

New intracellular and molecular aspects in pathophysiology of colorectal cancer

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ABSTRACT

Colorectal cancer is one of the most common malignancy in the world and the second cancer-related death, many molecular and genetic aspects of this disease have been cleared as chromosomal instability and the role of some key proteins as WNT/ β catenin, trypsin and others. Also recently the role of folate turnover and some neurotransmitters as serotonin were also considered. The scope of this review is to describe some details about new molecular pathways suggested for occurrence or progress of this disease.

Keywords: *Colorectal cancer, Molecular pathology, WNT protein, Trypsin, Serotonin.*

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Introduction

Colorectal cancer disease (CRC) is the fourth most common malignancy and the second cause of cancer-related deaths. For example only in 2008, 148,810 cases of colorectal cancer have been diagnosed and 49,960 were died from this malignancy (1). Considerable progress in curing this disease has been achieved during 12 the past years, and some new therapeutic agents have been introduced by which the survival rate of patients has been improved (1). Pathologic stage represents the most important prognostic factor for patients with colorectal cancer. According to tumor-node-

metastasis (TNM) system, as defined by the American Joint Committee on Cancer, considering regional lymph node involvement, and presence of distant sites of disease (2, 3), the stage of disease is divided from T1 (invasion of the submucosa) to T4 (invasion into the serosa or adjacent structures) for tumors and N₀ to N₃ for nodes involvement and M₀ to M₁ for metastasis (table 1). In patients with resectable colorectal cancer, many other pathologic and clinical aspects have been clarified that are associated with an increased risk for tumor recurrence. These include poorly differentiated histology, lymphovascular invasion, perineural invasion, T4 tumor penetration, bowel perforation, clinical bowel obstruction and an increased preoperative plasma level of carcinoembryonic

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antigen (4,5). During recent years, some advances have been happening in the treatment of CRC (colorectal cancer) and control of its' metastatic stages. The first line for the treatment of this malignancy is surgery. Patients with obstruction, perforation and/ or expression of some biomarkers such as heterozygosity in chromosome q 18 or mutation in TGF- β gene need chemotherapy after surgery. The metastatic patients have some limitations in treatment and their treatments with current methods are difficult and they are recommended for surgery and are given some palliative chemotherapy (6).

Table 1. Classification of stages of CRC according to tumor-node -metastasis (TNM) system

T ₀	Not present any lesion
T _{is}	Invasion limited to mucosa
T ₁	Invasion to submucosa
T ₂	Invasion to muscularis propria
T ₃	Invasion into subserosa or fats around colon
T ₄	Invasion into serosa and adjacent structure
N ₀	Not any invasion into the nodes
N ₁	1 to 3 nodes around the colon are positives
N ₂	4 nodes or more around the colon are positives
N ₃	Any node as long as a blood vessel is positive
M ₀	Not present any metastases
M ₁	Presence of any distal metastases

Microsatellite instability and loss of heterozygosity

Microsatellite instability and loss of heterozygosity at chromosome q18 are the 2 best-defined molecular prognostic markers (7) (table 2). Microsatellite instability results from mutations or promoter hypermethylation of DNA mismatch repair genes leading to errors in DNA replication and changes in short and repeated sequences of DNA. It is present in the most majority of tumors from patients with hereditary nonpolyposis colon cancer, but also is found in 15 to 20% of patients with sporadic colon cancer (7, 8). Patients with tumors possessing a high degree of microsatellite

instability have a more favorable prognosis than those patients whose tumors are microsatellite stable (8, 9). Loss of heterozygosity at chromosome q18 has been reported in approximately 50% of colon cancers and has been associated with a worse prognosis (9,10).

