Inflammation is one of the risk factors for development of colon cancer. Interestingly immune cells as mast cells and inflammatory mediators as LTC_4 play an important role in colon cancer. Genetic predisposition, which might lead to either activation of oncogenes or inhibition of tumor suppressor genes, are risk factors of colon cancer development.

The aim of my thesis was to evaluate the clinical significance of the tumor suppressors 15-PGDH and WNT5A in colon cancer patients. I investigated the underlying mechanisms/signaling triggered by these tumor suppressors in colon cancer cells and whether the re-expression of these tumor suppressors could be an attractive therapeutic strategy for treatment of colon cancer patients.

I found that presence of mast cells in colon cancer tissue was associated with better prognosis of colon cancer patients, and the presence of mast cells in polyps/tumors in a colitis-associated colon cancer mouse model was also beneficial. I found that the tumor suppressor gene 15-PGDH is down-regulated in colon cancer patients as well as in colon cancer cell lines. This down-regulation is often seen in parallel with down-regulated WNT5A, the non-canonical Wnt/β-catenin signaling ligand. I found that down-regulation of both these proteins is associated with poor prognosis for colon cancer patients. My results show that treatment of colon cancer cells with Foxy-5, a WNT5A mimicking peptide, leads to up-regulation of 15-PGDH through JNK/AP-1 pathway. In addition, I also found that the pro-inflammatory mediator LTC_4 via CysLTR2 can induce the expression of 15-PGDH through the JNK pathway, which indicate that LTC_4 via CysLTR2 has an anti-tumor effect.

In conclusion, these findings provide important information for better understanding of the tumor microenvironment and the tumor suppressor genes in colon cancer and might help to identify new therapeutic targets for colon cancer patients.

Key words Colon cancer, Mast cells, 15-PGDH, WNT5A, LTC_4		
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To my parents, my husband, my kids

لوالدي و والدتي, لزوجي واطفالي.. اهدي عملي هذا

وَمَا أُوتِيتُم مِنَ الْعِلْمِ إِلاَّ قَلِيلاً سورة الاسراء الاية 85

"And mankind have not been given of knowledge except a little"
Quran, chapter Al-isra, verse no. 85

Content

Abbreviations

15-PGDH	15-hydroxy prostaglandin dehydrogenase
5-ASA	5 aminosalicylic acid
5-FU	5-Fluorouracil
5-LOX	5-Lipooxygnase
AA	Arachidonic acid
AJCC	American Joint Committee on Cancer
AOM	Azoxymethane
APC	Adenomatous polyposis coli
CAC	Colitis associated cancer
COX-2	Cyclooxygenase-2
CysLTR1	Cysteinyl leukotriene receptor 1
CysLTR2	Cysteinyl leukotriene receptor 2
CysLTs	Cysteinyl leukotrienes
DSS	Dextran sulphate sodium
EGFR	Epidermal growth factor receptor
FAP	Familial adenomatous polyposis
GALT	Gut associated lymphoid tissue
GPCRs	G-protein coupled receptors
GSK-3β	Glycogen synthase kinase 3β
IBD	Inflammatory bowel disease
ISC	Intestinal stem cell
JNK	C-jun N-terminal Kinase
MCD	Mast cell density

MCS	Mast cells
Muc-2	Mucin-2
NSAID	Non steroidal anti-inflammatory drugs
PCK	Protein kinase C
PGE_2	Prostaglandin E_2
PPAR-α	Peroxisome proliferator activated receptor-α
SI	Sucrose isomaltase
SRS	Slow reacting substance
TAM	Tumor associated macrophages
TGF-β	Transforming growth factor β
TMA	Tissue microarray
TNF-α	Tumor necrosis factor- α
VEGR	Vascular endothelial growth factor

List of Papers

The following papers are included in this thesis.

I. **Lubna Mehdawi**, Janina Osman, Geriolda Topi and Anita Sjölander. High tumor mast cell density is associated with longer survival of colon cancer patients. 2016. Acta Oncologica. Doi: 10.108070284186X.2016.1198493. *

II. **Lubna M. Mehdawi,** Chandra Prakash Prasad[1], Roy Ehrnström[2], Tommy Andersson and Anita Sjölander. Non-canonical WNT5A signaling up-regulates the expression of the tumor suppressor 15-PGDH and induces differentiation of colon cancer cells. 2016. Molecular oncology. Doi: 10.1016. *

III. **Lubna M. Mehdawi,** Annika Gustafsson, Kent Lundholm, Maria Alvarado-Kristensson and Anita Sjölander. A possible anti-tumor effect of Leukortine C_4 by inducing 15-hydroxyprostaglandin dehydrogenase expression in colon cancer cells. (Manuscript)

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Introduction

Cancer is an ancient disease, with the earliest records dating to ancient Egypt (circa 3000 BC). The Greek physician Hippocrates (460-375 BC) first described the growth of cancer as a crab attaching to the surrounded tissue by its claws. Later, the Roman physician Celsus (25 BC-50 AD), who created the Latin language of medicine, translated the word *Crab* to *Cancer* [1]. Cancer became defined as abnormal cell growth, invading surrounding tissues and able to metastasize to other parts of the body. Cancer treatment has improved from surgery in the 19th century to today's use of adjuvant chemotherapy and radiotherapy. Although progress in cancer treatment is ongoing, it remains a major cause of death worldwide [2]. Various factors, such as chemical carcinogens, ionizing radiation and viruses, can cause non-inherited cancer by inducing genetic damage, leading to the identification of genes that are essential in cancer development. In the 1970s, these genes were classified into two families: proto-oncogenes and tumor suppressor genes. Accumulation of mutations in these genes is believed to cause cellular alterations, and subsequently lead to cancer development [3]. The essential cellular alterations required for neoplastic transformation, referred to as the hallmarks of cancer, include replicative ability, sustained proliferative, evasion of growth suppression and cell death, and induction of angiogenesis and invasion/metastasis [4]. More recently, evasion of immune destruction and reprogramming of energy metabolism have emerged as new hallmarks of cancer. The inflammatory milieu has also been appreciated as one of these hallmarks [5]. Inflammation, particularly chronic inflammation such as in inflammatory bowel disease (IBD), has been established as a connection between inflammatory disease and colorectal cancer [6]. In colon cancer, mutation of oncogenes and tumor suppressor genes leads to activation of several oncogenic pathways and deactivates tumor suppressor pathways [7]. Overexpression of cyclooxygenase-2 (COX-2), which has been shown in the majority of cancers, especially colorectal tumors [8], leads to overproduction of prostaglandin E_2, a proinflammatory lipid mediator, which induces proliferation, neovascularization, inhibition of cell death and motility of tumor cells [9]. 15-hydroxyprostaglandin dehydrogenase (15-PGDH) is the catabolic enzyme that leads to degradation of the prostaglandins [10]. It is considered a tumor suppressor gene and is known to be downregulated in colorectal cancer [11].

Here, we investigated the possible mechanisms regulating 15-PGDH in colon cancer cells, and we found that WNT-5A and its mimicking peptide Foxy-5 can induce 15-PGDH expression in colon cancer by activating the JNK/AP-1 pathway and inhibiting the β-catenin pathway. We also found that the proinflammatory mediator LTC_4 could induce the expression of 15-PGDH through CysLTR2 by activating the JNK pathway. However, the presence of mast cells in colon cancer patient material is associated with good prognosis for colon cancer patients.

Background

The intestinal tract and its function

The intestinal tract is one of the most complex and regenerative organs, and it is the largest immunological organ in the human body [12]. It is divided into the small intestine and large intestine. The small intestine is divided into three parts: the duodenum, jejunum and ileum, whereas the large intestine is subdivided into the cecum, ascending, transverse, descending and sigmoid colon (Figure 1).

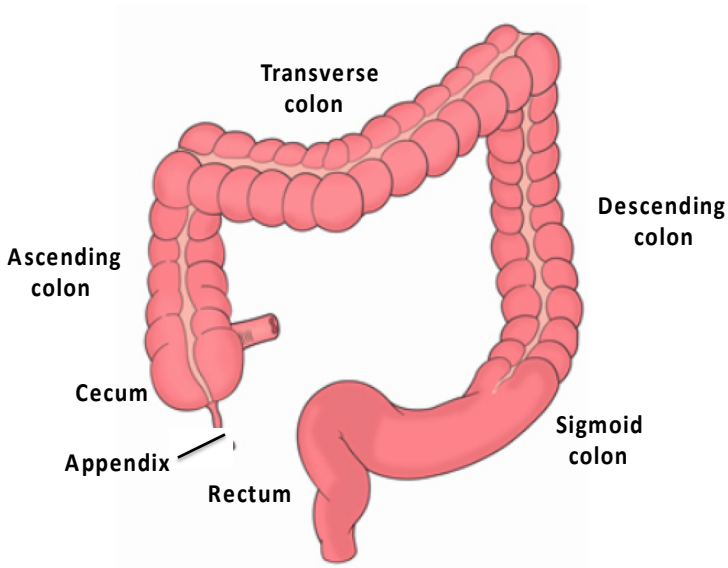

Figure 1: Schematic illustration of colorectal anatomy

The intestinal wall is composed of three layers. The outermost layer is the muscular layer, which consists of smooth muscles, the middle layer is a layer of stromal tissue, and the innermost layer is the mucosal layer, which is composed of epithelial cells. Epithelial cells of the intestine are simple, non-ciliated columnar cells, arranged in a layer, that form the luminal surface. Due to the presence of

"gut-friendly" bacteria, the human intestine is normally exposed to a low degree of inflammation [13]. The intestinal epithelium is protected from external pathogens and microorganisms by cells in the lamina propria, which contains many types of immune cells; it is rich in macrophages, lymphoid cells, and dendritic cells, making it the primary location for immune reactions (Figure 2, 3) [14]. Other intestinal defense mechanisms come from the intestine's own immune system, known as gut-associated lymphoid tissue (GALT). Impairment of this system leads to unbalanced inflammatory responses in the gut and predisposes people to inflammatory bowel disease (IBD) [15]. The absorptive surface of the small intestine is increased because of the presence of the villi, invaginations into the submucosal layer and the crypt of Lieberkun [16]. The epithelial surface of the large intestine is flat, as it lacks villi and contains only crypts (Figure 2).

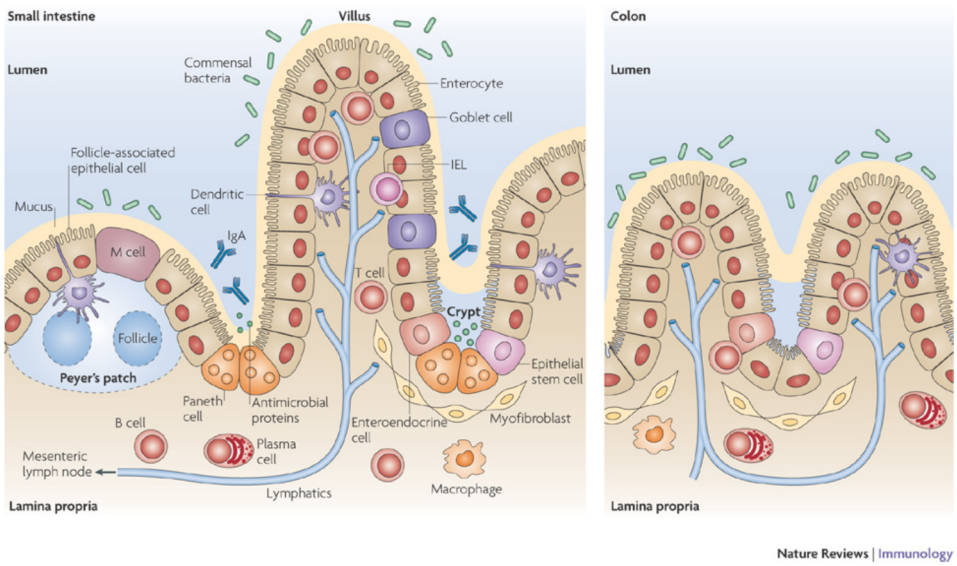

Figure 2: Comparison of histology between small and large intestine, adepted from Abreu,Maria T, et al., 2010 [17]

There are four types of differentiated cells in the intestine. Enterocytes, which are the majority, are absorptive in nature, having brush border microvilli on their apical surface. Goblet cells and Paneth cells secrete mucus and antimicrobial proteins, respectively, providing a substantial physical and biochemical barrier. Enteroendocrine cells secrete hormones such as secretin and pancreozymin. Intestinal epithelial homeostasis is maintained by three mechanisms. First, cell production by the division of the crypt progenitors every 12 h produces 200 cells per crypt. Second, all epithelial cells move continuously upward and reach the top of the villus in approximately 5 days, except for the Paneth cells, which escape the

movement upward. Third, proliferation dictated by the crypt niche is maintained as cells move along the crypt-villus axis [16, 18]. The epithelial cell renewal process within the intestine appears to be totally dependent on a limited number of long-lived intestinal multipotent stem or progenitor cells. Intestinal stem cells (ISC) have the following two properties: the capacity to maintain themselves for long periods of time (self-renewal) and the ability to generate all differentiated cell types, including enterocytes, entero-endocrine, and Paneth cells [19].

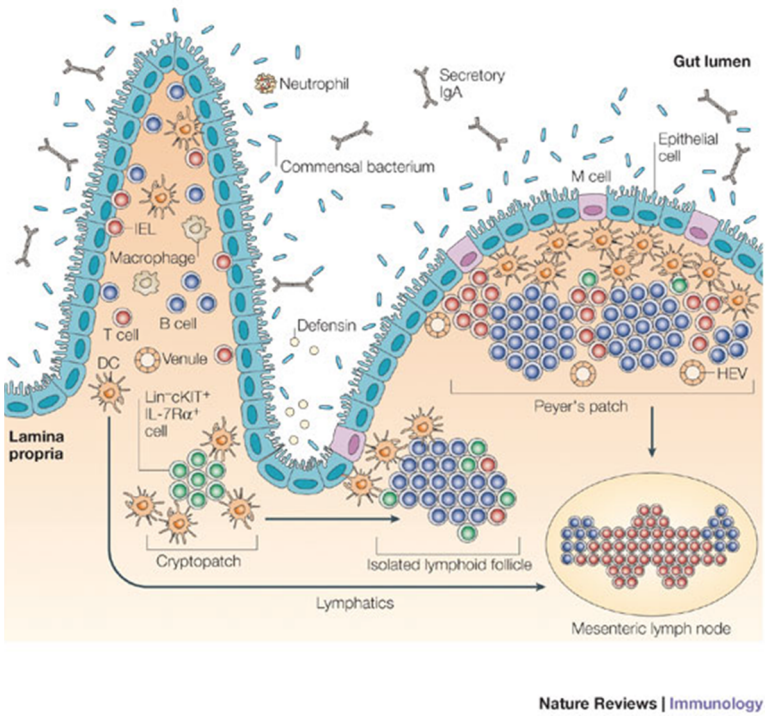

Figure 3: Representative intestinal mucosal surface, adapted from Eberl, Gerard, et al., 2005 [20]

Inflammation and cancer

In 1863, Virchow reported the presence of leukocyte infiltration in cancer tissue; he hypothesized that chronic inflammation can lead to cancer. Now, the understanding of the inflammatory microenvironment of cancer tissue supports Virchow's findings [21]. Tissue injury and inflammation lead to increased cell proliferation; although, it is clear now that proliferation of the cell alone cannot cause cancer. However, DNA damage and sustained cell proliferation in an

inflammatory milieu rich in inflammatory cells and growth factors can promote cancer [22]. The hallmarks of cancer include evading growth suppressors, sustaining proliferative signaling, resisting cell death, enabling explicative immortality, including angiogenesis, and activating invasion and metastasis. In addition, inflammation and genome instability have recently been established as hallmarks of cancer [5] (Figure 4).

