Cell. Mol. Life Sci. 63 (2006) 663–671
1420-682X/06/060663-9
DOI 10.1007/s00018-005-5425-4


Cellular and Molecular Life Sciences

# Review

# Colorectal carcinoma: from tumorigenesis to treatment

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Received 15 September 2005; received after revision 3 November 2005; accepted 25 November 2005
Online First 23 February 2006

**Abstract.** Colorectal carcinoma (CRC) is a complicated and often fatal genetic disease. Fortunately, owing to rapid expansion of knowledge and technology development in oncology, much progress has been made regarding the diagnosis, understanding of the molecular genetics and malignant progression, as well as the novel regimens of CRC. In this review, we summarize the staging system, the most critical genetic and epigenetic alterations, the pleiotropic effects of MMP-7, the controversial roles of Hedgehog signaling, the intriguing involvement of thymosin $\beta$-4, and the possible contribution of the putative colon (cancer) stem cells in CRC tumorigenesis. Current treatments as well as several potentially applicable therapeutic strategies for CRC are also discussed.

**Key words.** Colorectal carcinoma; tumorigenesis; staging; matrix metalloproteinase; hedgehog; thymosin $\beta$-4; stem cells; treatment.

## Introduction

Colorectal carcinoma (CRC) is one of the leading causes of cancer death in much of the developed world despite the fact that colonic malignancies can be effectively managed if detected early and that chemoprevention has shown some success in reducing the disease [1]. Crucial for treatment guidance and prognosis prediction, the CRC staging system is continuously being modified to become a better tool [2]. Genetically, CRC tumorigenesis appears to be the result of a progressive transformation of colorectal epithelial cells due primarily to the accumulation of mutations in a number of oncogenes as well as tumor suppressor genes [3]. Matrix metalloproteinase (MMPs) play an important role in the growth and invasion of CRC, and the levels of certain MMPs can be used to estimate the metastatic capacity and recurrence of this disease as well as prognosis of patients [4]. However, for effective CRC therapy using MMP inhibitors, timely and highly selective administration may be required, because MMPs also play a negative role in the ultimate malignancy [5, 6]. Aberrant expression of the genes encoding Hedgehog (Hh) proteins [7, 8] and thymosin $\beta$-4 (T$\beta$4) [9] have recently been found to be associated with CRC progression. The former might exert their effect by altering the growth and differentiation of colonic enterocytes [7, 8], whereas the latter may increase the motility and invasion of tumor cells [9, 10]. Extensive investigations are currently being conducted to elucidate the precise mechanisms of these proteins. The perpetual stem cells residing in the crypt base of the colon have been hypothesized to be the ones responsible for CRC development because the balance of asymmetrical cell division and cell proliferation in them could be disrupted by various genetic and/or epigenetic alterations [11]. If colon cancer stem cells do exist and the differences between these and other 'regular' cancer cells can be identified, more effective chemotherapy and/or targeted therapy for CRC may someday be realized.

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## Staging

The early Dukes pathologic staging system introduced more than 50 years ago classified colorectal malignancies into three groups: Lesions contiguous with the bowel wall but not penetrating the muscularis was designated Dukes' A. Those penetrating the muscularis into the surrounding fat or adventitia were designated Dukes' B, and any with positive lymph node involvement were designated Dukes' C [12]. A number of modifications in this system have subsequently been introduced, including the addition of a so-called Dukes' stage D for patients with metastatic disease. Recently, a more specific TNM (Tumor-Nodal-Metastasis) staging system developed by the American Joint Commission on Cancer (AJCC) was widely used and recommended by the WHO. Even more recently, the revised AJCC sixth edition cancer staging system further stratified colon cancer stages II (T3 or T4 N0 M0) and III (Any T N1 M0) defined by the AJCC fifth edition system to stages IIa (T3 N0 M0), IIb (T4 N0 M0), IIIa (T1 or T2 N1 M0) and IIIb (T3 or T4 N1 M0). This system for colon cancer stratifies survival more distinctly than the fifth edition system by providing more substages. Interestingly, the association of stage IIIa colon cancer with a statistically significantly better survival than stage IIb in the new system may reflect current clinical practice, in which stage III patients receive adjuvant chemotherapy, but stage II patients generally do not [13].