Table 2. Prognostic markers for CRC

Microsatellite instability	>Mutations or promoter hypermethylation of DNA mismatch repair genes	>Errors in DNA replication and changes in short, repeated sequences of DNA
loss of heterozygosity	>Loss of heterozygosity at chromosome q18	> has been reported in approximately 50% of colon cancers>worse prognosis

Metastases

The progression of tumor beyond the colorectal and regional lymph nodes defines the M stage of the American Joint Committee on Cancer classification system, with M1 indicating the presence of tumor metastases to distant sites. Approximately 20% of patients with metastatic disease and 30% to 40% of patients with localized disease ultimately develop metastases. The liver reflects the most common initial site of disease spread, but metastases to other organs during the course of the disease are common, including lungs, peritoneum and intra-abdominal lymph nodes. Patients with a small number of isolated, organ-confined metastases may be cured of their disease by surgical resection; many patients with metastatic disease are candidates for systemic chemotherapy to palliate symptoms and prolong life (11). As the stage of disease increases from stage I to stage IV, the year overall survival rates declines dramatically: stage I, greater than 90%; stage II, 70%–85%; stage III, 25%–80%; and stage IV, less than 10% (12,13).

Molecular pathology from stem cells to cancer

Stem cells of intestine are localized towards the bottom of the crypt in the large intestine, and superior to the Paneth cells in the small intestine (14). Their differentiating progeny migrate upwards through the transit amplifying zone in the lower-to-middle region of the crypt, before becoming terminally differentiated, and are eventually led into the lumen (15) (figure 1). The Paneth cell is one of five cell types into which the small intestinal epithelial stem cell can differentiate; the other cell types are the absorptive enterocyte, hormone secreting endocrine cells, mucus producing goblet cells and 'M' cells. M cells are involved in the transport of antigens from the gut lumen into Peyer's patches (16).

Regulation of intestinal stem cells functions by WNT pathways

WNT/ β -catenin pathway is important for regulating gut epithelial stem cell function. WNT genes encode a large family of secreted molecules that have important roles in the regulation of proliferation and apoptosis of epithelial cells. In humans, there are at least 19 members of the WNT family and at least 10 members of its receptor family, FZ (frizzled) (17). The Wnt receptor complex is combined with FZ plus LRP5 and LRP6 (LDL receptor related proteins), which are homologs of the LDL receptor (17) binding of WNT to FZ activates one of two pathways (table 3): the canonical pathway, which involves β -catenin (Figure 2) and controls cellular proliferation; and the planar pathway, which