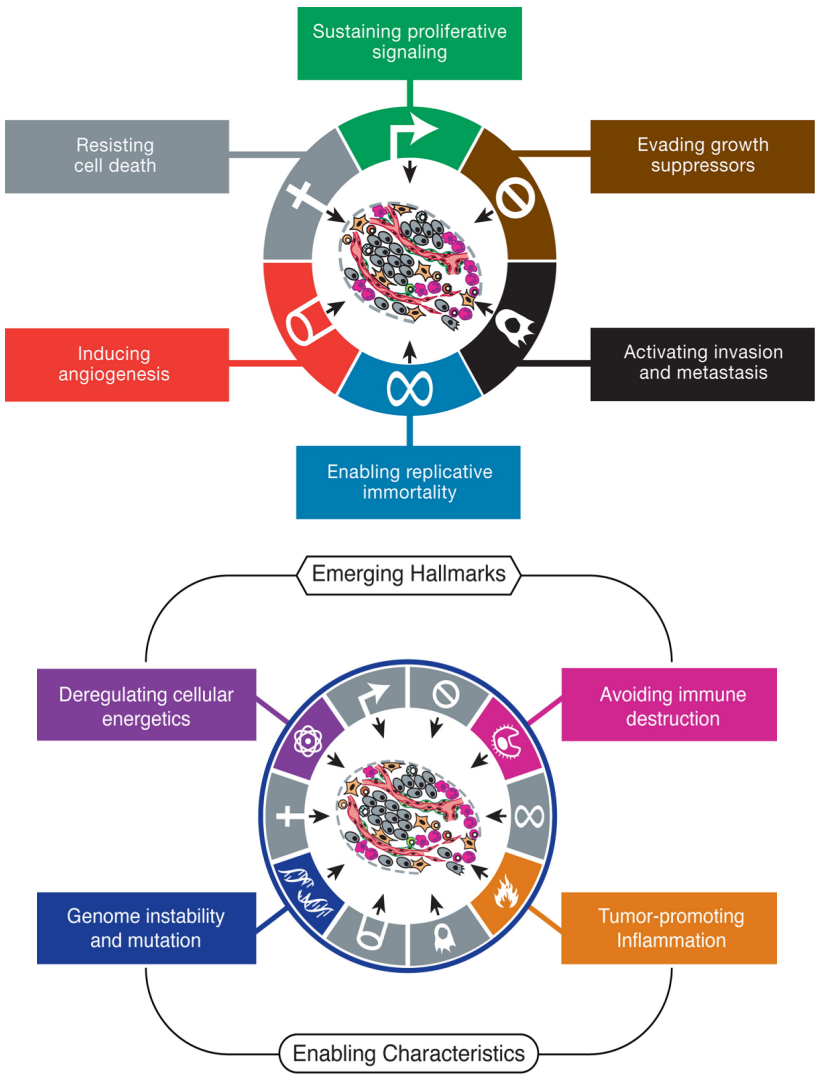

Figure 4: Hallmark capability of cancer, adapted from Hanahan, et al., 2011 [5]

Inflammatory bowel disease and colitis associated colon cancer

Inflammatory bowel disease (IBD) defined as chronic intestinal disorder caused by a number of factors that contribute to the development of mucosal inflammation, including environmental and genetic factors as well as a dysregulated immune response [23]. IBD includes Crohn's disease, which affects any part of the gastrointestinal tract from the mouth to the anus in a irregular pattern, and ulcerative colitis (UC), which affects the rectum and may involve part or all of the colon in a continuous pattern [24]. The inflammation in Crohn's disease is often transmural, whereas it is typically confined to the mucosa in UC [25]. The risk of developing colon cancer is highly increased among these patients, perhaps due to prolonged exposure to inflammation in the bowel. Colitis-associated cancer (CAC) is colon cancer occurs in patients with clinically detectable inflammatory bowel disease [26-29]. The increased risk of developing cancer in IBD patients depends on many factors, such as disease duration and severity and the effectiveness of treatment [30-32]. Treatment of IBD patients with anti-inflammatory drugs such as 5 aminosalicylic acid (5-ASA) has been linked to a decreased risk of developing CAC [33-35], as has a tumor necrosis factor-α (TNF-α) antagonist [36]. In animal studies, treatment with 5-ASA in an AOM/DSS mouse model of CAC shows reduction in dysplastic colon lesions in treated mice [37]

Colon cancer

Colorectal cancer is the second most common cancer in women and the third most common cancer in men worldwide, and the fourth leading cause of cancer-related deaths [38, 39]. Etiological factors of colon cancer include a low fiber and high fat diet and high alcohol intake [40] [41]. Genetic alteration can lead to the activation of oncogenes as *BRAF* and *KRAS*, which are mutated in approximately 50% of CRC [42], inactivation of tumor suppressor genes, such as *APC* and *P53* [43, 44]. The majority (approximately 65-85%) of colon cancer is sporadic; hereditary colon cancer, a genetic disorder and the occurrence of CRC in first- and second-degree relatives, accounts for approximately 10-30% [45, 46]. Familial adenomatous polyposis (FAP) is an autosomal dominant disease characterized by the presence of countless adenomatous polyps throughout the colon and rectum. If not treated, the polyps can progress to cancer at a young age [47, 48]. FAP patients have a germ line mutation in the tumor suppressor gene APC; however, 70% of sporadic colon cancers have somatic mutations in the APC gene [49-51]. APC is a multifunctional protein that participates in many cellular processes, including cell-

cell adhesion, cell migration, and apoptosis; it also regulates β-catenin degradation [52, 53] (Figure 5).

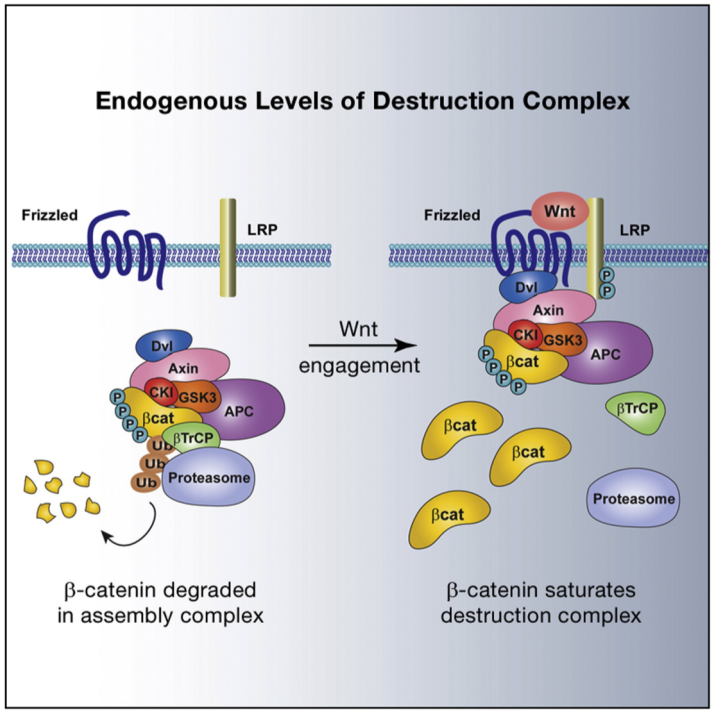

Figure 5: β-catenin destruction complex, adapted from Li, Vivian, et al., 2012 [54]

Adenoma-carcinoma sequence

Adenomas are important precursor lesions for carcinomas. The adenoma-carcinoma sequence describes the stepwise progression from normal to dysplastic epithelium to carcinoma, with the accumulation of specific mutations at specific stages [55]. These mutations affect genes and pathways that are important for the regulation of cell growth and differentiation [56]. *APC* mutations occur early during the progression from adenoma to carcinoma [57, 58]. *KRAS* mutations also occur early in the adenoma-carcinoma sequence; however, *KRAS* mutations are less common in small adenomas. *P53*, *SMAD2*, and *SMAD4* are tumor suppressors; mutations in these genes can lead to colon cancer progression [55].

Colon cancer classification and staging

In general, cancer staging provides critical information about the extent of the disease, helping in treatment decisions and prognosis determination. In 1932, Dukes proposed a classification of colorectal cancer based on the extent of the disease, which is evaluated by the degree of the tumor infiltration through the wall of the bowel and the presence or absence of lymph node involvement [59]. In 1987, the American Joint Committee on Cancer (AJCC) and the International Union Against Cancer introduced the cancer staging system based on the size and the extent of the tumor (T), number and degree of lymph node involvement (N), and presence or absence of distant metastasis (M) [60] (Table 1). This staging system of CRC was updated in 2009 and is the most common staging system used in clinical practice [61, 62].

Tumor, node, metastasis (TNM) staging of colorectal cancer

T - primary tumor

TX - primary tumor cannot be assessed

T0 - No evidence of primary tumor

Tis - Carcinoma in situ: intraepithelial or invasion of lamina propria

T1 - Tumor invades the submucosa

T2 - Tumor invades the muscularis propria

T3 - Tumor invades through the muscularis propria into the subserosa or non-peritonealized pericolic or perirectal tissues

T4 - Tumor directly invades other organs or structures and/or perforated visceral peritoneum

N - regional lymph nodes

NX - Regional lymph node cannot be assessed

N0 - No regional lymph node metastasis

N1 - Metastasis in one to three regional lymph nodes

N2 - Metastasis in four or more regional lymph nodes

M- distant metastasis

MX - Distant metastasis cannot be assessed

M0 - No distant metastasis

M1 - Distant metastasis

Table 1. Comparison of TNM and Duckes classification

TNM Classification American Joint Committee on Cancer (AJCC)				**Duckes staging**
Stages	**T**	**N**	**M**	**Stages**
Stage 0	Tis	N0	M0	-
Stage I	T1	N0	M0	A
	T2	N0	M0	A
Stage II	T3	N0	M0	B
	T4	N0	M0	B
Stage III	T1, T2	N1 or N2	M0	C
	T3, T4	N1 or N2	M0	C
Stage IV	Any T	Any N	M1	-

Treatment of colorectal cancer

In general, CRC treatment depends on many factors, such as the age of the patient and disease stage. The four key approaches currently used for CRC treatment are surgery, which is the main approach, chemotherapy, radiotherapy, which is mostly for rectal cancer, and targeted therapies [63]. To reduce symptoms and increase survival in metastatic CRC, chemotherapy and targeted therapies are given [64]. The most commonly used chemotherapy is 5-fluorouracil (5-FU); antibodies targeting EGFR (epithelial growth factor receptor) or VEGF (vascular endothelial growth factor) are commonly used [65]. Colorectal cancer patients with mutant *KRAS* have no benefit with anti-EGFR therapy, which make it a promising predictive biomarker for personalized treatment [66].

Signaling pathways in colon cancer

Wnt signaling pathway

Wnt proteins are a family of secreted cysteine-rich glycoproteins approximately 40 kDa in size [67]. The name Wnt is derived from a fusion of wingless, which is a *Drosophila* segment polarity gene, and integrated or int-1, the vertebrate homolog [68, 69]. Wnt protein signaling pathways play an important role in many cellular processes, including cell fate, cell polarity, cell motility, primary axis formation and organogenesis; most recently, this pathway has been shown to play a role in stem cell renewal [70, 71].

To induce and regulate intracellular transduction signaling, Wnt proteins bind to cell surface G protein coupled receptors, called frizzled receptors (FZ), in the presence or absence of the co-receptors LRP 5/6, ROR or RYK [72-75]. Wnt malformation can lead to various diseases, including cancer and degenerative diseases [76]. The Wnt signaling pathway can be divided into the canonical signaling pathway (Wnt/β-catenin) and the non-canonical signaling pathways (Wnt/Ca^{+2} signaling, planar cell polarity (PCP)) [74, 77].

In canonical Wnt/β-catenin signaling, in the absence of a Wnt ligand, such as Wnt1 and Wnt3a, the free cytoplasmic β-catenin binds to a destruction complex consisting of Axin, APC (adenomatous polyposis coli), and GSK-3β (glycogen synthase kinase-3β-catenin), leading to β-catenin phosphorylation, ubiquitination and degradation by proteasomes. Thus, the Wnt/β-catenin target genes remain inactive (Figure 6a). When Wnt ligand binds to frizzled receptors, a cascade of events involving Dishevelled (Dvl) leads to the phosphorylation of the co-receptor LRP5/6 and the recruitment of Axin complex to the receptor. These events lead to the inhibition of Axin-mediated β-catenin phosphorylation, whereby β-catenin is stabilized, accumulates in the cytoplasm and translocates to the nucleus where it activates Wnt target genes, such as COX-2, C-myc and Cyclin-D1 (Figure 6b) [78-80].

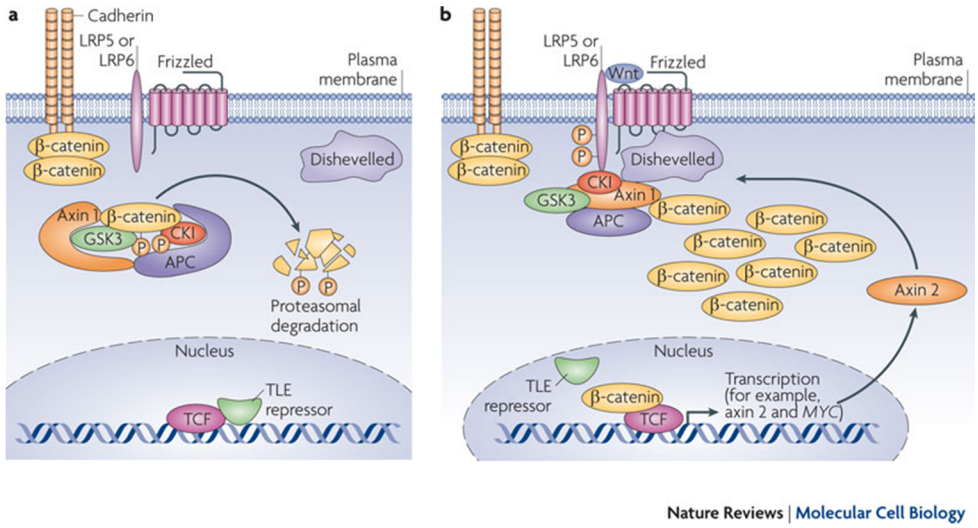

Figure 6: β-catenin destruction complex, adapted from McNeill, H, et al., 2012 [81]

Non-canonical Wnt signaling (β-catenin independent Wnt signaling) is an umbrella term for all β-catenin independent pathways. In contrast to the canonical Wnts, non-canonical Wnts, such as Wnt-4, Wnt-11 and WNT5A, are non-transforming, and they are involved in processes such as polarized cellular movements [82-84].