## Oncogenes and tumor suppressor genes

*k-ras* is the best-studied and most common oncogene involved in colorectal carcinogenesis [14, 15]. Even though only 9% of the small adenomas exhibit *k-ras* mutations, they are detected in 58% of adenomas larger than 1 cm and 47–50% of colorectal cancers [16]. Mutated k-ras appears to be capable of stimulating Wnt signaling in colon cancer through suppression of glycogen synthetase kinase-3$\beta$ (GSK-3$\beta$) [17]. By contrast, ras inhibition leads to the transcriptional activation of *p53* and downregulation of *Mdm2*, thus increasing the function of this checkpoint protein in colon cancer cells [18], which might activate p53-dependent apoptosis and antagonize tumor growth [19]. Recently, hypermethylation of the promoter of a novel ras-effector gene, *RASSF2A,* has been identified as an early event in colon carcinogenesis which correlates inversely with *k-ras* mutations [20]. Owing to the high frequency of *k-ras* mutation in CRC, a polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) analysis for this gene has been developed as a powerful tool for detecting isolated tumor cells in patients' liver, lymph node and bone marrow specimens [21]. The *TGF-$\beta$RII* (TGF-$\beta$ receptor type II) is the other gene whose inactivating mutations are also frequently found in CRC with microsatellite instability [22]. On the other hand, mutational activation of this gene has been shown to occur early and in a subset of ulcerative colitis-associated neoplasms and commonly in sporadic CRC [23]. c-Src is a non-receptor protein tyrosine kinase whose activation is reported as an early event in the development of preneoplastic colonic adenomas and also detected in >70% of colon carcinomas [24]. By activating Akt-mediated survival pathways that decrease the sensitivity of detached cells to anoikis, overexpression of *c-src* plays an important role in invasive behavior as well as metastasis of experimental colon epithelial cells [25]. Concomitantly, attenuation of c-Src signaling has been reported to sensitize human metastatic colon cancer cells to apoptosis induced by anticancer drugs and also to the activation of the Fas death receptor [26]. The 'gate-keeping' event for initiation of colorectal neoplasia is the inactivation of both copies of the adenomatous polyposis coli (APC) gene [27], which may lead to the disruption of normal adherens junction by interfering with the association between catenins and cell adhesion molecule E-cadherin, thus disturbing normal tissue architecture [28, 29]. Defects in directed cell migration may also be caused by APC loss [30] due to aberrant cytoskeletal regulation that affects both microtubules [31, 32] and F-actin [33, 34]. Moreover, dysfunction of this tumor suppressor protein may result in an increased incidence of mitotic errors [35]. More important, by functioning as a scaffold to promote complex formation between GSK-3$\beta$, $\beta$-catenin, axin, and various kinases and phosphatases, APC plays a crucial role in Wnt signaling [36]. Therefore, it is not surprising that mutations or loss of *APC* were found in a majority of FAP (familial adenomatous polyposis) patients [37] as well as in > 80% of sporadic CRCs and adenomas [38]. Approximately 15% of sporadic CRCs are caused by somatic inactivation of 'mismatch repair' (MMR) genes, which leads to a 'microsatellite instability' (MIN) phenotype [39]. Interestingly, a much higher incidence (~85%) of MIN has been found in CRC patients with hereditary nonpolyposis colorectal cancer (HNPCC) tumors [40, 41]. The predominant mechanism responsible for the inactivation of *MMR* in these tumors is epigenetic silencing through promoter methylation [42] with the additional involvement of somatic mutations [39]. Intriguingly, MIN-positive CRC patients seemed to have a significantly better prognosis compared with those with an intact MMR system [43], suggesting that this system has multiple roles in CRC progression. In addition to genetic changes, epigenetic silencing resulting from promoter methylation and/or alterations in histone modification (acetylation, methylation, phosphorylation, ubiquitination and SUMOylation) is now recognized as a 'third pathway' in Knudson's model of tumor-suppressor gene inactivation in cancer [44]. In this regard, the biologic relevance of a CpG island methylator phenotype (CIMP) (i.e. hyper methylation of several genes simultaneously) in

colon cancer has recently been established even though its expression was profoundly affected by the presence or absence of MIN [45]. In addition, DNA methylation was postulated to be closely associated with histone modification [46] because protein complexes containing histone deacetylases and histone methylases have recently been shown to be recruited by several methyl DNA-binding proteins localized to the methylated promoters [47–49]. Accordingly, 'epigenetic therapy' using demethylating agents and/or inhibitors of histone deacetylases shows promise as an approach to cancer prevention and therapy [50].