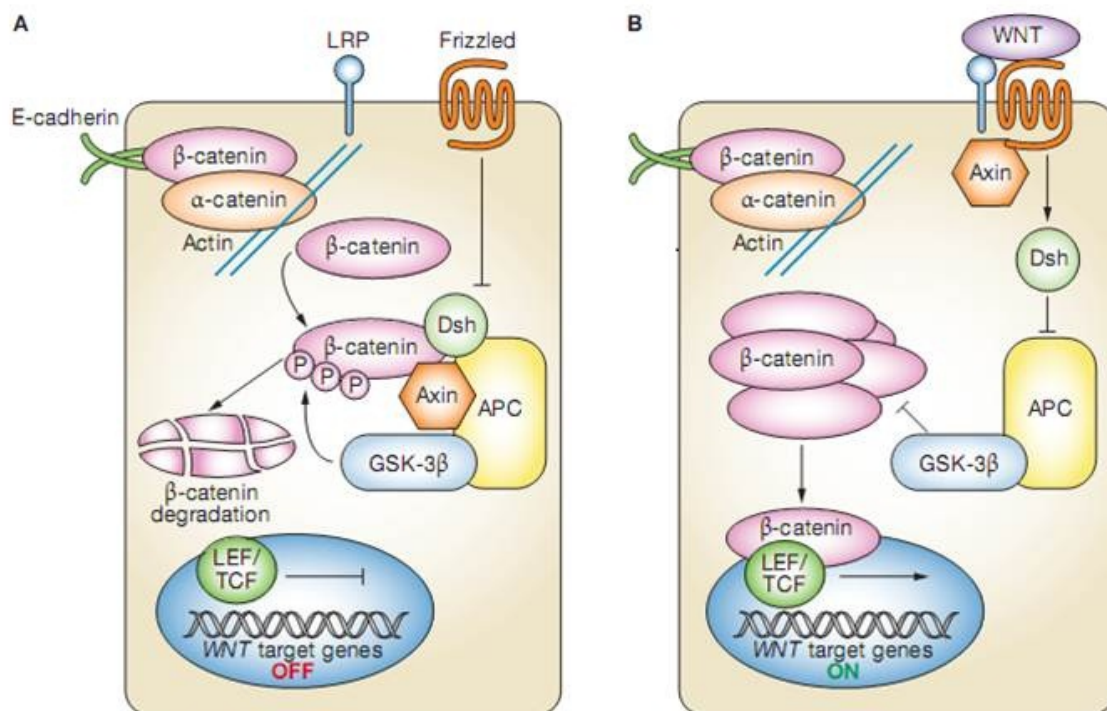


Figure 2. The Wnt signaling pathway. **(A)** In the absence of WNT binding to frizzled, dishevelled remains unactivated and β -catenin is targeted for degradation via the adenomatous polyposis coli/axin/glycogen-synthase-kinase 3 β pathway. **(B)** When Wnt binds to frizzled (right), dishevelled is activated and uncouples β -catenin from the adenomatous polyposis coli; β -catenin can then associate with transcription factors and mediate transcription of the target gene. PC, adenomatous polyposis coli; Dsh, dishevelled; GSK-3 β , glycogen synthase kinase 3 β ; LEF, lymphoid enhancer-binding factor, LRP, LDL-receptor-related protein; P, phosphorylation; TCF, T-cell factor.

involves Ca^{2+} and is important in cellular movement and polarity (18).

Table 3. WNT signaling pathway in pathophysiology of colon cancer

Canonical	Involves β -Catenin	Controls cell proliferation
Planar	Involves Ca^{++}	Control cellular movement and polarity

Briefly, in the canonical pathway, in the absence of WNT, cytosolic β -catenin, which is normally bound to membranous E-cadherin, interacts with the 'destruction complex', which is composed of the tumor-suppressor protein APC (adenomatous polyposis coli), glycogen synthase kinase 3β and axin (19). This results in serine phosphorylation of β -catenin, its recognition by an E3 ubiquitin ligase, and its degradation (19). But if WNT is present, it couples with FZ receptor then the kinase activity of the destruction complex is blocked, and β -catenin remains unphosphorylated, resulting in accumulation of β -catenin in the nucleus (19). This enables β -catenin to bind to the transcription factor TCF4 that can activate downstream target genes such as the protooncogene MYC that promotes entry of the cell into the S-phase of the cell cycle (20). Data indicate that the TCF4/ β -catenin pathway also has a role in the maintenance of intestinal progenitor cells in adult mammalian crypts. According to Van de Wetering and colleagues' reports of a DNA microarray study in human colon adenocarcinoma cell lines that had inducible dominant-negative TCF4 mutations: by inhibiting TCF4- β -catenin complex formation, growth was halted in the G1 phase of the cell cycle. It is possible that the TCF4- β -catenin complex acts as a master switch that controls the balance of proliferation and differentiation in healthy and malignant intestinal epithelial cells (19).

Cancer and stem cells

Cancer is the disease of stem cells, because only stem cells have the ability to self-renew and

neoplasia is essentially dysregulated of self renewal (21). In addition, stem cells are one of the few cell types that are sufficiently long-lived to acquire the necessary number of sequential mutations to convert a cell from the normal to malignant state (21). Tumors themselves probably contain stem cells—so-called cancer stem cells (CSCs) (22).

In a heterogeneous population of cells, it is only CSCs that have the ability to self-renew and therefore, sustain and increase the tumor cell population. It was not until more recently that CSCs were described in the NOD/SCID (nonobese diabetic/severe combined immunodeficient) mouse model by Dick and colleagues (23). As yet, CSCs have not been isolated from colorectal cancers but have been successfully isolated from some breast and brain tumors (24-26).