The Wnt/Ca^{2+} pathway, a non-canonical Wnt signaling pathway, involves the binding of ligands, such as Wnt5a and Wnt-11, to the FZ receptor, a G-protein coupled receptor, leading to activation of phospholipase C (PCL), which subsequently triggers the intracellular release of calcium ions [85, 86]. Calcium activates protein kinase C (PKC) [87], which regulates cellular processes such as cell adhesion and tissue separation during gastrulation via Cdc42. Intracellular accumulation of Ca^{2+} also activates calmodulin-dependent protein kinase II [88], which in turn can antagonize canonical Wnt signaling by activating TAK1 (TGFβ activated kinase) and NLK (Nemo-like kinase) [89, 90].

The planar cell polarity pathway (PCP) is another non-canonical pathway, first described in *Drosophila*, that regulates cytoskeletal polarization. The Wnt/jun N-terminal Kinase (JNK) pathway in vertebrates is similar to the PCP pathway in *Drosophila.* Binding of a non-canonical Wnt ligand, such as Wnt-11, to the FZ receptor activates Dvl, which mediates cytoskeletal rearrangements through activation of small GTPases, such as Rho and Rac [84, 91].

WNT5A

WNT5A is one of the most extensively studied non-canonical Wnts, and it is a representative of β-catenin-independent, non-canonical Wnt signaling. WNT5A plays essential roles in organ development, cell proliferation, tissue orientation and cell migration [92]. In addition to signaling through non-canonical pathways [82, 93], WNT5A can also activate or inhibit canonical Wnt signaling depending on the presence or absence of certain receptors/co-receptors [89, 94, 95]. Recent studies have shown that any misregulation of WNT5A signaling results in cancerous growth of certain tissues, but it is debatable whether WNT5A has a tumor suppressor or tumor promoting effect; this depends on the type of tissue and additional factors, regulators and receptors available [82, 93]. In many studies, Wnt5a has a tumor suppressor effect, as it is downregulated in many cancers, including colorectal cancer [96], invasive ductal breast carcinomas [97, 98], neuroblastoma [99], hepatocellular carcinoma and leukemia [100, 101]. In colon cancer, loss of WNT5A is associated with poor outcomes in stage Dukes B colon cancer patients [96].

Eicosanoids

Eicosanoids are a class of lipid mediators derived from 20-carbon polyunsaturated fatty acids (the Greek word *eicosa* means 20). The main eicosanoid precursor in the mammalian system is arachidonic acid (AA), which belongs to the ω-6 family of polyunsaturated fatty acids [102]. Three main enzymatic pathways metabolize arachidonic acid. Eicosanoids and prostanoids (prostaglandins and thromboxanes) are synthesized via the COX pathway, leukotrienes and hydroxyl eicosatetraenoic acids (HETEs) are generated via the lipoxygenase (LOX) pathway, and epoxides are generated via the cytochrome P-450 epoxygenase pathway [103] (Figure 7).

In the 1930s, the vasodepressor and smooth muscle-stimulating activities of prostaglandins were discovered, although the origin and the structures of these compounds were not yet reported. In 1982, Samuelson and Bergström demonstrated the 5-LOX pathway and the discovery of cysteinyl leukotrienes as the slow-reacting substance (SRS) of anaphylaxis. These mediators were responsible for the contraction of the smooth muscles of the guinea pig lung after treatment with cobra venom. They were also found to be mediators in asthma and other immediate hypersensitivity reactions [104, 105]. In addition to their role in acute systemic inflammation [103], the role of eicosanoids in the maintenance of the hemostasis of the intestine [106] and their dysregulated functions in

pathological conditions, such as chronic inflammation and cancer, are well established [107, 108].

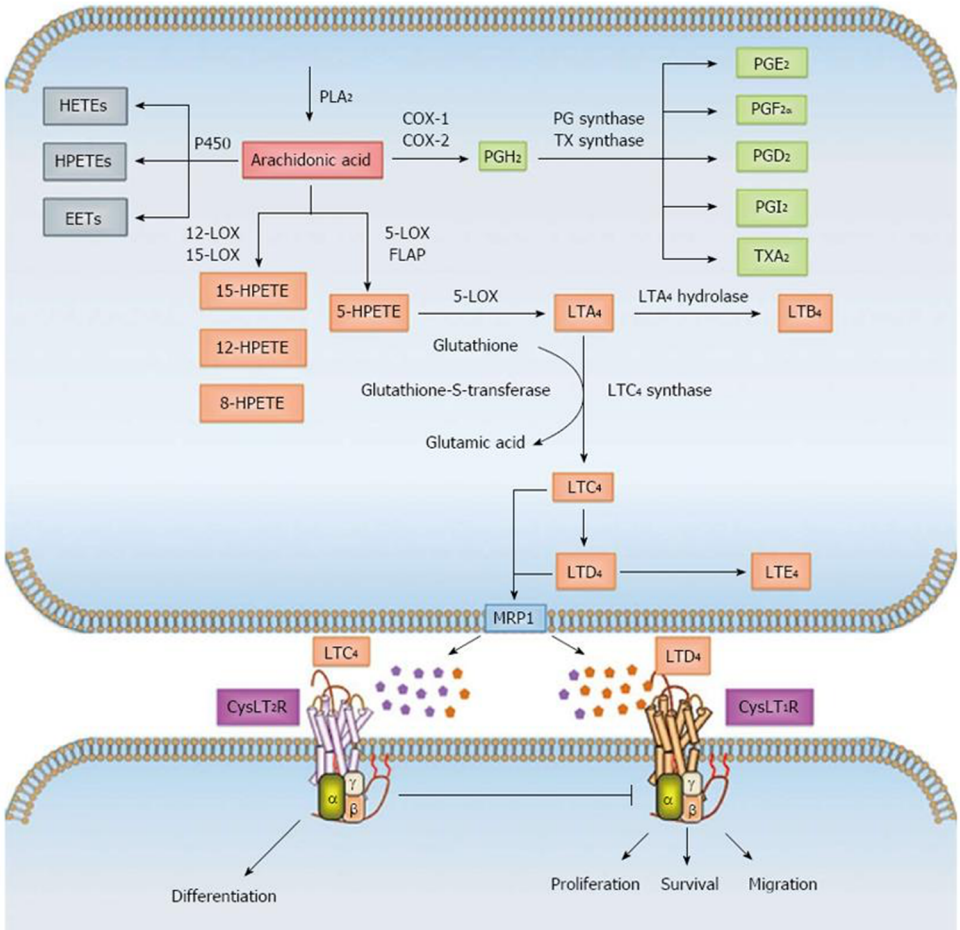

Figure 7: Arachidonic acid metabolism and cysteinyl leukotriene signaling. Adapted from Savari et al., 2014 [109]

The COX pathway

Cyclooxygenase (COX) is the key enzyme required to convert AA to prostanoid. The prostanoid-synthesizing enzyme COX exists in two isoforms, COX-1 and COX-2.

COX-1, which is expressed by most cells, is considered to have a housekeeping function in cells via the production of homeostatic prostanoids. COX-2, which is induced by stress and cytokines, is the source of prostanoid formation in

inflammation and cancer [110]. Prostanoids include prostaglandins and thromboxane A_2. In addition to their role in inflammation, prostaglandins are involved in many physiological functions, including blood clotting, bone metabolism, wound healing kidney function and immune response [111]. Prostaglandins include PGE_2, $PGF_{2\alpha}$, PGD_2 and PGI_2, which mediate their action via the activation of receptors, including EP1-4 for PGE_2, prostaglandin receptor $F_{2\alpha}$ for $PGF_{2\alpha}$, DP_1 and DP_2 for PGD_2, and prostaglandin I_2 (IP receptor) for PGI_2 [112]. Thromboxane A_2 is an activating and recruiting mediator for platelets, mediating its action through the specific G-protein coupled receptor TBXA2R [113].

Elevated levels of COX-2 have been identified in colon [114, 115], breast

[116], lung [117], pancreatic, esophageal and ovarian cancers [118-120]. PGE_2 is the most common prostaglandin found in many human malignancies, including colon and breast cancer, and it is associated with poor prognosis [107]. COX-2 produces PGE_2, the only prostanoid demonstrated to induce proliferation, angiogenesis, cell death inhibition and motility of tumor cells [9, 121], and more recently, to expand the number of colon cancer stem cells [122]. 15-Hydroxyprostaglandin dehydrogenase (15-PGDH) is the enzyme responsible for the degradation of PGE_2, converting it into its inactive metabolites [123]. 15-PGDH is highly expressed in normal colon mucosa but is lost in many CRCs [124], and its decreased expression has also been implicated in many other cancers, such as lung, bladder, pancreatic and gastric cancer [125-128]. Deletion of the 15-PGDH gene increases colonic PGE_2 levels and enhances tumorigenesis *in vivo* [124], while treatment of colon cancer cells with indomethacin (NSAID) decreases the expression of COX-2 and induces 15-PGDH up-regulation in colon cancer cells [129].

The 5-Lipoxygenase pathway (5-LOX)

Leukotrienes (LTs)

In contrast to prostaglandins, which are produced by most cells, LTs are mainly produced by leukocytes, such as mast cells, macrophages, basophils and eosinophils. The term *leuko-* is given according to the cell type (leukocytes) where they were originally identified, and *triene* refers to the presence of three conjugated double bonds [130]. The 5-LOX pathway is involved in the biosynthesis of leukotrienes, it interacts with 5-LOX activating protein (FLAP) converting AA to LTA_4. Thereafter, LTA_4 is metabolized to biologically active LTB_4 or to cysteinyl leukotrienes (CysLTs): LTC_4, LTD_4 and LTE_4 [107, 109] (Figure 7). In addition to its role in inflammatory diseases such as asthma [131],

LTB_4 has chemotactic activity and pro-tumorigenic effects in breast cancer and melanoma [132, 133].

Leukotrienes, particularly, CysLTs, mediate their actions through the following two cysteinyl leukotriene receptors (CysLTRs): CysLTR1, which is sensitive to classical antagonists, and CysLTR2, which is not inhibited by classical antagonists. Both receptors belong to the G-protein coupled receptor (GPCR) family [134, 135]. Binding of CysLT ligand to the receptor induces conformational changes leading to G-protein activation, converting GDP to GTP; GTP hydrolysis and many intracellular events occur, such as increased concentrations of Ca^{2+} in the cytosol [135]. CysLTR1 is the high affinity CysLT receptor and has a higher affinity for LTD_4 than LTC_4 [136], whereas CysLTR2 has a lower overall affinity but an equal affinity for LTD_4 and LTC_4 [137]. The link between alteration of CysLTRs and cancer has been documented. High expression of CysLTR1 has been reported in many cancers, including the brain, bladder, prostate, breast, and colon [138-141]. High expression of CysLTR1 is associated with poor prognosis of breast and CRC patients. Patients expressing low CysLTR1 and high CysLTR2 have a good prognosis, which is linked to higher survival of CRC patients [142]. CysLTR1 has been shown to induce the accumulation of free β-catenin through LTD_4, thereby inducing proliferation and migration by activating target genes associated with carcinogenesis, such as CyclinD1, C-myc and COX-2 [143]. Moreover, we found that LTD_4 and PGE_2 can induce the promotion of cancer stem cells in both colon cancer cells and xenograft mouse models [144, 145]. In contrast, a CysLTR1 antagonist can prevent the growth of colon cancer xenograft via reducing proliferation and inducing apoptosis of tumor cells [146]. *Cysltr1*$^{-/-}$ mice have significantly reduced tumor formation compared to wild type mice, which indicates the role of CysLTR1 in tumorigenesis [147]. However, LTC_4 acting through CysLTR2 does not induce proliferation of colon cancer cells; it is involved in the terminal differentiation of colon cancer cells, implicating the anti-tumorigenic activity of CysLTR2 compared to CysLTR1 [148].

Inflammatory cells and colorectal cancer

Colorectal cancers, like other solid tumors, are infiltrated by different types of immune cells. Innate immune cells, such as mast cells, macrophages, natural killer cells, neutrophils and dendritic cells, have been found in these tumors [149]. Adaptive immune system cells, such as T-lymphocytes, are also detected in colorectal tumors, where they are either pro- or antitumorigenic. T cells are important for inflammation, cancer development and progression, and also for

anticancer immunity [150-152]. Macrophages, especially tumor-associated macrophages (TAM), are also found in solid tumors and are believed to have an M2-like phenotype [153]. In colon cancer, we found that TAMs promote colon cancer cell migration and subsequent metastasis [154]. In my thesis, I have focused on the role of mast cells in colon cancer.

Mast cells (MCs)

Mast cells (MCs) were first discovered by the German scientist Ehrlich in 1878, who observed connective tissue cells that appeared "well fed", referring to the presence of filled secretory granules that he called "Mastzellen" [155]. However, Kitamura and co-workers reported that MCs are long-lived, hematopoietic cells derived from pluripotent precursors of the bone marrow [156, 157]. MCs are divided into mucosal and connective tissue mast cells. They normally leave the bone marrow as immature cells that circulate through vascular system as immature progenitors; they complete their development either within mucosal or connective tissues [157]. MC granules contain different types of mediators, such as histamine, serotonin, tryptase, chymase and tumor necrosis factor (TNF-α) [158]. In humans, MCs have been categorized into two groups depending on their protease content; one mainly contains tryptase or (more rarely) chymase, and the other group has both proteases. In mice, MCs are divided into connective tissue mast cells, which contain heparin, histamine, tryptase and chymase in their granules, and mucosal mast cells, which contain chymase and small amounts of histamine [159] (Figure 8).

In general, MCs are best known for their involvement in allergic reactions. When mast cells are activated, either through recognition receptors or associated with IgE receptor, they degranulate and release their contents into the microenvironment [160] (Figure 8). Activating MCs can also produce inflammatory mediators de novo, including cysteinyl leukotrienes (LTC_4, LTD_4 and LTE_4) [161]. In addition to their role in allergy and anaphylaxis, MCs also have a role in tissue remodeling, angiogenesis and immune modulation [162].