## MMPs

Death in the majority of CRC patients is caused by tumor metastasis [51], a complicated multistage process requiring degradation of extracellular matrix (ECM) components by proteolytic enzymes [52]. The principal enzymes responsible for ECM turnover are matrix metalloproteinases (MMPs), a large group of secreted proteinases that require divalent cations for their catalytic activities [53]. Regarding the involvement of MMPs in CRC tumorigenesis, elevated levels of MMP-1, -2, -3, -7, -9, and -13 were found in tumors compared with healthy mucosa [4]. Moreover, upregulated expression of MMP-1 and -7 has also been found in the invasive fronts of CRC [54, 55]. In addition to degrading ECM, MMPs could promote cancer progression by mechanisms such as liberating growth factors and/or cytokines, suppressing the immune response and modulating angiogenesis [53]. For example, MMP-7, one of the most important MMPs in colorectal tumorigenesis, has been shown to induce angiogenesis directly by accelerating the proliferation of endothelial cells [56]. Additionally, MMP-7 may enhance the metastatic potential of CRC by processing a cell surface protein(s) and thereby inducing loose and then tight aggregation of tumor cells [57]. Proteolysis of the insulin-like growth factor binding protein 3 (IGFBP-3) by this protease has been shown to play a crucial role in promoting the survival of colon cancer cells via regulating IGF-I bioavailability [58]. MMP-7 may play a role in EGF receptor activation in colon cancer cells [59]. Finally, MMP-7 may confer resistance to FasL-induced apoptosis to colon cancer cells by cleaving Fas, the cognate death receptor for this ligand [60]. Since immune systems rely heavily on the FasL/Fas signaling to fight cancers [61], downregulation of this death receptor could help CRC to escape immunosurveillance.

## Hedgehog proteins

*Hedgehog* (*Hh*), first identified in a screen for genes implicated in the embryonic development of *Drosophila melanogaster* [62], encodes a secreted protein important in regulating proliferation and establishing cell fate in flies [63]. Three mammalian homologues, Sonic, Indian and Desert hedgehogs (Shh, Ihh, Dhh), with functions similar to their fly counterpart have subsequently been identified [63, 64]. In human, the Hh signaling pathway is crucial for normal development and patterning of various organs, including the gut [65]. These proteins, are also involved in adult gastric gland development and gastric epithelial differentiation [66]. Recently, Ihh signaling has been reported to restrict the expression of two Wnt targets, Engrailed-1 and BMP-4, to the colonic precursor cell compartment *in vivo* and repress the Wnt signaling in colon cancer cells *in vitro*. Moreover, mutual antagonism between the Ihh and the $\beta$-catenin/Tcf pathways demonstrated *in vitro* might actually occur *in vivo* [8]. Hence, the loss of *Ihh* expression could be part of the reason for colonic dysplasia resulting from uncontrolled proliferation of enterocytes initiated by APC mutations. In contrast, Shh, Patched (Ptch), the receptor for Hh, as well as Smoothened (Smoh), a Ptch-associated transmembrane protein, have been found to be upregulated in hyperplastic polyps, adenomas and adenocarcinomas of the colon [7]. Moreover, exogenous Shh seems to be capable of promoting the growth of primary murine colonocytes [7], suggesting that the signal triggered by Shh might facilitate CRC progression. A positive role for the Hh pathway in CRC tumorigenesis was also postulated by a recent finding that approximately 23% of CRC patients carry truncating mutations in *EDD* whose product is a putative negative regulator of Hh signaling [67, 68].