Trypsin in colorectal cancer

Recently, it has been shown that Trypsin (a digestive enzyme) has considerable role in developing of neoplasia, and invasion and metastasis in several cancers (27, 28). Trypsin has the potential as a prognosticator (29, 30) and patients with trypsin-positive colorectal cancers (CRCs) have shorter overall and disease-free survival than patients with trypsin-negative CRC (28). However, the biological mechanisms by which trypsin contributes to the poor outcome in cancer in general and in CRC in particular, are not well cleared.

Trypsin is one of characterized serine proteinases. Proteinases play essential roles not only in many physiological processes (eg food digestion, blood coagulation, fibrinolysis, and control of blood pressure) but also in a wide range of important pathological processes (eg atherosclerosis, inflammation and cancer) (31, 32). Trypsin exhibits selective proteolytic activity against the peptide bonds in protein molecules that have carboxyl groups donated by

the amino acids arginine and lysine. For physiological protection against premature activity, as known from pancreatic physiology, trypsin is secreted as an inactive zymogen (trypsinogen) in the pancreatic juice, and is activated by conversion to trypsin by an enteropeptidase in the alkaline milieu of the duodenal lumen. Secondly, trypsinogen may be activated into active trypsin by an enteropeptidase found in duodenal enterocytes (33, 34). Interestingly, adenocarcinoma cells of the duodenum (32), as well as other tissues expressing trypsin, have a trypsin-activating enteropeptidase (29,30). Lastly, the antiprotease mediator pancreatic secretory trypsin inhibitor (PSTI) protects from premature activity. An imbalance in this 'proteinase-antiproteinase-system' plays a pathophysiological role in the development of pancreatitis and seems to present an increased risk for developing pancreatic adenocarcinoma (29-31). PSTI is excreted by the mucosa of the normal gastrointestinal tract, where it serves to protect the cells from proteolytic breakdown. The same peptide is secreted by tumour cells, and is often referred to as 'tumour associated trypsin inhibitor' (TATI), which is identical to PSTI. Trypsin expression is increased in human cancer cells of the ovary, prostate, lung, stomach, colon, and others. Trypsin in cancer is often referred to as 'tumour-associated trypsin', and generally represents trypsin-1 and trypsin-2 (35). The contributing role of the tumor environment and its constituents (ie stroma cells, signal molecules, matrix enzymes) in the invasive and metastatic process is emerging (Figure 3, 4) (36). Recently evidence declare that the adverse effects of trypsin are mediated through interplay with other proteinases systems (figure 4), such as the MMPs (37) and the recently explored PAR-2 (37-40). The current a mechanism by which trypsin induces invasion and metastasis are manifold. Firstly, as a

proteolytic enzyme, trypsin may directly degrade extracellular proteins by itself, by attacking type 1 collagen of the basal membrane (39). Secondly, its effect is likely to be mediated indirectly through the activation and effect of other latent proteolytic cascades, the most important being the MMPs. Lastly, recent evidence points to the activation of signal molecules, such as PAR-2.

Overexpression of trypsin associated with poor prognosis in CRC patients has been reported with several evidences. Yamamoto et al. found that trypsin Immunoreactivity was more intense at the invasive front than in the superficial part of the tumor (28). Trypsin positivity (defined as > 30% positive cells at the invasive front of CRCs) correlated significantly with the depth of invasion, lymphatic and venous invasion, lymph node and distant metastasis, advanced pathological tumor-node-metastasis stage, as well as recurrence. Trypsin activates matrilysin (MMP-7), which plays an important role in CRC progression, and may explain the adverse effect of trypsin on CRC prognosis.