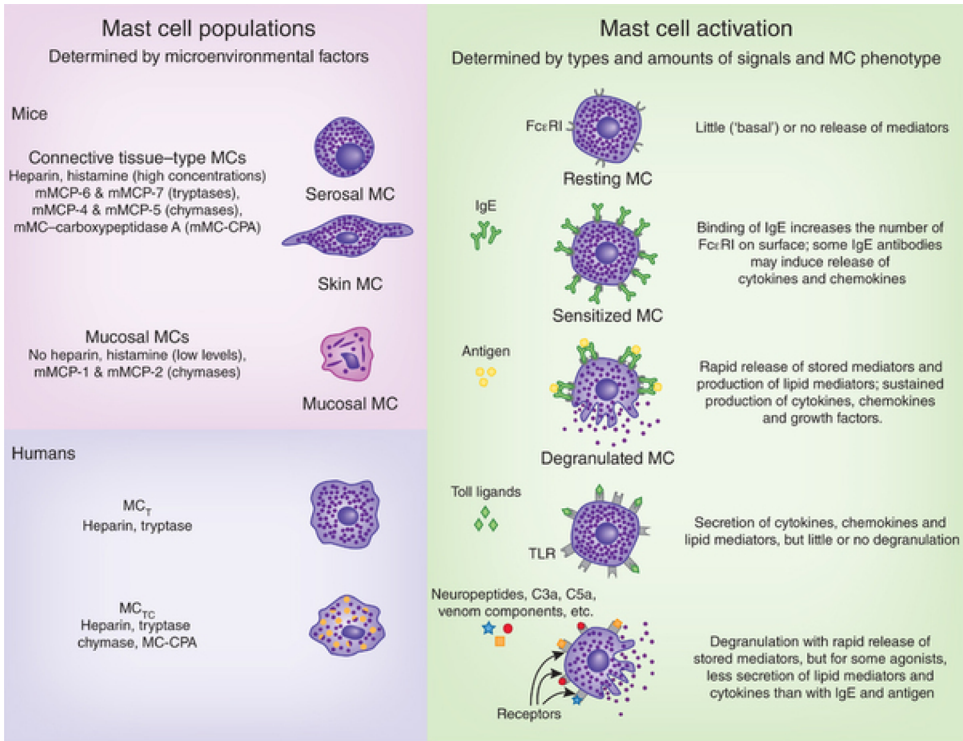

Figure 8: Mast cell population and patterns of functional activation. Adapted from Galli et al. 2011 [159]

The role of MCs in cancer is debatable, including whether they have tumor suppressor effects or tumor promoting effects [163]. MCs are long-lived cells; therefore, they can influence the microenvironment for a long time. Many studies have reported the correlation of the presence of MCs and tumor development with poor patient outcome [164]. High MC density (MCD) together with angiogenesis was associated with poor prognosis in patients with colon [165-167], lung [168] and pancreatic cancer [169]. However, many other studies have shown a protective role of MCs in human cancers. A multivariate analysis of colorectal cancer patients, including Dukes stage, sex, age, perioperative blood transfusion, tumor location, and counts of MCs, predicted good survival [163]. MCs activate nuclear peroxisome proliferator-activated receptor-γ (PPAR-γ), which is associated with better clinical outcome in colon cancer [170]. Low MC density is associated with worse prognosis in non-small lung cancer patients [171, 172]. A positive correlation between MC density and better clinical outcomes in prostate cancer has been documented [173]. In our study, we have also shown that high MCD is associated with good prognosis in colon cancer patients [174].

Aim

The general aim of the thesis is to evaluate the clinical significance of various tumor suppressors in colon cancer patients. Additionally, we investigated the underlying mechanisms or signaling triggered by these tumor suppressors in colon cancer cells, and whether the re-expression of these tumor suppressors could be an attractive therapeutic strategy for the treatment of colon cancer patients.

1. To explore the relevance of mast cells for the overall survival of colon cancer patients and a colitis-associated colon cancer mouse model
2. To investigate the effect of WNT-5A and its mimicking peptide Foxy-5 on 15-PGDH expression in colon cancer
3. To investigate the effect of LTC_4, through its receptor CysLT2, on 15-PGDH expression in colon cancer cell lines and colon cancer tissue

Methodology

Cell lines

All cell lines used in my projects were from ATCC. HT-29 colon cancer cells (ATCC# HTB-38™), grown in McCoy's 5A medium with glutamine, have a wild-type *KRAS* gene and mutant carboxy-truncated *APC* gene. Caco-2 colon cancer cells (ATCC# HTB-37™) have a wild-type *KRAS* gene, wild-type *BRAF* gene and mutated *APC* gene. MDA-MB-468 (ATCC# HTB-132) breast cancer cells, grown in DMEM, have mutated *P53* and *BR1* genes. The media was supplemented with 10% fetal bovine serum and 100 µg/ml penicillin/streptomycin. Cells were maintained at 37°C in a humidified atmosphere containing 5% CO_2. The cells were routinely screened for the absence of mycoplasma contamination.

Western blotting and cell fractionation

After washing the cells twice with ice-cold PBS, whole cell lysates were prepared with lysing in lysis buffer. The resulting lysates were homogenized by 10 passages through a syringe and then centrifuged at 10,000 × g for 10 min at 4°C. The supernatant was considered to represent the whole cell lysate. For nuclear fractions, a nuclear extraction kit from Thermo Scientific was used according to the manufacturer's instructions. Laemmle buffer (4X) was added to the samples. The samples were adjusted to contain equal amounts of protein and boiled for 10 min in sample buffer (0.5 M Tris-base, 10% SDS, glycerol, bromophenol blue, 15 mg/ml DTT) before being loaded onto 10% SDS polyacrylamide gels and analyzed by electrophoresis (SDS-PAGE). After separation, the proteins were transferred to a PVDF membrane (Bio-Rad, Hercules, CA, USA). The membranes were blocked for 1 h with 3% BSA/PBS at room temperature, followed by incubation with a primary antibody overnight at 4°C. The membranes were washed extensively and incubated with the corresponding secondary antibody for 1 h at RT. The membranes were washed and incubated with Immobilon™ Western Chemiluminescent HRP Substrate (Immobilon™ Western, Merck Millipore, Billerica, MA, USA), and the proteins were detected using the Bio-Rad ChemiDoc

XRS+ system. The densitometric analyses were conducted using Image Lab 3.0 software.

Patients

All patients underwent surgery for colorectal cancer at Malmö hospital in 1990. The tumor material was retrospectively collected, and no matching or stratification was performed. All patients had a pathologically confirmed diagnosis of adenocarcinoma before surgery. All tumors with available slides or paraffin blocks were histologically re-evaluated on hematoxylin and eosin (H&E) stained slides. Clinical and histopathological data were retrieved from patient charts and pathology records. Tumors were staged according to TNM classification for malignant tumors, and tumor grade was determined as high, medium or low. The follow up started at the time of diagnosis and ended at death, emigration or December 31, 2000. The study was approved by the ethical committee of Lund University. In project II, tumor tissues and corresponding mucosa from 23 colon cancer patients were from the Department of Clinical Science, Sahlgrenska Academy, Gothenburg, Sweden. Tumors and large bowel tissue samples were collected down to the serosa level, kept fresh frozen in liquid nitrogen, and stored at -80°C until analysis. A certified pathologist staged all tumors. Tumor samples contained approximately 70-80% tumor cells based on visual inspection [175].

Tumor tissue microarray (TMA) and immunohistochemistry

From the tumor tissue, two to three 1.5 mm tissue cores were placed in a new paraffin block using an automated Beecher Micro-Arrayer (Beecher Instruments,Sun Prairie, WI,USA). The normal areas were chosen from the distal parts of the safe margins. Immunohistochemical staining was performed as previously described [142]. Briefly, the formalin-fixed and paraffin-embedded sections were cut into 1 μm sections, dried, de-paraffinized and rehydrated, then stained with H&E for re-evaluation by a pathologist (RE). Tumor sections were then stained for 15-PGDH or WNT5A, as previously described. All immunohistochemical procedures were performed using a Dako automatic slide stainer (Dako) according to the manufacturer's instructions.

Real-time qPCR

Total RNA from the different cell samples was isolated using Qiagen RNeasy Plus Mini Kits (Qiagen GmbH, Hilden, Germany). The cDNA synthesis was performed using RevertAid H Minus M-MuLV reverse transcriptase (ThermoFisher Scientific, USA). The following primers were used: *HPRT1* (HS99999909), *HPGD* (15-PGDH) HS00168359_m1), *SI* (SI, HS00356112_m1), *MUC2* (mucin-2, HS00159374_m1) and *CCND1* (cyclin D1, Hs00765553_m1). Amplifications were performed in an Mx3005P system (Agilent Technologies, Inc., CA, USA). The reactions were normalized to the housekeeping gene HPRT1 and analyzed with MxPro qPCR software (Agilent Technologies, Santa Clara, CA, USA).

Luciferase assay

A Dual-Luciferase Reporter Assay System (Promega, Madison, WI, USA) was used. 15-PGDH promoter plasmids [141] (a gift from Professor Birgit Gellersen, University of Hamburg, Germany) at a final concentration of 1 μg/ml, together with a control *Renilla* luciferase reporter plasmid of 50 ng/ml, were used for the transfections. The DNA plasmids were allowed to form complexes with PolyFect Transfection Reagent (Qiagen) (ratio 4:1) in Opti-MEM (Gibco/Thermo Fisher Scientific, Waltham, MA, USA), and the cells were treated with the DNA-PolyFect mixture at 37°C for 24 h. The medium was changed to a serum-containing medium, and the cells were allowed to recover for 24 h. Thereafter, the cells were incubated for 2 h with a serum-free medium before treatments. The cells were washed twice with PBS and lysed by the addition of the passive lysis buffer from the Dual-Luciferase Reporter Assay System. The lysed samples were cleared by centrifugation at 1000 x g for 5 min according to the manufacturer's instructions. Firefly and the control *Renilla* luminescence were measured on a MiniLumat LB 9506 luminometer (Berthold Technologies GmbH, Dusseldorf, Germany) according to the protocol, and the ratio was calculated. Triplicate samples were prepared in every set of experiments.

Immunofluorescence

In project II, cells were seeded to grow on coverslips for three days, washed with PBS, and fixed in 4% paraformaldehyde for 15 min. Next, cells were washed with

PBS, permeabilized in 0.1% Triton X-100 in PBS for 5 min, and washed six times in PBS. Cells were thereafter incubated at RT in 3% goat serum in PBS for 45 min and washed, followed by 1 h incubation with anti-β-catenin antibody (1:500) in 1% goat serum/PBS. Cells were then stained with DAPI for 3 min (1:1000). The coverslips were washed and mounted in Zeiss fluorescent mounting medium. Fluorescent images were taken using the Zeiss LMS 700 confocal microscope (Carl Zeiss Microscopy GmbH, Jena, Germany). In project III, cells were cultured on coverslips for 3 days. Then, cells were fixed and permeabilized in methanol/acetone (1:1; -20^{o} C for 4 min). Cells were washed with PBS and incubated for 30 min in PBS staining buffer (PBS, 1% FCS, 0.5% BSA) to prevent non-specific antibody binding. Cells were incubated 1 h with primary antibody, washed, and incubated 1 h with Alexa 480 or Alexa 568 secondary antibody (Invitrogen). Cover slips were washed and mounted on glass slides with a fluorescent mounting medium. Fluorescence images were captured using a Zeiss LSM 700 confocal microscope (Carl Zeiss).

Colitis-associated colon cancer (CAC) mouse model

The chemically induced CAC model was generated using 6- to 8-week-old female mice during a 90-day period (n=5 mice/genotype). The CAC model was generated through an intraperitoneal injection of the carcinogen azoxymethane (10 mg/kg body weight), followed by three cycles of the irritant dextran sodium sulfate in the drinking water, provided *ad libitum*, for five days. Mice with a targeted *Cysltr1* gene disruption (*Cysltr1*$^{-/-}$) on the C57BL/6N background were a kind gift from Professor Frank Austen (Harvard Medical School, Brigham and Women's Hospital, Boston, MA, USA) [176]. *Cysltr1*$^{-/-}$ mice and their control littermates were bred and maintained at Lund University Animal Facility in Malmö according to ethical permit M262-12, which was approved by the Regional Ethical Committee for Animal Research at Lund University, Sweden. The breeding colony was genotyped for the presence of the *Cysltr1* allele by PCR assays using the following three primers: *Cysltr1* sense: 5'-AAAACAATGACGTGCACTATAAAG-3', *Cysltr1* antisense: 5'-AATCATGTATACTTGGAAGAAGGCTGA-3', and Neo antisense: 5'-ATCTTGTTCAATGGCCGATCCCAT-3', generating a product of 284 bp for the wild type and 333 bp for the null *Cysltr1* allele.

All mouse colons were first rinsed with a physiological salt solution and subsequently fixed in formalin using the "swiss roll" method, followed by embedding in paraffin.

MDA-MB-468 cell xenografts

The generation of MDA-MB-468 breast cancer cells stably expressing a WNT5A vector or an empty control vector was previously described by Prasad et al [177]. Four- to five-week-old female athymic nude mice were purchased from Harlan Laboratories Inc. (Boxmeer, Netherlands). The cells (2 x 10^6 cells in 100 µl of serum-free medium) were inoculated subcutaneously into both flanks of the mice. The animals were sacrificed 24 days after they had been inoculated, and the tumors were removed, measured and weighed. The primary tumors were dissected, fixed in 4% paraformaldehyde and embedded in paraffin. Sections (4 µm) were cut and subsequently stained with the 15-PGDH-specific antibody. The stained tissue sections were scanned with the Scanscope CS System (Aperio, Bristol, UK) and analyzed with the Aperio Image Scope software. These animal experiments were performed at Pharmatest Services Ltd (Turku, Finland) under the ethical permission no. 3257/04.10.07/2014.

Statistical analysis

GraphPad Prism software 5.0 (San Diego, CA, USA) was used for the statistical analyses. The differences between groups of data were considered statistically significant if $P \leq 0.05$ by the two-tailed Student's *t*-test. All means were calculated based on data from at least three different experiments. Overall survival (OS) was the primary endpoint. Kaplan-Meier estimates were used to illustrate survival, and log rank test was used to assess for the equality of survival curves. Hazard ratios were estimated using a Cox proportional hazards model for OS in uni- and multivariate analyses. SPSS version 19.0 (SPSS, IBM, Armonk, NY, USA) was used for the statistical analyses of all the immunostaining data.