## Thymosin $\beta$-4

Thymosin $\beta$-4 ($T\beta_4$), a small (43 amino acids) acidic peptide isolated originally from calf thymus, was initially postulated to be a thymic hormone [69]. However, along with other members of this peptide family, $T\beta_4$ was identified later, as intracellular G-actin sequestering molecules are present in almost every type of cell [70]. By preventing the formation of actin microfilaments via complexing with monomeric G-actin and supplying a pool of actin monomers for polymerization when the cell needs filaments [71, 72], $T\beta_4$ plays a key role in modulating actin dynamics, tissue remodeling, cell differentiation and wound healing [73].

Participation of $\beta$-thymosins in carcinogenesis was postulated years ago because of the aberrant expression of $T\beta_4$ and $T\beta_{10}$ in malignant renal tumors [74]. Later on, overexpression of these genes in human colon carcinomas and a variety of other tumors has been reported [75]. Upregulated $T\beta_4$ expression has been detected in highly metastatic melanoma cells [76] as well as in breast cancer cells [77]. Increased tumorigenicity and metastasis in fibrosar-

coma cells by $T\beta_4$ overexpression were demonstrated by an *in vivo* study [78]. In human SW480 colon carcinoma cells, overexpression of T$\beta_4$ gene resulted in a decrease of E-cadherin, accumulation of $\beta$-catenin in the nucleus, activation of the Tcf/LEF pathway and consequential malignant progression [10]. Meanwhile, enforced $T\beta_4$ expression in mouse melanoma cells increased their tumorigenicity, metastasis, as well as angiogenesis-stimulating activity [79]. More recently, upregulation of this gene was found to correlate with increased invasion of colon carcinoma cells as well as liver metastasis in CRC patients [9]. The latter may be explained in part by downregulation of Fas in tumor tissues, which could render them less susceptible to attack by FasL-bearing immune cells [61]. Another possible explanation for the positive correlation between T$\beta_4$ expression and distant metastasis of CRC is increased expression of Survivin, an antiapoptotic factor [80, 81]. Upregulation of Survivin is not only associated with poor prognosis of stage II [82] and III [unpublished data] CRC patients, but it also confers drug resistance (doxorubicin and etoposide) to T$\beta_4$- overexpressing colon cancer cells [83]. With the successful cloning of the functional human T$\beta_4$ gene and delineation of its promoter [84], dissecting the molecular mechanism of aberrant $T\beta_4$ expression during the metastatic progression of CRC became feasible.

## Colon (cancer) stem cells

The intestinal epithelium lining the gastrointestinal tract has a well-defined architecture with the simple columnar epithelium folded to form a number of invaginations, or crypts [85]. In the colon, the crypt progenitors proliferate rapidly, and the dividing cells migrate to the intercrypt table at the top of the colonic crypt during which they differentiate into enterocytes, mucus-secreting Goblet cells and the peptide hormone-secreting enteroendocrine cells [86]. In other words, cell replacement and production in colonic crypts are likely to be accomplished by the stem cells (SCs) located at their bases [87, 88], but the existence of these cells has as yet not been demonstrated. Interestingly, two independent studies have reported a preferential expression of Musashi-1 (Msi-1), an RNA-binding protein involved possibly in asymmetric divisions during *Drosophila* neural development, in the early generations of the cell lineages in the intestinal epithelium [89, 90]. Since strong Msi-1 expression was also detected in the early dysplastic crypts and adenomas in $Apc^{Min/+}$ mice [91], this protein may be used in conjunction with other markers to identify the actual SC population of the colon as well as early colon cancer lesions.

Recently, an increase in the number of cells expressing the crypt base cell phenotype was found in both FAP adenomas and sporadic CRC, supporting the hypothesis that SC overproduction may underlie CRC initiation and promotion, including the upward proliferative shift (the 'bottom up' theory [92]) in early colon tumorigenesis and adenoma formation [11]. On the other hand, spontaneous microadenoma development was also explained by the lateral and downward expansion of a mutant clone derived from the proliferation of a mutated stem cell migrating from the crypt base to the intercryptal zone (a modified 'top down' hypothesis [93]). However, to demonstrate unequivocally the existence of colon cancer stem cells (CSCs), it is essential to identify a subpopulation from either the primary tumors of patients or the established CRC cell lines that exhibits self-renewal capacity and generates faithfully phenocopied tumors during serial transplantations as those found in other types of tumors [94]. If CSCs were indeed the culprits of CRC formation, then therapeutic interventions that target only the main tumor mass are unlikely to succeed, or at best they leave patients at a high risk of cancer recurrence because most of the CSCs (or so-called side populations) identified thus far express high levels of various ABC transporter proteins which could efflux a number of anticancer drugs [95].