Trypsin as a target for the therapy of cancer cells show as an analogy to the natural protective mechanisms of trypsin by expressing TATI (Tumor associated trypsin inhibitor) at increased levels in CRCs compared with adjacent normal mucosa (41). This suggests that an innate protective mechanism occurs even in tumors (42). Although TATI is highly co-expressed in CRC, experimental inhibition of trypsin with TATI is only partially successful (43), and TATI is not a useful tumor marker compared with carcinoembryonic antigen. Inhibition of trypsin-mediated invasion is feasible with derivatives of tetracycline (43). However, trypsin interacts with other cascades, making single targeted inhibition difficult and probably ineffective (table 4).

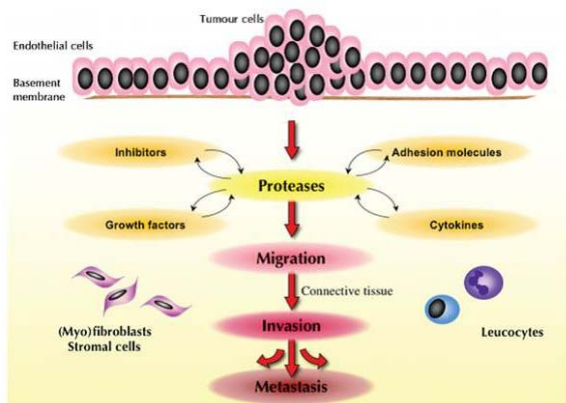


Figure 3. General model illustrating the stepwise influence of proteinase systems and related co-factors from tumour cells, extracellular matrix and immune system on cancer invasion and metastasis. Proteinases, such as trypsin, may play a major role in this process by direct and indirect activation of other proteinase cascades and related factors.

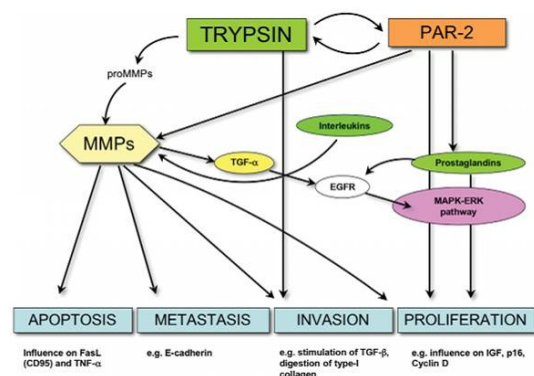


Figure 4. Simplified model showing current knowledge of trypsin interaction with proteinase-activated receptor 2 (PAR-2) and the matrix metalloproteinase (MMPs). The arrows indicate activation.

Polymorphisms in the one carbonated metabolic pathway

In recent years it has been shown that one-carbon (e.g., folate) metabolism has a crucial role in the etiology of colorectal cancer (CRC). Cytosolic serine hydroxymethyltransferase (cSHMT), methylenetetrahydrofolate dehydrogenase (MTHFD1)

and glutamate carboxypeptidase (HGCPII) are key genes involved in this pathway (44). Low intake or low levels of circulating folate, a key component of one-carbon metabolism, has been consistently associated with increased risk of colorectal cancer (CRC) (45, 46). Functional polymorphisms in folate-metabolizing genes are capable of modifying the risk of CRC as shown by (Jia Chen et al) (46) studies on 2 such genes, the methylenetetrahydrofolate reductase (MTHFR) and the thymidylate synthase (TS). Specifically, it was reported that the 677TT genotype of the MTHFR 677C3T polymorphism (47, 48) and the 2R/2R genotype of the tandem repeated polymorphism in the TS promoter (48) were associated with reduced risk of CRC. The TS 2R/2R genotype, associated with low TS expression, was also associated with better survival after CRC diagnosis (48). Additionally, both polymorphisms modified plasma folate and total homocysteine (tHcy) levels. Serine hydroxymethyltransferase (SHMT), methylenetetrahydrofolate dehydrogenase (MTHFD1) and glutamate carboxypeptidase II (GCPII) are 3 of the constellation of genes involved in folate-dependent one-carbon metabolism. Several polymorphisms in these genes have recently been clarified and their functionality has also been implicated.