Results and discussion

Paper I

Chronic inflammation is a risk factor for colon cancer. The key players in the inflammation–neoplasia relationship are the immune cells and the products released by the innate immune system. Chronic inflammation is dominated by the presence of cells, macrophages and MCs at the site of inflammation [178]. MCs induce T-lymphocyte activation and recruitment through the production of chemotactic cytokines such as C-C motif chemokine ligand 5 (CCL5), which bind to G-protein coupled receptor C-C chemokine receptor type 5 (CCR5). MCs, eosinophils and basophils also produce cytokines such as interleukin 4 (IL-4), which induce the apoptosis of tumor cells. Macrophages and MCs secrete tumor necrosis factor α (TNF-α), which induces both tumor cell death and leukocyte infiltration [179]. MCs are a primary source of intratumoral prostaglandin D2 (PGD_2), which limits tumor growth through the suppression of angiogenesis and proliferation and promotes apoptosis [107]. Although MCs have a clear role in inflammation and allergic reaction, their role in cancer is not yet clear. Several studies have shown that MCs can promote tumor development and progression. MCs have been shown to play a role in primary breast cancer angiogenesis [180] and lung cancer angiogenesis [181]. Furthermore, other studies have shown that high MCD was correlated with low overall survival of colon cancer patients [165, 167]. In contrast, still other studies have reported the correlation between high MCD and longer survival of colon cancer patients [163, 166, 182]. The aim of my study was to explore the relevance of mast cells in CRC.

In our study, tumor samples from 72 colon cancer patients were evaluated for the presence of MCs by immunohistochemistry in normal and cancer tissues using anti-tryptase and anti-chymase antibodies. MCD was evaluated in normal and cancer tissue. We found that in normal tissue, the mean MCD±SEM was 15±1.6 for tryptase-positive MCs and 101±11.5 for chymase-positive MCs; in cancer tissue, the mean MCD was 8±1.1 tryptase-positive and 52±7.4 chymase-positive MCs. For both tryptase- and chymase-positive cells, the MCD was significantly higher in normal tissue compared to cancer tissue ($P=<0.001$). For survival analysis, we found that colon cancer patients with high MCD have significantly longer survival ($n=70$, $P=0.031$). We observed the same result in patients without

distant metastasis at the time of diagnosis (n=56, P=0.004) and in a subgroup of patients younger than 75 years (n=33, P=0.015). We preformed univariate and multivariate Cox regression analysis. In univariate analysis, we found that high MCD in cancer tissue significantly reduced the risk of death by 46% (HR 0.539; 95% CI 0.302-0.961); in multivariate analysis, our data showed that MCD was independently associated with longer overall survival in colon cancer patients (HR 0.380; 95% CI 0.202-0.713). Previously, we have shown that patients with few M2 macrophages in their cancer tissues have a better prognosis[154]; here, we found that patients having low M2 and high MCD in cancer tissue had statistically significantly longer survival compared to patients with high M2 and low MCD.

We have previously shown that low CysLTR1 expression in CRC patients is associated with better overall survival [142]. In this study, we investigated the correlation between MCs and cytoplasmic CysLTR1. We found a significant negative correlation between the number of MCs in cancer tissue and CysLTR1 expression (Pearson's correlation, r = 0.3; P = 0.028). Based on this result, we investigated the presence of MCs in normal and tumor tissue of wild type and *Cysltr1*$^{-/-}$ mice in a colitis associated colon cancer (CAC) model, a model known to have fewer polyps/tumors [147]. Our result showed that *Cysltr1*$^{-/-}$ mice had a significantly higher number of tryptase-positive MCs in polyps/tumors compared with wild-type mice (P = < 0.05). Taken together, these results indicate that mast cells have an anti-tumor effect, possibly by creating an anti-tumor environment in colon cancer [174].

Paper II

PGE_2 plays an important role in cancer, especially colorectal cancer, where its levels are elevated [183], and can influence many of the hallmarks of cancer [5, 107] (see figure 4). This elevated level of PGE_2 is due to the up-regulation of COX-2, the enzyme responsible for converting arachidonic acid into PGH2, which is subsequently converted to prostaglandins via prostaglandin synthase [184]. In normal tissue, PGE_2 is degraded by 15-hydroxyprostaglandin dehydrogenase (15-PGDH) by converting it into a biologically less active metabolite. 15-PGDH is downregulated in colon cancer, which suggests that 15-PGDH could be a tumor suppressor gene [185]. Elevated levels of PGE_2 were previously attributed to COX-2 up-regulation, but more recently to the down-regulation of 15-PGDH. The WNT signaling pathway plays an important role in the homeostasis of the intestinal epithelium, and it is activated in 90% of sporadic CRC [186]. Canonical Wnt/β-catenin signaling has been shown to interact with the 15-PGDH promoter and repress 15-PGDH expression in colon cancer [123]. The aim of the present study

was to investigate the effect of non-canonical Wnt/β-catenin signaling, via WNT5A, on the expression of 15-PGDH.

We have investigated the expression of both 15-PGDH and WNT5A using tissue microarrays (TMA) with human colon cancer tissues and matched normal tissues. The paired samples were stained with both 15-PGDH and WNT5A specific antibodies. Both proteins were expressed at high levels in normal mucosa and significantly downregulated in cancer tissues. For further analysis, tumor samples from 84 patients with primary colon cancer were used to generate TMAs, which were stained with 15-PGDH for immunohistochemical evaluation. Out of 84 patients, 74 (88%) had low expression and 10 (12%) had high expression of 15-PGDH. Of the 84 patient samples analyzed for 15-PGDH, 66 were analyzed for the expression of WNT5A, and our results showed 26 (39%) of these samples have no or low expression of WNT5A, based on the positive correlation found between 15-PGDH and WNT-5A (Pearson correlation $r = 0.3$; $P = 0.027$). Kaplan-Meier survival analysis shows that patients with high levels of 15-PGDH tended to have better survival compared to those expressing low levels ($P = 0.08$). For WNT5A, patients expressing high levels of WNT5A had statistically significantly better survival compared to the patients with low expression levels ($P = 0.002$). All 26 patients with low expression WNT5A levels also had low expression of 15-PGDH, indicating that loss of WNT5A protein expression in CRC correlated with loss of 15-PGDH protein expression. Moreover, patients expressing low levels of both proteins had reduced overall survival compared to patients with high levels of both proteins ($P = 0.005$).

We next investigated whether WNT5A signaling can lead to re-expression of 15-PGDH protein in colon cancer cells (HT-29 and Caco-2) that are negative for WNT5A. After treating cells with either recombinant WNT5A or its mimicking peptide Foxy-5, the cells showed significantly increased expression of 15-PGDH at both the protein and mRNA levels. To investigate the signaling pathway by which WNT5A mediates 15-PGDH expression, we analyzed the c-jun N-terminal kinase (JNK) pathway, a well-studied target of WNT5A signaling. We observed an up-regulation of phospho-JNK after stimulating the cells with Foxy-5. We also observed down-regulation of 15-PGDH promoter activity in the presence of a JNK inhibitor after stimulation with both rWNT5A or Foxy-5 in cells transfected with the 15-PGDH promoter. Furthermore, we also found that JNK inhibition down-regulates 15-PGDH mRNA levels. These results indicate that WNT5A mediates 15-PGDH expression through the JNK pathway. We further investigated whether WNT5A suppressed canonical Wnt/β-catenin signaling, and whether that suppression could affect the expression of 15-PGDH. We found that rWNT5A or Foxy-5 reduced the non-phospho-active form of β-catenin and induced the phosphorylation of β-catenin, which reduced the total level of β-catenin in both HT-29 and Caco-2 colon cancer cell lines. Moreover, we investigated whether

inhibition of canonical Wnt/β-catenin signaling could influence 15-PGDH expression. To this end, we used XAV-939, an inhibitor of Wnt/β-catenin signaling that stabilizes axin, leading to increased degradation of β-catenin. Our results showed that XAV-939 can induce 15-PGDH expression and reduce non-phospho-active β-catenin and the total level of β-catenin to a similar level as rWNT5A. We also observed that in the presence of rWNT5A and XAV-939, the increase of 15-PGDH expression and reduction of active form or total β-catenin were more pronounced. Our finding showed that non-canonical WNT5A can inhibit canonical Wnt/β-catenin signaling, and as a result, an up-regulation of 15-PGDH was observed. In breast cancer cells, we noticed the up-regulation of 15-PGDH after stimulating MDA-MB-468 cells with rWNT5A or Foxy-5. In mice, we injected WNT5A-transfected MDA-MB-468 cells into mice flanks; as a control, we injected MDA-MB-468 cells transfected with empty vector (control). We found a significant reduction in tumor growth and tumor volume in mice with WNT5A-transfected cells compared to the control mice. The tumors were immunostained with anti-15-PGDH antibody, and we observed a significant increase in 15-PGDH intensity in tumors with WNT5A-transfected cells compared to the control. These results confirm our previous finding that WNT5A induces the expression of 15-PGDH in both colon and breast cancer cells *in vivo* and *in vitro*. Cancer cells are proliferative cells in their nature, and they are poorly differentiated. To investigate whether our finding could affect the differentiation of colon cancer cells, we studied the effect of WNT5A on differentiation by measuring the level of two differentiation markers, mucin-2 (Muc-2) and sucrose isomaltase (SI). A significant up-regulation in both markers was observed in both cell lines after treatment with WNT5A and Foxy-5. This up-regulation could be inhibited in the presence of both a JNK inhibitor and the GSK-3β inhibitor (CHIR-99021), indicating that WNT5A could induce differentiation in colon cancer cells via activating the JNK pathway and inhibiting Wnt/β-catenin signaling. We believe that this could be due to the ability of WNT5A to induce 15-PGDH expression.

In conclusion, our findings reveal a novel interaction between two tumor suppressor proteins, 15-PGDH and WNT5A, through two different pathways. The benefit of this finding is that the level of 15-PGDH in tumor tissue could be used as biomarker in future clinical studies with WNT5A-mimicking peptide Foxy-5 [187].

Paper III

We previously showed that high expression of CysLTR2 in colon cancer patients is associated with better prognosis, which indicates that CysLTR2 has an anti-tumor effect [148]. Based on these data, the aim of this study was to investigate the effect of LTC_4, via CysLTR2, on the tumor suppressor 15-PGDH. We found that tumor tissue from 23 colon cancer patients expressed low levels of both CysLTR2 and 15-PGDH. Next, we examined whether LTC_4 through CysLTR2 can induce the expression of 15-PGDH. Our results showed that after treatment with LTC_4, a significant induction of 15-PGDH expression was observed in HT-29 and Caco-2 cells. This up-regulation was inhibited by pretreatment with CysLTR2 antagonist. Our results showed that LTC_4 via CysLTR2 could induce the expression of 15-PGDH. The 15-PGDH promoter has binding sites for AP-1 [188]. Therefore, we elucidated whether LTC_4 can induce AP-1 activity, and which pathway is involved in this process. We transfected HT-29 cells with a 15-PGDH promoter construct with different binding sites for AP-1. The cells were pretreated with different pathway inhibitors, including MAPK inhibitor, PI3K inhibitor, JNK1 inhibitor and CysLTR2 antagonist. We found that LTC_4 induced 15-PGDH promoter activation, which was inhibited by the CysLTR2 antagonist. We also found that except for the JNK1 inhibitor, none of the other inhibitors could inhibit the promoter activity of 15-PGDH. Furthermore, phosphorylation of JNK by LTC_4 was detected by Western blot in HT-29 cells. We speculated that LTC_4, via CysLTR2, acted through JNK/AP-1 pathway to induce the expression of 15-PGDH in colon cancer cells.

Independently, both CysLTR2 and 15-PGDH were previously shown to inhibit the proliferation of colon cancer cells [148, 189], but their role in inducing the differentiation of colon cancer cells is not clear. Here, we investigated their effect on differentiation by studying two differentiation markers, sucrase isomaltase (SI) and mucin-2 (Muc-2). After stimulating the cells with LTC_4, we found significant up-regulation of Muc-2 mRNA levels. This up-regulation was inhibited in the presence of both the CysLTR2 antagonist and the JNK1 inhibitor. Furthermore, we confirmed the results by confocal microscopy. We also observed a significant up-regulation of SI at the protein and mRNA levels. These results indicated that LTC_4 signaling through a CysLTR2/JNK dependent pathway could induce differentiation of colon cancer cells.

Conclusions

The conclusions of my thesis are as follows:

- The presence of mast cells in the tumor microenvironment is associated with longer survival of CRC patients by creating an antitumor microenvironment

- Re-expression of the tumor suppressor gene 15-PGDH by WNT5A or its mimicking peptide Foxy-5 leads to better and longer survival of CRC patients

- CysLTR2 shows antitumor effects by re-expressing 15-PGDH in combination with a more differentiated colon cancer cell

- These findings provide an important clue to better understand the tumor microenvironment and the tumor suppressor genes in colon cancer, and can help to identify new therapeutic targets for colon cancer patients

Popularized summary

Cancer is uncontrolled cell growth with the ability to invade or spread to other parts of the body. Colorectal cancer (CRC) is cancer of colon and rectum. CRC is the third most common cancer and the fourth leading cause of cancer-related deaths worldwide. CRC is a multifactorial disease, with environmental causes such as excessive consumption of alcohol, diets rich in red meat or low in fiber, and low physical activity, as well as genetic causes, which involve either the activation of oncogenes, the genes that have the potential to cause cancer, or the inhibition of the tumor suppressor genes, the genes that protect the cell from cancer. CRC is closely linked to chronic inflammation. Patients with inflammatory bowel disease (IBD) have an increased risk of developing CRC. Many studies have shown that host immune cells that have infiltrated the tumor can enforce a massive inflammatory response that can kill tumor cells and inhibit growth of the tumor . The milieu which contains cellular infiltration surrounding the tumor is called the tumor microenvironment.

The aim of this thesis was to evaluate the clinical significance of the tumor microenvironment, including mast cells (MCs), and tumor suppressor genes, including 15-hydroxyprostaglandin dehydrogenase (15-PGDH) and WNT5A, in colon cancer, and whether the re-expression of these tumor suppressors could be an attractive therapeutic strategy for the treatment of colon cancer patients.

Mast cells (MCs) are immune cells derived from bone marrow. They are well known in allergic reactions, but their role in cancers, including colon cancer, is still under debate. Past studies have shown that MCs in the tumor microenvironment could have tumor promoting effects, whereas other studies showed that MCs have tumor suppressor effects. In our study, we found that the presence of MCs in the tumor microenvironment is beneficial for colon cancer patients; patients with more MCs have a better prognosis and longer survival, indicating that MCs have an antitumor effect.

15-PGDH is a tumor suppressor known to be downregulated in colon cancer. In our study, we found that colon cancer patients expressing higher levels of 15-PGDH have good prognosis compared to those who have low expression. We also found that WNT5A, another tumor suppressor, interacts with 15-PGDH. Patients expressing high levels of both proteins have even better prognosis and longer survival than patients expressing low levels of both proteins. We also found that

WNT5A and its mimicking peptide, Foxy-5, can suppress β-catenin, which is often mutated in colon cancer. We also found that the eicosanoid LTC_4, which is an inflammatory mediator present in the tumor microenvironment, has an anti-tumor effect by inducing the expression of 15-PGDH and inducing the differentiation of colon cancer cells by changing the phenotype of cancer cells to more normal-like cells.

In summary, these new findings provide a better understanding of both the tumor microenvironment and tumor suppressor genes and may help to identify new therapeutic targets for colon cancer treatment.