## Treatment

Surgical resection of the primary tumor as well as regional lymph nodes is the mainstay and the only curative therapy for CRC, whereas conservative palliative resection or bypass is usually indicated for patients having unresectable metastatic disease at the time of surgery. There has long been interest in preoperative (neoadjuvant) therapy of operable rectal cancer. Despite a lack of randomized data demonstrating clinical benefit, preoperative chemoradiation has been increasingly used in patients with T3 disease in North America, and preoperative radiation therapy is more frequently used in Europe [96]. Since mesorectal tissue is an ideal substrate for the spreading of rectal cancer cells, the addition of total mesorectal excision to anterior resection may provide adequate block dissection of the lymphatics of the rectum with a lower rate of local recurrence, even in node-positive disease [97]. For patients with stage I CRC, no additional treatment is needed after curative surgery (cure rate >90%). For patients with stage III disease, the combination of 5-fluorouracil (5-FU) and leucovorin (LV) for 6 months can significantly reduce tumor relapse and improve survival, in comparison with surgery alone [98]. Even though the value of adjuvant chemotherapy for stage II CRC has as yet been clearly established, a higher 3-year disease-free survival rate was detected in stage II/III CRC patients treated with oxaliplatin plus infusional 5-FU/LV (FOLFOX) than those with 5-FU/LV alone after receiving curative surgery [99].

For patients with disseminated recurrent disease who are ineligible for surgical treatment, regional therapy with hepatic arterial infusion (HAI) of floxuridine has been employed for liver metastasis. Although tumor regression rates can be improved by HAI, overall patient survival has not changed in comparison with systemic chemotherapy [100]. Irinotecan, a topoisomerase I inhibitor, and oxaliplatin are drugs commonly used in the management of metastatic disease (MCRC). Since 5-FU-based combination therapy with irinotecan or oxaliplatin has similar efficacy for MCRC in a first-line setting [101, 102], and oxaliplatin is effective in adjuvant therapy for stage II/III CRC, further studies exploring the impact of irinotecan in the adjuvant setting are warranted. More recently, the combination of several target therapeutic agents with chemotherapy has been shown to have a superior efficacy compared with chemotherapy alone in MCRC patients. For example, combination of cetuximab, a human/mouse chimeric epidermal growth factor receptor (EGFR) monoclonal antibody, with irinotecan was suggested for treating EGFR-expressing MCRC patients who did not show improvement after irinotecan-based chemotherapy [103]. In addition, bevacizumab, an anti-VEGF (vascular endothelial growth factor) antibody, was approved for patients with previously untreated MCRC [104]. Further studies need to focus on identifying patient groups most likely to benefit from the anti-angiogenic agents and designing optimal sequences and therapeutic combinations.

## Concluding remarks

Owing to the efforts of numerous investigators during the past several decades, loss of genomic stability has been identified as a key molecular and pathophysiologic step in colorectal carcinogenesis since it creates a permissive environment for the occurrence of alterations in tumor suppressor genes (*APC*, *MMR* and others) and oncogenes (*k-ras*, *TGFBR2*, *c-src* and others). Taking advantage of this progress, molecular staging has been developed and was found to identify patient prognosis more accurately than traditional clinical staging, particularly for intermediate Dukes' stage B and C patients [105]. A more versatile staging system based on a combination of pathological, biological and genetic technologies may be available in the near future. In theory, MMPs seem to be good targets for developing new anticancer drugs. Surprisingly, however, most of the clinical results using broad-spectrum MMP inhibitors to treat patients with advanced cancer were disappointing, suggesting a necessity to reformulate MMP inhibition strategies. Since MMP-7 is the most critical MMP for CRC progression (fig. 1), developing selective inhibitors against this protease and their administration in the early stage of disease may be worth trying. Involvement