Role of serotonin and serotonin receptors in CRC

Serotonin (5-hydroxytryptamine, 5-HT) is a neurotransmitter that mediates a wide variety of physiological effects including peripheral and central action through the binding of multiple receptor subtypes. Mitogenic effects of serotonin receptors in prostate, bladder and breast cancer, especially 5-HT_{1A-D} and 5HT_{2A} receptors had been shown before. Recently we have shown that also 5HT can have mitogenic role in CRC and role of 5HT_{1B} receptors is with much more importance (49-51). It was suggested that mitogenic role of

Table 4. Role of trypsin in colorectal cancer

Trypsin, a digestive enzyme has considerable role in developing of neoplasia, invasion and metastasis	> Has potential as a prognosticator	> trypsin-positive(CRCs) : shorter overall and disease-free survival
PSTI excreted by the mucosa of the normal gastrointestinal tract, serves to protect the cells from proteolytic breakdown	> The same peptide secreted by tumour cells, and is often referred to as 'tumour associated trypsin inhibitor' (TATI), which is identical to PSTI	
Trypsin expression in human cancer cells of the ovary, prostate, lung, stomach, colon, and others	> tumor-associated trypsin', generally represents trypsin-1 and trypsin-2	
Recently evidences: adverse effects of trypsin are mediated through MMPs and the recently explored PAR-2	> as a proteolytic enzyme, directly degrade extracellular proteins by itself, by attacking type 1 collagen of the basal membrane.	> indirectly through the activation other latent proteolytic cascades, the most important, the MMPs. Lastly, activation of signal molecules, such as PAR-2
Trypsin activates Matrilysin(MMP-7)	> plays an important role in CRC progression, may explain the adverse effect of trypsin on CRC prognosis	
Trypsin as a target for therapy of cancer cells	> Natural protective mechanisms of trypsin by expressing TATI (Tumor associated trypsin inhibitor) > increased levels in CRCs compared with adjacent normal mucosa (Innate protective mechanism occurs even in tumors).	> Although TATI is highly co-expressed in CRC, experimental inhibition of trypsin with TATI is only partially successful.

serotonin receptors in cancer is via the activation of (Phospholipase C- β /protein Kinase C) and also the activation of MAPKinase and extracellular Kinase controlled with signal, which can induce some intracellular phosphorylation events leading to the activation of some transcription factors as TCF4 (49,50).

Conclusion

According to a recent review about pathophysiology of colorectal cancer we can consider this malignancy as a multifactorial disease in which many biochemical and genetic

factors are involved and the role of stem cells and their regulatory proteins as WNT/ β catenin are with importance and some chromosomal and genetic mutations as microsatellite instability and loss of heterozygosity at chromosome 18q are associated with poor prognosis. Also we can consider a correlation between trypsin overexpression and matrilysin protein activation with CRC progression. Recently the role of folate turnover and serotonin receptors and their signaling pathways are also considered. Our knowledge about accurate pathophysiology of this disease is incomplete and needs more

investigations and more molecular studies especially on intracellular and extracellular pathways and intermediate proteins. This disease is correlated closely with our diets and with our living habits; as with much more exercises and better diet regimes (low fat and free radicals foods and high bran, folate and antioxidants diets) we can prevent much better this malignancy even in old ages.

Future Perspectives

As role of folate and antioxidants have been shown effective in the prevention of colorectal cancer, it can be suggested to use these agent especially curcumin and melatonin as potent antioxidants in diets regimes for high risk people. Also research developments on trypsin inhibitors and monoclonal antibodies against carcinoembryonic antigen and anti serotonergic drugs can be novel approaches in chemotherapy of CRC. Further researches on stem cells for exploring other genetical and molecular signaling pathways are important for better understanding of molecular pathophysiology of this malignancy.

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