Populärvetenskaplig sammanfattning

Cancer kan definieras som okontrollerad celltillväxt med förmågan att invadera eller spridas till andra delar av kroppen. Kolorektalcancer är cancer i tjocktarmen eller ändtarmen. Tjocktarmscancer är den tredje vanligaste cancerformen och den fjärde ledande orsaken till cancerrelaterade dödsfall i världen. Tjocktarmscancer är en multifaktoriell sjukdom, med miljömässiga orsaker som överdriven konsumtion av alkohol, kost rik på rött kött eller låg i fiber, låg fysisk aktivitet, liksom genetiska orsaker, som innebär antingen aktivering av onkogener, de gener som har potential att orsaka cancer, eller hämning av tumörundertryckande gener (tumorsuppressorgener), de gener som skyddar cellen från cancer. Tjocktarmscancer är nära kopplad till kronisk inflammation i tarmen. Patienter med inflammatorisk tarmsjukdom har en ökad risk att utveckla tjocktarmscancer. Många studier har också visat att värdimmunceller som har infiltrerat tumören kan ge en massiv inflammatorisk respons som kan döda tumörceller och hämma tillväxt av tumören. Den miljö som innehåller cellulär infiltration kring tumören kallas tumörens mikromiljö.

Syftet med min avhandling var att utvärdera den kliniska betydelsen av tumörens omgivande miljö, som mastceller, och tumörsuppressorgener, som 15-hydroxyprostaglandin dehydrogenas (15-PGDH) och WNT5A i tjocktarmscancer, och om re-expression av dessa tumörsuppressorer skulle kunna vara en attraktiv terapeutisk strategi för behandling av tjocktarmscancer.

Mastceller är immunceller som produceras i benmärgen. De är välkända i allergiska reaktioner, men deras roll i cancer, inklusive koloncancer, är fortfarande inte klarlagda. Tidigare studier har visat att mastceller i tumörens mikromiljö kan ha tumörbefrämjande effekterna, medan andra studier visade att mastceller har tumörhämmande effekter. I vår studie fann vi att närvaron av mastceller i tumören mikromiljön är fördelaktigt för koloncancerpatienter; patienter med fler mastceller har en bättre prognos och längre överlevnad, vilket indikerar att mastceller har en antitumöreffekt.

15-PGDH är en tumörsuppressor som ofta är nedreglerad i tjocktarmscancer. I vår studie fann vi att tjocktarmscancerpatienter som uttrycker högre nivåer av 15-PGDH har god prognos jämfört med patienter som har lågt uttryck. Vi fann också att WNT5A en annan tumörsuppressor, interagerar med 15-PGDH. Patienter som uttrycker höga nivåer av båda proteinerna har ännu bättre prognos och längre

överlevnad än patienter som uttrycker låga nivåer av dessa proteiner. Vi fann också att WNT5A eller dess peptid, Foxy-5, kan undertrycka β-catenin, som ofta är förändrad i tjocktarmscancer. Vi fann också att leukotrien C4, som är en inflammatorisk mediator som finns i tumörens mikromiljö, har en anti-tumöreffekt genom att öka mängden av tumörsuppressor proteinet 15-PGDH samt att inducera differentiering av koloncancerceller genom att ändra fenotypen av cancerceller till en mer ”normal-liknande cell”.

Sammanfattningsvis, kan dessa nya rön ge en bättre förståelse för både tumörens mikromiljö och tumörsuppressor proteinerna 15-PGDH och WNT5A som kan bidra till att identifiera nya terapeutiska mål för behandling av tjocktarmscancer.

دور الجينات الكابحة لسرطان القولون، آليات و فرص العلاج

السرطان هو نمو الخلايا غير المنضبط مع القدرة على الغزو أو الإنتشار إلى أجزاء أخرى من الجسم. سرطان القولون هو ثالث أكثر أنواع السرطان شيوعاً و رابع سبب رئيسي للوفيات المرتبطة بالسرطان في جميع أنحاء العالم، و هو مرض متعدد العوامل و الأسباب، منها أسباب بيئية مثل الإستهلاك المفرط للكحول، و الوجبات الغنية باللحوم الحمراء أو منخفضة الألياف، بالإضافة إلى انخفاض النشاط البدني، فضلاً عن الأسباب الوراثية، والتي تنطوي إما على تفعيل الجينات المسرطنة و هي الجينات التي يمكن أن تسبب السرطان، أو تثبيط الجينات الكابحة للورم. ويرتبط سرطان القولون ارتباطا وثيقاً بالإلتهابات المزمنة

كما أن المرضى الذين يعانون من التهابات القولون المزمنة يزيد لديهم خطر الاصابة بسرطان القولون. وقد أظهرت العديد من الدراسات أن الأورام السرطانية تحتوي على خلايا مناعية ذات تأثير التهابي واسع النطاق مع خاصية منع أي نمو غير طبيعي والقدرة على القضاء على الخلايا السرطانية

وكان الهدف من هذه الرسالة تقييم الأهمية السريرية للخلايا المناعية و الجينات الكابحة للورم، بغية استخدامها كاستراتيجية علاجية لمرضى سرطان القولون مستقبلاً

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References

1. Hajdu SI. A note from history: landmarks in history of cancer, part 1. Cancer. 2011;117(5):1097-102.
2. DeVita VTJ, Rosenberg SA. Two Hundred Years of Cancer Research. New England Journal of Medicine. 2012;366(23):2207-14.
3. Zhu K, Liu Q, Zhou Y, Tao C, Zhao Z, Sun J, et al. Oncogenes and tumor suppressor genes: comparative genomics and network perspectives. BMC genomics. 2015;16 Suppl 7:S8.
4. Hanahan D, Weinberg RA. The hallmarks of cancer. Cell. 2000;100(1):57-70.
5. Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. Cell. 2011;144(5):646-74.
6. Triantafillidis JK, Nasioulas G, Kosmidis PA. Colorectal cancer and inflammatory bowel disease: epidemiology, risk factors, mechanisms of carcinogenesis and prevention strategies. Anticancer Research: International Journal of Cancer Research and Treatment. 2009;29(7):2727-37.
7. Markowitz SD, Bertagnolli MM. Molecular origins of cancer: Molecular basis of colorectal cancer. The New England journal of medicine. 2009;361(25):2449-60.
8. Sinicrope FA, Gill S. Role of cyclooxygenase-2 in colorectal cancer. Cancer metastasis reviews. 2004;23(1-2):63-75.
9. Turini ME, DuBois RN. Cyclooxygenase-2: a therapeutic target. Annual review of medicine. 2002;53:35-57.
10. Ensor CM, Tai HH. 15-Hydroxyprostaglandin dehydrogenase. Journal of lipid mediators and cell signalling. 1995;12(2-3):313-9.
11. Backlund MG, Mann JR, Holla VR, Buchanan FG, Tai HH, Musiek ES, et al. 15-Hydroxyprostaglandin dehydrogenase is down-regulated in colorectal cancer. The Journal of biological chemistry. 2005;280(5):3217-23.
12. Wittkopf N, Neurath MF, Becker C. Immune-epithelial crosstalk at the intestinal surface. Journal of gastroenterology. 2014;49(3):375-87.
13. Garrett WS, Gordon JI, Glimcher LH. Homeostasis and inflammation in the intestine. Cell. 2010;140(6):859-70.
14. Varol C, Vallon-Eberhard A, Elinav E, Aychek T, Shapira Y, Luche H, et al. Intestinal lamina propria dendritic cell subsets have different origin and functions. Immunity. 2009;31(3):502-12.
15. Chandran P, Satthaporn S, Robins A, Eremin O. Inflammatory bowel disease: dysfunction of GALT and gut bacterial flora (I). The surgeon : journal of the Royal Colleges of Surgeons of Edinburgh and Ireland. 2003;1(2):63-75.

16. Sancho E, Batlle E, Clevers H. Signaling pathways in intestinal development and cancer. Annual review of cell and developmental biology. 2004;20:695-723.
17. Abreu MT. Toll-like receptor signalling in the intestinal epithelium: how bacterial recognition shapes intestinal function. Nat Rev Immunol. 2010;10(2):131-44.
18. Peterson LW, Artis D. Intestinal epithelial cells: regulators of barrier function and immune homeostasis. Nat Rev Immunol. 2014;14(3):141-53.
19. Umar S. Intestinal Stem Cells. Current gastroenterology reports. 2010;12(5):340-8.
20. Eberl G. Inducible lymphoid tissues in the adult gut: recapitulation of a fetal developmental pathway? Nat Rev Immunol. 2005;5(5):413-20.
21. Balkwill F, Mantovani A. Inflammation and cancer: back to Virchow? Lancet (London, England). 2001;357(9255):539-45.
22. Coussens LM, Werb Z. Inflammation and cancer. Nature. 2002;420(6917):860-7.
23. Hanauer SB. Inflammatory bowel disease: epidemiology, pathogenesis, and therapeutic opportunities. Inflammatory bowel diseases. 2006;12(5):S3-S9.
24. Podolsky DK. Inflammatory bowel disease. New England Journal of Medicine. 1991;325(13):928-37.
25. Abraham C, Cho JH. Inflammatory bowel disease. The New England journal of medicine. 2009;361(21):2066-78.
26. Feagins LA, Souza RF, Spechler SJ. Carcinogenesis in IBD: potential targets for the prevention of colorectal cancer. Nature reviews Gastroenterology & hepatology. 2009;6(5):297-305.
27. Saleh M, Trinchieri G. Innate immune mechanisms of colitis and colitis-associated colorectal cancer. Nat Rev Immunol. 2011;11(1):9-20.
28. Rubin DC, Shaker A, Levin MS. Chronic intestinal inflammation: inflammatory bowel disease and colitis-associated colon cancer. Frontiers in immunology. 2012;3:107.
29. Kinugasa T, Akagi Y. Status of colitis-associated cancer in ulcerative colitis. World journal of gastrointestinal oncology. 2016;8(4):351-7.
30. Herrinton LJ, Liu L, Levin TR, Allison JE, Lewis JD, Velayos F. Incidence and mortality of colorectal adenocarcinoma in persons with inflammatory bowel disease from 1998 to 2010. Gastroenterology. 2012;143(2):382-9.
31. Nguyen GC, Bressler B. A tale of two cohorts: are we overestimating the risk of colorectal cancer in inflammatory bowel disease? Gastroenterology. 2012;143(2):288-90.
32. Axelrad JE, Lichtiger S, Yajnik V. Inflammatory bowel disease and cancer: The role of inflammation, immunosuppression, and cancer treatment. World journal of gastroenterology : WJG. 2016;22(20):4794-801.
33. Rubin DT, Cruz-Correa MR, Gasche C, Jass JR, Lichtenstein GR, Montgomery EA, et al. Colorectal cancer prevention in inflammatory bowel disease and the role of 5-aminosalicylic acid: a clinical review and update. Inflamm Bowel Dis. 2008;14(2):265-74.

34. Rubin DT, LoSavio A, Yadron N, Huo D, Hanauer SB. Aminosalicylate therapy in the prevention of dysplasia and colorectal cancer in ulcerative colitis. Clinical gastroenterology and hepatology : the official clinical practice journal of the American Gastroenterological Association. 2006;4(11):1346-50.
35. Tang J, Sharif O, Pai C, Silverman AL. Mesalamine protects against colorectal cancer in inflammatory bowel disease. Dig Dis Sci. 2010;55(6):1696-703.
36. Triantafillidis JK, Merikas E, Georgopoulos F. Current and emerging drugs for the treatment of inflammatory bowel disease. Drug Design, Development and Therapy. 2011;5:185-210.
37. Clapper ML, Gary MA, Coudry RA, Litwin S, Chang WC, Devarajan K, et al. 5-aminosalicylic acid inhibits colitis-associated colorectal dysplasias in the mouse model of azoxymethane/dextran sulfate sodium-induced colitis. Inflamm Bowel Dis. 2008;14(10):1341-7.
38. Ferlay J, Soerjomataram I, Dikshit R, Eser S, Mathers C, Rebelo M, et al. Cancer incidence and mortality worldwide: sources, methods and major patterns in GLOBOCAN 2012. International journal of cancer Journal international du cancer. 2015;136(5):E359-86.
39. Ferlay J, Shin HR, Bray F, Forman D, Mathers C, Parkin DM. Estimates of worldwide burden of cancer in 2008: GLOBOCAN 2008. International journal of cancer Journal international du cancer. 2010;127(12):2893-917.
40. Huxley RR, Ansary-Moghaddam A, Clifton P, Czernichow S, Parr CL, Woodward M. The impact of dietary and lifestyle risk factors on risk of colorectal cancer: a quantitative overview of the epidemiological evidence. International Journal of Cancer. 2009;125(1):171-80.
41. Slattery ML. Diet, lifestyle, and colon cancer. Seminars in gastrointestinal disease. 2000;11(3):142-6.
42. Ahn DH, Ciombor KK, Mikhail S, Bekaii-Saab T. Genomic diversity of colorectal cancer: Changing landscape and emerging targets. World journal of gastroenterology : WJG. 2016;22(25):5668-77.
43. Fearon ER. Molecular genetics of colorectal cancer. Annual review of pathology. 2011;6:479-507.
44. Rivlin N, Brosh R, Oren M, Rotter V. Mutations in the p53 Tumor Suppressor Gene: Important Milestones at the Various Steps of Tumorigenesis. Genes & cancer. 2011;2(4):466-74.
45. Munkholm P. Review article: the incidence and prevalence of colorectal cancer in inflammatory bowel disease. Alimentary pharmacology & therapeutics. 2003;18 Suppl 2:1-5.
46. Taylor DP, Burt RW, Williams MS, Haug PJ, Cannon-Albright LA. Population-based family history-specific risks for colorectal cancer: a constellation approach. Gastroenterology. 2010;138(3):877-85.
47. Hagen CE, Setia N, Lauwers GY. Familial adenomatous polyposis: a review of gastrointestinal manifestations. Diagnostic Histopathology. 2015;21(4):152-60.