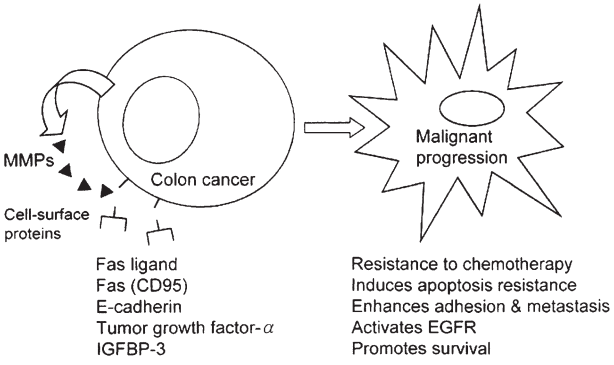


Figure 1. Cleavage of cell-surface proteins other than the ECM components is crucial for MMP-mediated CRC tumorigenesis.

of Hh signaling in CRC tumorigenesis appears to be controversial [7, 8], and is complicated further by a recent finding that, unlike CRC, cells of the upper digestive tract (i.e. Oesophagus, stomach, biliary tract and pancreas) tumors exhibit increased Hh pathway activity [106]. More work is required to resolve this question. In the meantime, discovery of the involvement of $T\beta_4$ in CRC progression is intriguing (fig. 2). Besides modulating the organization of micro filaments, $T\beta_4$ has recently been shown to promote the migration and survival of cardiomyocytes as well as cardiac repair by stimulating Akt via its complexing with PINCH and integrin-linked kinase (ILK) [107]. Since ILK has been postulated to play a positive role in CRC progression [108, 109], it will be of interest to examine whether ILK expression/activity is upregulated by $T\beta_4$ overexpres-

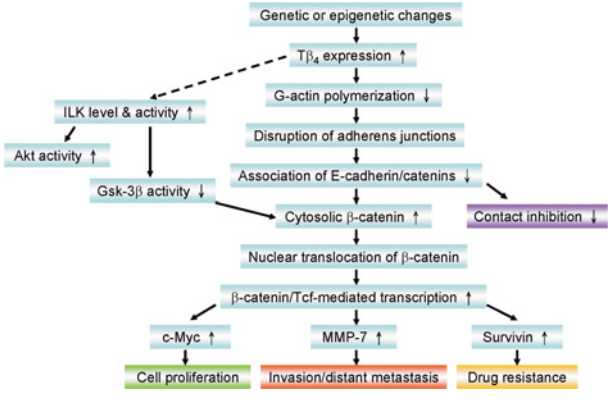


Figure 2. Overview of the action mechanisms of $T\beta_4$ in promoting CRC progression. $T\beta_4$, upregulated by unknown mechanism, disrupts F-actins as well as the adherens junctions supported by these microfilaments, which may result in the dissociation between E-cadherin and catenins. Free $\beta$-catenin molecules, if not being phosphorylated by GSK-3$\beta$ due to its inactivation by the $T\beta_4$-stimulated ILK, will translocate into nucleus and form complexes with Tcf to activate the expression of factors critical for CRC progression such as c-Myc, MMP-7 and Survivin. Arrows with dotted lines indicate evidence from our unpublished work.

sion in colon cancer cells and whether $\beta$-catenin/Tcf signaling is consequentially stimulated, because ILK-dependent activation of $\beta$-catenin-mediated gene transcription has previously been reported [110]. Even though neither colon SCs nor colon CSCs have been identified thus far, involvement of SCs in CRC development has nonetheless been proposed. Accordingly, isolation and *in vitro* propagation of colon CSCs from primary CRC tumor lesions and/or from established CRC cell lines may be crucial not only for designing studies to better understand how tumorigenic pathways operate but also for developing therapeutic strategies aimed at eradicating this silent but lethal subpopulation within CRC. In combination with novel target-specific treatments, this approach might greatly reduce the mortality of CRC patients.

*Acknowledgements.* We would like to thank our colleague Ms. Szuyuan Chen for editing the manuscript and preparing Fig. 2. This work has been supported in part by the National Science Council of Taiwan, R.O.C. (grant no. NSC93-2320-B-010-047 and NSC94-2320-B-010-050) to Y. Su.

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