48. Plawski A, Banasiewicz T, Borun P, Kubaszewski L, Krokowicz P, Skrzypczak-Zielinska M, et al. Familial adenomatous polyposis of the colon. Hereditary Cancer in Clinical Practice. 2013;11(1):1-9.
49. Christie M, Jorissen RN, Mouradov D, Sakthianandeswaren A, Li S, Day F, et al. Different APC genotypes in proximal and distal sporadic colorectal cancers suggest distinct WNT/beta-catenin signalling thresholds for tumourigenesis. Oncogene. 2013;32(39):4675-82.
50. Luchtenborg M, Weijenberg MP, Roemen GM, de Bruine AP, van den Brandt PA, Lentjes MH, et al. APC mutations in sporadic colorectal carcinomas from The Netherlands Cohort Study. Carcinogenesis. 2004;25(7):1219-26.
51. Segditsas S, Tomlinson I. Colorectal cancer and genetic alterations in the Wnt pathway. Oncogene. 2006;25(57):7531-7.
52. Aoki K, Taketo MM. Adenomatous polyposis coli (APC): a multi-functional tumor suppressor gene. Journal of cell science. 2007;120(19):3327-35.
53. Fodde R. The APC gene in colorectal cancer. European journal of cancer. 2002;38(7):867-71.
54. Li Vivian SW, Ng Ser S, Boersema Paul J, Low Teck Y, Karthaus Wouter R, Gerlach Jan P, et al. Wnt Signaling through Inhibition of β-Catenin Degradation in an Intact Axin1 Complex. Cell.149(6):1245-56.
55. Leslie A, Carey FA, Pratt NR, Steele RJ. The colorectal adenoma-carcinoma sequence. The British journal of surgery. 2002;89(7):845-60.
56. Berg M, Soreide K. Genetic and epigenetic traits as biomarkers in colorectal cancer. International journal of molecular sciences. 2011;12(12):9426-39.
57. Traverso G, Shuber A, Levin B, Johnson C, Olsson L, Schoetz DJ, Jr., et al. Detection of APC mutations in fecal DNA from patients with colorectal tumors. The New England journal of medicine. 2002;346(5):311-20.
58. Kinzler KW, Vogelstein B. Lessons from hereditary colorectal cancer. Cell. 1996;87(2):159-70.
59. Puppa G, Sonzogni A, Colombari R, Pelosi G. TNM staging system of colorectal carcinoma: a critical appraisal of challenging issues. Archives of pathology & laboratory medicine. 2010;134(6):837-52.
60. Hutter RV. At last--worldwide agreement on the staging of cancer. Archives of surgery (Chicago, Ill : 1960). 1987;122(11):1235-9.
61. Torricelli P. Rectal cancer staging. Surg Oncol. 2007;16 Suppl 1:S49-50.
62. Edge SB, Compton CC. The American Joint Committee on Cancer: the 7th edition of the AJCC cancer staging manual and the future of TNM. Annals of surgical oncology. 2010;17(6):1471-4.
63. Hagan S, Orr MC, Doyle B. Targeted therapies in colorectal cancer-an integrative view by PPPM. The EPMA journal. 2013;4(1):3.
64. Cersosimo RJ. Management of advanced colorectal cancer, Part 1. American journal of health-system pharmacy : AJHP : official journal of the American Society of Health-System Pharmacists. 2013;70(5):395-406.

65. Schmoll HJ, Van Cutsem E, Stein A, Valentini V, Glimelius B, Haustermans K, et al. ESMO Consensus Guidelines for management of patients with colon and rectal cancer. a personalized approach to clinical decision making. Annals of oncology : official journal of the European Society for Medical Oncology / ESMO. 2012;23(10):2479-516.
66. Tan C, Du X. KRAS mutation testing in metastatic colorectal cancer. World journal of gastroenterology : WJG. 2012;18(37):5171-80.
67. Clevers H, Nusse R. Wnt/β-Catenin Signaling and Disease. Cell. 2012;149(6):1192-205.
68. Wodarz A, Nusse R. Mechanisms of Wnt signaling in development. Annual review of cell and developmental biology. 1998;14:59-88.
69. Nusse R, Brown A, Papkoff J, Scambler P, Shackleford G, McMahon A, et al. A new nomenclature for int-1 and related genes: the Wnt gene family. Cell. 1991;64(2):231.
70. Komiya Y, Habas R. Wnt signal transduction pathways. Organogenesis. 2008;4(2):68-75.
71. Cadigan KM, Nusse R. Wnt signaling: a common theme in animal development. Genes & development. 1997;11(24):3286-305.
72. Huelsken J, Behrens J. The Wnt signalling pathway. Journal of cell science. 2002;115(21):3977-8.
73. Logan CY, Nusse R. The Wnt signaling pathway in development and disease. Annual review of cell and developmental biology. 2004;20:781-810.
74. Miller JR. The Wnts. Genome biology. 2002;3(1):Reviews3001.
75. Clevers H, Nusse R. Wnt/beta-catenin signaling and disease. Cell. 2012;149(6):1192-205.
76. Nusse R. Wnt signaling in disease and in development. Cell research. 2005;15(1):28-32.
77. Kestler HA, Kühl M. From individual Wnt pathways towards a Wnt signalling network. Philosophical Transactions of the Royal Society of London B: Biological Sciences. 2008;363(1495):1333-47.
78. MacDonald BT, Tamai K, He X. Wnt/β-Catenin Signaling: Components, Mechanisms, and Diseases. Developmental cell. 2009;17(1):9-26.
79. Novellasdemunt L, Antas P, Li VSW. Targeting Wnt signaling in colorectal cancer. A Review in the Theme: Cell Signaling: Proteins, Pathways and Mechanisms. American Journal of Physiology - Cell Physiology. 2015;309(8):C511-C21.
80. Najdi R, Holcombe RF, Waterman ML. Wnt signaling and colon carcinogenesis: beyond APC. Journal of carcinogenesis. 2011;10:5.
81. McNeill H, Woodgett JR. When pathways collide: collaboration and connivance among signalling proteins in development. Nat Rev Mol Cell Biol. 2010;11(6):404-13.
82. McDonald SL, Silver A. The opposing roles of Wnt-5a in cancer. British journal of cancer. 2009;101(2):209-14.
83. Rao TP, Kuhl M. An updated overview on Wnt signaling pathways: a prelude for more. Circ Res. 2010;106(12):1798-806.

84. Veeman MT, Axelrod JD, Moon RT. A second canon. Functions and mechanisms of beta-catenin-independent Wnt signaling. Developmental cell. 2003;5(3):367-77.
85. Slusarski DC, Corces VG, Moon RT. Interaction of Wnt and a Frizzled homologue triggers G-protein-linked phosphatidylinositol signalling. Nature. 1997;390(6658):410-3.
86. Slusarski DC, Yang-Snyder J, Busa WB, Moon RT. Modulation of Embryonic Intracellular Ca2+Signaling byWnt-5A. Developmental Biology. 1997;182(1):114-20.
87. Sheldahl LC, Park M, Malbon CC, Moon RT. Protein kinase C is differentially stimulated by Wnt and Frizzled homologs in a G-protein-dependent manner. Current biology : CB. 1999;9(13):695-8.
88. Kuhl M, Sheldahl LC, Malbon CC, Moon RT. Ca(2+)/calmodulin-dependent protein kinase II is stimulated by Wnt and Frizzled homologs and promotes ventral cell fates in Xenopus. The Journal of biological chemistry. 2000;275(17):12701-11.
89. Ishitani T, Ninomiya-Tsuji J, Nagai S, Nishita M, Meneghini M, Barker N, et al. The TAK1-NLK-MAPK-related pathway antagonizes signalling between beta-catenin and transcription factor TCF. Nature. 1999;399(6738):798-802.
90. Ishitani T, Kishida S, Hyodo-Miura J, Ueno N, Yasuda J, Waterman M, et al. The TAK1-NLK mitogen-activated protein kinase cascade functions in the Wnt-5a/Ca(2+) pathway to antagonize Wnt/beta-catenin signaling. Molecular and cellular biology. 2003;23(1):131-9.
91. Kohn AD, Moon RT. Wnt and calcium signaling: beta-catenin-independent pathways. Cell calcium. 2005;38(3-4):439-46.
92. Yamaguchi TP, Bradley A, McMahon AP, Jones S. A Wnt5a pathway underlies outgrowth of multiple structures in the vertebrate embryo. Development. 1999;126(6):1211-23.
93. Pukrop T, Binder C. The complex pathways of Wnt 5a in cancer progression. Journal of molecular medicine (Berlin, Germany). 2008;86(3):259-66.
94. Mikels AJ, Nusse R. Purified Wnt5a protein activates or inhibits beta-catenin-TCF signaling depending on receptor context. PLoS biology. 2006;4(4):e115.
95. Topol L, Jiang X, Choi H, Garrett-Beal L, Carolan PJ, Yang Y. Wnt-5a inhibits the canonical Wnt pathway by promoting GSK-3-independent beta-catenin degradation. The Journal of cell biology. 2003;162(5):899-908.
96. Dejmek J, Dejmek A, Safholm A, Sjolander A, Andersson T. Wnt-5a protein expression in primary dukes B colon cancers identifies a subgroup of patients with good prognosis. Cancer research. 2005;65(20):9142-6.
97. Dejmek J, Leandersson K, Manjer J, Bjartell A, Emdin SO, Vogel WF, et al. Expression and signaling activity of Wnt-5a/discoidin domain receptor-1 and Syk plays distinct but decisive roles in breast cancer patient survival. Clinical cancer research : an official journal of the American Association for Cancer Research. 2005;11(2 Pt 1):520-8.
98. Jonsson M, Dejmek J, Bendahl PO, Andersson T. Loss of Wnt-5a protein is associated with early relapse in invasive ductal breast carcinomas. Cancer research. 2002;62(2):409-16.

99. Blanc E, Roux GL, Benard J, Raguenez G. Low expression of Wnt-5a gene is associated with high-risk neuroblastoma. Oncogene. 2005;24(7):1277-83.
100. Liu XH, Pan MH, Lu ZF, Wu B, Rao Q, Zhou ZY, et al. Expression of Wnt-5a and its clinicopathological significance in hepatocellular carcinoma. Digestive and liver disease : official journal of the Italian Society of Gastroenterology and the Italian Association for the Study of the Liver. 2008;40(7):560-7.
101. Liang H, Chen Q, Coles AH, Anderson SJ, Pihan G, Bradley A, et al. Wnt5a inhibits B cell proliferation and functions as a tumor suppressor in hematopoietic tissue. Cancer cell. 2003;4(5):349-60.
102. Smith WL. The eicosanoids and their biochemical mechanisms of action. The Biochemical journal. 1989;259(2):315-24.
103. Funk CD. Prostaglandins and leukotrienes: advances in eicosanoid biology. Science. 2001;294(5548):1871-5.
104. Samuelsson B. Leukotrienes: mediators of immediate hypersensitivity reactions and inflammation. Science. 1983;220(4597):568-75.
105. Samuelsson B. The discovery of the leukotrienes. American journal of respiratory and critical care medicine. 2000;161(2 Pt 2):S2-6.
106. Ferrer R, Moreno JJ. Role of eicosanoids on intestinal epithelial homeostasis. Biochemical pharmacology. 2010;80(4):431-8.
107. Wang D, Dubois RN. Eicosanoids and cancer. Nature Reviews Cancer. 2010;10(3):181-93.
108. Cathcart MC, Lysaght J, Pidgeon GP. Eicosanoid signalling pathways in the development and progression of colorectal cancer: novel approaches for prevention/intervention. Cancer metastasis reviews. 2011;30(3-4):363-85.
109. Savari S, Vinnakota K, Zhang Y, Sjolander A. Cysteinyl leukotrienes and their receptors: Bridging inflammation and colorectal cancer. World journal of gastroenterology : WJG. 2014;20(4):968-77.
110. Smyth EM, Grosser T, Wang M, Yu Y, FitzGerald GA. Prostanoids in health and disease. Journal of lipid research. 2009;50 Suppl:S423-8.
111. Dubois RN, Abramson SB, Crofford L, Gupta RA, Simon LS, Van De Putte LB, et al. Cyclooxygenase in biology and disease. FASEB journal : official publication of the Federation of American Societies for Experimental Biology. 1998;12(12):1063-73.
112. Bos CL, Richel DJ, Ritsema T, Peppelenbosch MP, Versteeg HH. Prostanoids and prostanoid receptors in signal transduction. The international journal of biochemistry & cell biology. 2004;36(7):1187-205.
113. Paul BZ, Jin J, Kunapuli SP. Molecular mechanism of thromboxane A(2)-induced platelet aggregation. Essential role for p2t(ac) and alpha(2a) receptors. The Journal of biological chemistry. 1999;274(41):29108-14.
114. DuBois RN, Radhika A, Reddy BS, Entingh AJ. Increased cyclooxygenase-2 levels in carcinogen-induced rat colonic tumors. Gastroenterology. 1996;110(4):1259-62.

115. Eberhart CE, Coffey RJ, Radhika A, Giardiello FM, Ferrenbach S, DuBois RN. Up-regulation of cyclooxygenase 2 gene expression in human colorectal adenomas and adenocarcinomas. Gastroenterology. 1994;107(4):1183-8.
116. Singh-Ranger G, Mokbel K. The role of cyclooxygenase-2 (COX-2) in breast cancer, and implications of COX-2 inhibition. European Journal of Surgical Oncology (EJSO). 2002;28(7):729-37.
117. Wolff H, Saukkonen K, Anttila S, Karjalainen A, Vainio H, Ristimaki A. Expression of cyclooxygenase-2 in human lung carcinoma. Cancer research. 1998;58(22):4997-5001.
118. Tucker ON, Dannenberg AJ, Yang EK, Zhang F, Teng L, Daly JM, et al. Cyclooxygenase-2 Expression Is Up-Regulated in Human Pancreatic Cancer. Cancer research. 1999;59(5):987-90.
119. Ratnasinghe D, Tangrea J, Roth MJ, Dawsey S, Hu N, Anver M, et al. Expression of cyclooxygenase-2 in human squamous cell carcinoma of the esophagus; an immunohistochemical survey. Anticancer research. 1999;19(1a):171-4.
120. Matsumoto Y, Ishiko O, Deguchi M, Nakagawa E, Ogita S. Cyclooxygenase-2 expression in normal ovaries and epithelial ovarian neoplasms. International journal of molecular medicine. 2001;8(1):31-6.
121. Wang D, DuBois RN. The Role of Prostaglandin E2 in Tumor-Associated Immunosuppression. Trends in Molecular Medicine. 2016;22(1):1-3.
122. Wang D, Fu L, Sun H, Guo L, DuBois RN. Prostaglandin E2 Promotes Colorectal Cancer Stem Cell Expansion and Metastasis in Mice. Gastroenterology. 2015;149(7):1884-95.e4.
123. Smartt HJ, Greenhough A, Ordonez-Moran P, Talero E, Cherry CA, Wallam CA, et al. beta-catenin represses expression of the tumour suppressor 15-prostaglandin dehydrogenase in the normal intestinal epithelium and colorectal tumour cells. Gut. 2012;61(9):1306-14.
124. Myung SJ, Rerko RM, Yan M, Platzer P, Guda K, Dotson A, et al. 15-Hydroxyprostaglandin dehydrogenase is an in vivo suppressor of colon tumorigenesis. Proceedings of the National Academy of Sciences of the United States of America (PNAS). 2006;103(32):12098-102.
125. Tai HH, Tong M, Ding Y. 15-hydroxyprostaglandin dehydrogenase (15-PGDH) and lung cancer. Prostaglandins & other lipid mediators. 2007;83(3):203-8.
126. Tseng-Rogenski S, Gee J, Ignatoski KW, Kunju LP, Bucheit A, Kintner HJ, et al. Loss of 15-hydroxyprostaglandin dehydrogenase expression contributes to bladder cancer progression. The American journal of pathology. 2010;176(3):1462-8.
127. Pham H, Chen M, Li A, King J, Angst E, Dawson DW, et al. Loss of 15-hydroxyprostaglandin dehydrogenase increases prostaglandin E2 in pancreatic tumors. Pancreas. 2010;39(3):332-9.
128. Song HJ, Myung SJ, Kim IW, Jeong JY, Park YS, Lee SM, et al. 15-hydroxyprostaglandin dehydrogenase is downregulated and exhibits tumor suppressor activity in gastric cancer. Cancer investigation. 2011;29(4):257-65.

129. Gustafsson A, Andersson M, Lagerstedt K, Lönnroth C, Nordgren S, Lundholm K. Receptor and enzyme expression for prostanoid metabolism in colorectal cancer related to tumor tissue PGE2. International journal of oncology. 2010;36(2):469-78.
130. Peters-Golden M, Henderson WRJ. Leukotrienes. New England Journal of Medicine. 2007;357(18):1841-54.
131. Sampson AP. The role of eosinophils and neutrophils in inflammation. Clinical and experimental allergy : journal of the British Society for Allergy and Clinical Immunology. 2000;30 Suppl 1:22-7.
132. Earashi M, Noguchi M, Tanaka M. In vitro effects of eicosanoid synthesis inhibitors in the presence of linoleic acid on MDA-MB-231 human breast cancer cells. Breast cancer research and treatment. 1996;37(1):29-37.
133. Bachi AL, Kim FJ, Nonogaki S, Carneiro CR, Lopes JD, Jasiulionis MG, et al. Leukotriene B4 creates a favorable microenvironment for murine melanoma growth. Molecular cancer research : MCR. 2009;7(9):1417-24.
134. Fredriksson R, Lagerstrom MC, Lundin LG, Schioth HB. The G-protein-coupled receptors in the human genome form five main families. Phylogenetic analysis, paralogon groups, and fingerprints. Molecular pharmacology. 2003;63(6):1256-72.
135. Singh RK, Gupta S, Dastidar S, Ray A. Cysteinyl leukotrienes and their receptors: molecular and functional characteristics. Pharmacology. 2010;85(6):336-49.
136. Lynch KR, O'Neill GP, Liu Q, Im DS, Sawyer N, Metters KM, et al. Characterization of the human cysteinyl leukotriene CysLT1 receptor. Nature. 1999;399(6738):789-93.
137. Heise CE, O'Dowd BF, Figueroa DJ, Sawyer N, Nguyen T, Im DS, et al. Characterization of the human cysteinyl leukotriene 2 receptor. The Journal of biological chemistry. 2000;275(39):30531-6.
138. Matsuyama M, Funao K, Hayama T, Tanaka T, Kawahito Y, Sano H, et al. Relationship Between Cysteinyl-Leukotriene-1 Receptor and Human Transitional Cell Carcinoma in Bladder. Urology. 2009;73(4):916-21.
139. Matsuyama M, Hayama T, Funao K, Kawahito Y, Sano H, Takemoto Y, et al. Overexpression of cysteinyl LT1 receptor in prostate cancer and CysLT1R antagonist inhibits prostate cancer cell growth through apoptosis. Oncology reports. 2007;18(1):99-104.
140. Magnusson C, Bengtsson AM, Liu M, Liu J, Ceder Y, Ehrnström R, et al. Regulation of cysteinyl leukotriene receptor 2 expression--a potential anti-tumor mechanism. PloS one. 2011;6(12):e29060.
141. Ohd JF, Nielsen CK, Campbell J, Landberg G, Löfberg H, Sjölander A. Expression of the leukotriene D4 receptor CysLT1, COX-2, and other cell survival factors in colorectal adenocarcinomas. Gastroenterology. 2003;124(1):57-70.
142. Magnusson C, Mezhybovska M, Lorinc E, Fernebro E, Nilbert M, Sjolander A. Low expression of CysLT1R and high expression of CysLT2R mediate good prognosis in colorectal cancer. European journal of cancer. 2010;46(4):826-35.
143. Salim T, Sand-Dejmek J, Sjolander A. The inflammatory mediator leukotriene D4 induces subcellular beta-catenin translocation and migration of colon cancer cells. Experimental cell research. 2014;321(2):255-66.

144. Bellamkonda K, Sime W, Sjolander A. The impact of inflammatory lipid mediators on colon cancer-initiating cells. Molecular carcinogenesis. 2015;54(11):1315-27.
145. Bellamkonda K, Chandrashekar NK, Osman J, Selvanesan BC, Savari S, Sjolander A. The eicosanoids leukotriene D4 and prostaglandin E2 promote the tumorigenicity of colon cancer-initiating cells in a xenograft mouse model. BMC cancer. 2016;16:425.
146. Savari S, Liu M, Zhang Y, Sime W, Sjolander A. CysLT(1)R antagonists inhibit tumor growth in a xenograft model of colon cancer. PloS one. 2013;8(9):e73466.
147. Savari S, Chandrashekar NK, Osman J, Douglas D, Bellamkonda K, Jonsson G, et al. Cysteinyl leukotriene 1 receptor influences intestinal polyp incidence in a gender-specific manner in the ApcMin/+ mouse model. Carcinogenesis. 2016;37(5):491-9.
148. Magnusson C, Ehrnstrom R, Olsen J, Sjolander A. An increased expression of cysteinyl leukotriene 2 receptor in colorectal adenocarcinomas correlates with high differentiation. Cancer research. 2007;67(19):9190-8.
149. Terzic J, Grivennikov S, Karin E, Karin M. Inflammation and colon cancer. Gastroenterology. 2010;138(6):2101-14.e5.
150. Erdman SE, Sohn JJ, Rao VP, Nambiar PR, Ge Z, Fox JG, et al. CD4+CD25+ regulatory lymphocytes induce regression of intestinal tumors in ApcMin/+ mice. Cancer research. 2005;65(10):3998-4004.
151. Izcue A, Coombes JL, Powrie F. Regulatory lymphocytes and intestinal inflammation. Annual review of immunology. 2009;27:313-38.
152. Dunn GP, Old LJ, Schreiber RD. The three Es of cancer immunoediting. Annual review of immunology. 2004;22:329-60.
153. Biswas SK, Mantovani A. Macrophage plasticity and interaction with lymphocyte subsets: cancer as a paradigm. Nat Immunol. 2010;11(10):889-96.
154. Zhang Y, Sime W, Juhas M, Sjolander A. Crosstalk between colon cancer cells and macrophages via inflammatory mediators and CD47 promotes tumour cell migration. European journal of cancer. 2013;49(15):3320-34.
155. Amin K. The role of mast cells in allergic inflammation. Respiratory Medicine. 2012;106(1):9-14.
156. Wernersson S, Pejler G. Mast cell secretory granules: armed for battle. Nat Rev Immunol. 2014;14(7):478-94.
157. Okayama Y, Kawakami T. Development, migration, and survival of mast cells. Immunologic Research. 2006;34(2):97-115.
158. Marech I, Ammendola M, Gadaleta C, Zizzo N, Oakley C, Gadaleta CD, et al. Possible biological and translational significance of mast cells density in colorectal cancer. World journal of gastroenterology : WJG. 2014;20(27):8910-20.
159. Galli SJ, Borregaard N, Wynn TA. Phenotypic and functional plasticity of cells of innate immunity: macrophages, mast cells and neutrophils. Nat Immunol. 2011;12(11):1035-44.
160. Caughey GH. Mast cell tryptases and chymases in inflammation and host defense. Immunological reviews. 2007;217:141-54.

161. Kanaoka Y, Boyce JA. Cysteinyl leukotrienes and their receptors: cellular distribution and function in immune and inflammatory responses. Journal of immunology. 2004;173(3):1503-10.
162. Maltby S, Khazaie K, McNagny KM. Mast cells in tumor growth: angiogenesis, tissue remodelling and immune-modulation. Biochimica et biophysica acta. 2009;1796(1):19-26.
163. Nielsen HJ, Hansen U, Christensen IJ, Reimert CM, Brunner N, Moesgaard F. Independent prognostic value of eosinophil and mast cell infiltration in colorectal cancer tissue. The Journal of pathology. 1999;189(4):487-95.
164. Khazaie K, Blatner NR, Khan MW, Gounari F, Gounaris E, Dennis K, et al. The significant role of mast cells in cancer. Cancer and Metastasis Reviews. 2011;30(1):45-60.
165. Gulubova M, Vlaykova T. Prognostic significance of mast cell number and microvascular density for the survival of patients with primary colorectal cancer. Journal of gastroenterology and hepatology. 2009;24(7):1265-75.
166. Yodavudh S, Tangjitgamol S, Puangsa-art S. Prognostic significance of microvessel density and mast cell density for the survival of Thai patients with primary colorectal cancer. Journal of the Medical Association of Thailand = Chotmaihet thangphaet. 2008;91(5):723-32.
167. Acikalin MF, Oner U, Topcu I, Yasar B, Kiper H, Colak E. Tumour angiogenesis and mast cell density in the prognostic assessment of colorectal carcinomas. Digestive and liver disease : official journal of the Italian Society of Gastroenterology and the Italian Association for the Study of the Liver. 2005;37(3):162-9.
168. Imada A, Shijubo N, Kojima H, Abe S. Mast cells correlate with angiogenesis and poor outcome in stage I lung adenocarcinoma. The European respiratory journal. 2000;15(6):1087-93.
169. Strouch MJ, Cheon EC, Salabat MR, Krantz SB, Gounaris E, Melstrom LG, et al. Crosstalk between mast cells and pancreatic cancer cells contributes to pancreatic tumor progression. Clinical cancer research : an official journal of the American Association for Cancer Research. 2010;16(8):2257-65.
170. Ogino S, Shima K, Baba Y, Nosho K, Irahara N, Kure S, et al. Colorectal cancer expression of peroxisome proliferator-activated receptor gamma (PPARG, PPARgamma) is associated with good prognosis. Gastroenterology. 2009;136(4):1242-50.
171. Carlini MJ, Dalurzo MC, Lastiri JM, Smith DE, Vasallo BC, Puricelli LI, et al. Mast cell phenotypes and microvessels in non-small cell lung cancer and its prognostic significance. Human pathology. 2010;41(5):697-705.
172. Welsh TJ, Green RH, Richardson D, Waller DA, O'Byrne KJ, Bradding P. Macrophage and mast-cell invasion of tumor cell islets confers a marked survival advantage in non-small-cell lung cancer. Journal of clinical oncology : official journal of the American Society of Clinical Oncology. 2005;23(35):8959-67.

173. Fleischmann A, Schlomm T, Kollermann J, Sekulic N, Huland H, Mirlacher M, et al. Immunological microenvironment in prostate cancer: high mast cell densities are associated with favorable tumor characteristics and good prognosis. The Prostate. 2009;69(9):976-81.
174. Mehdawi L, Osman J, Topi G, Sjolander A. High tumor mast cell density is associated with longer survival of colon cancer patients. Acta oncologica (Stockholm, Sweden). 2016:1-9.
175. Lonnroth C, Andersson M, Arvidsson A, Nordgren S, Brevinge H, Lagerstedt K, et al. Preoperative treatment with a non-steroidal anti-inflammatory drug (NSAID) increases tumor tissue infiltration of seemingly activated immune cells in colorectal cancer. Cancer Immun. 2008;8:5.
176. Maekawa A, Austen KF, Kanaoka Y. Targeted gene disruption reveals the role of cysteinyl leukotriene 1 receptor in the enhanced vascular permeability of mice undergoing acute inflammatory responses. J Biol Chem. 2002;277(23):20820-4.
177. Prasad CP, Chaurasiya SK, Axelsson L, Andersson T. WNT-5A triggers Cdc42 activation leading to an ERK1/2 dependent decrease in MMP9 activity and invasive migration of breast cancer cells. Molecular oncology. 2013;7(5):870-83.
178. Atreya I, Neurath MF. Immune cells in colorectal cancer: prognostic relevance and therapeutic strategies. Expert review of anticancer therapy. 2008;8(4):561-72.
179. Conti P, Castellani ML, Kempuraj D, Salini V, Vecchiet J, Tete S, et al. Role of mast cells in tumor growth. Annals of clinical and laboratory science. 2007;37(4):315-22.
180. Ranieri G, Ammendola M, Patruno R, Celano G, Zito FA, Montemurro S, et al. Tryptase-positive mast cells correlate with angiogenesis in early breast cancer patients. Int J Oncol. 2009;35(1):115-20.
181. Ibaraki T, Muramatsu M, Takai S, Jin D, Maruyama H, Orino T, et al. The relationship of tryptase- and chymase-positive mast cells to angiogenesis in stage I non-small cell lung cancer. European journal of cardio-thoracic surgery : official journal of the European Association for Cardio-thoracic Surgery. 2005;28(4):617-21.
182. Elezoglu B, Tolunay S. The relationship between the stromal mast cell number, microvessel density, c-erbB-2 staining and survival and prognostic factors in colorectal carcinoma. Turk patoloji dergisi. 2012;28(2):110-8.
183. Rigas B, Goldman IS, Levine L. Altered eicosanoid levels in human colon cancer. The Journal of laboratory and clinical medicine. 1993;122(5):518-23.
184. Chell S, Kaidi A, Williams AC, Paraskeva C. Mediators of PGE2 synthesis and signalling downstream of COX-2 represent potential targets for the prevention/treatment of colorectal cancer. Biochimica et biophysica acta. 2006;1766(1):104-19.
185. Myung SJ, Rerko RM, Yan M, Platzer P, Guda K, Dotson A, et al. 15-Hydroxyprostaglandin dehydrogenase is an in vivo suppressor of colon tumorigenesis. Proceedings of the National Academy of Sciences of the United States of America. 2006;103(32):12098-102.
186. Barker N, Clevers H. Mining the Wnt pathway for cancer therapeutics. Nature reviews Drug discovery. 2006;5(12):997-1014.

187. Mehdawi LM, Prasad CP, Ehrnstrom R, Andersson T, Sjolander A. Non-canonical WNT5A signaling up-regulates the expression of the tumor suppressor 15-PGDH and induces differentiation of colon cancer cells. Molecular oncology. 2016.
188. Nandy A, Jenatschke S, Hartung B, Milde-Langosch K, Bamberger AM, Gellersen B. Genomic structure and transcriptional regulation of the human NAD+-dependent 15-hydroxyprostaglandin dehydrogenase gene. Journal of molecular endocrinology. 2003;31(1):105-21.
189. Choi SH, Kim BG, Robinson J, Fink S, Yan M, Sporn MB, et al. Synthetic triterpenoid induces 15-PGDH expression and suppresses inflammation-driven colon carcinogenesis. The Journal of clinical investigation. 2014;124(6):2472-82.

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