World Journal of Gastrointestinal Pathophysiology

Online Submissions: http://www.wjgnet.com/2150-5330office
wjgp@wjgnet.com
doi:10.4291/wjgp.v3.i1.1

World J Gastrointest Pathophysiol 2012 February 15; 3(1): 1-9
ISSN 2150-5330 (online)

EDITORIAL

Regulation of colon cancer recurrence and development of therapeutic strategies

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Author contributions: Kanwar SS carried out the review of literature, conception, analysis and wrote the first draft of the manuscript; Poolla A helped in the analysis and interpretation of the data; Majumdar APN, the principal investigator, was responsible for discussion, critical evaluation of the review and overall supervision of the final preparation of the manuscript.
Supported by The National Institutes of Health/National Institute on Aging, AG014343; and the Department of Veterans Affairs, to Adhip PN Majumdar
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Received: August 1, 2011 Revised: September 8, 2011
Accepted: February 8, 2012
Published online: February 15, 2012

Abstract

Recurrence of colon cancer still remains a major issue which affects nearly 50% of patients treated by conventional therapeutics. Although the underlying causative factor(s) is not fully understood, development of drug-resistance has been associated with induction of cancer stem or stem-like cells (CSCs) which constitute a small sub-population of tumor cells known to be highly resistant to chemotherapy. In fact, the discovery of CSCs in a variety of tumors (including colon cancer) has changed the view of carcinogenesis and therapeutic strategies. Emerging reports have indicated that to improve patient outcomes, conventional anticancer therapies should be replaced with specific approaches targeting CSCs. Thus, therapeutic strategies that specifically target CSCs are being sought to reduce the risk of relapse and metastasis. In order to specifically target colon CSCs (while sparing somatic intestinal stem cells), it is critical to identify unique deregulated pathways responsible for self-renewal of CSCs and colon cancer recurrence. Colon CSCs present a unique opportunity to better understand the biology of solid tumors. Thus, a better understanding of the clinical signs and symptoms of colon cancer patients (undergoing surgery or chemotherapy) during perioperative periods, along with the underlying regulatory events affecting the stem/progenitor cell self-renewal and differentiation of colon epithelial cells, is of immense importance. In this review we discuss the implication of clinical factors and the emerging role of CSCs during recurrence of colon cancer along with the development of new therapeutic strategies involving the use of natural agents.

Key words: Chemo-resistance; 5-Fluorouracil; Oxaliplatin; β-catenin; Cancer stem cells

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Kanwar SS, Poolla A, Majumdar APN. Regulation of colon cancer recurrence and development of therapeutic strategies. *World J Gastrointest Pathophysiol* 2012; 3(1): 1-9 Available from: URL: http://www.wjgnet.com/2150-5330/full/v3/i1/1.htm DOI: http://dx.doi.org/10.4291/wjgp.v3.i1.1

INTRODUCTION

Colorectal cancer represents one of the most common cancers in the United States and worldwide. The estimated new cases and deaths from colon and rectal cancer (colorectal cancer) in the United States in 2010 are as follows: 102 900 colon cancer, 39 670 rectal cancer and 51 370 deaths from colon and rectal cancer combined[1,2].

The lifetime risk of colorectal cancer is 1 in 18 with advancing age contributing to the risk[3].

Traditionally colorectal cancer is staged based on histology/tumor size/invasion either by tumor node metastasis (TNM) staging or Dukes method. The American Joint Committee on Cancer endorses TNM staging according to which, stage I consists of tumor with size T1-2, N0, M0; stage Ⅱ with T3-4, N0, M0; stage Ⅲ with T1-4, N1-2, M0 and stage Ⅳ with T any, N any, M1. Currently stages Ⅰ, Ⅱ, Ⅲ without any invasion of serosa are treated by surgical resection with or without adjuvant chemotherapy and stage Ⅳ with metastasis is treated with chemotherapy alone[3]. The main chemotherapeutic agent used is 5-flurouracil (5FU)/leucovorin + oxaliplatin, also known as the FOLFOX regimen. Though individual rates of recurrence for each stage are not known, surgically resected cases of colorectal cancer (CRC) are known to have a 40%-60% recurrence rate in the first 3 years after surgery with the majority in the second year[4]. Lymph node metastasis and/or adjacent organ involvement in stage Ⅱ is said to have a recurrence of 20%-30% and stage Ⅲ 50%-80% recurrence after surgery[3].

Various factors have been proposed to play a crucial role in the recurrence of cancer including: number of nodes positive at surgery, pre operative and operative conditions, body mass index (BMI)/obesity, physical activity post surgery and chemotherapy, certain tumor markers and genetic factors, *etc*. Research findings have delineated various factors at the molecular level that can potentially cause recurrence, the latest being the discovery of the association of cancer stem/stem-like cells (CSCs) with drug-resistance phenomenon. Similarly, newer therapeutic approaches are now under consideration with the advent of new chemotherapeutic or preventive agents and regimens. The following is a review of the various factors, at clinical and molecular level, that are being implicated in recurrence of CRC and current knowledge on developing potential therapeutic strategies to combat the recurrence of CRC.

DEVELOPMENT OF COLON CARCINOGENESIS

CRC is a result of a sequential process of carcinogenesis. There are 2 models to explain the occurrence of CRC. One model states that the initial step begins with somatic mutations in adenomatous polyposis coli (APC) gene, which is considered as the initiating step of transforming the normal mucosa to an adenoma (class Ⅰ) by hyper proliferation[5]. The hyper proliferation is brought about by accumulation of β-catenin that in turn enters the nucleus to trigger cell cycle leading to hyper proliferation[3]. The next step involved is the activation of *K-ras*, which is a proto-oncogene that results in the transformation of an early adenoma to an intermediate adenoma (class Ⅱ adenoma)[3,5]. The third step is the loss of function gene deleted in colorectal cancer gene on chromosome 18q resulting in the formation of a class Ⅲ adenoma[3]. The last step is the mutations in *p53* gene that finally transforms an adenoma into an invasive/early cancer[5]. It is predicted that the above 4 steps take approximately 10 years and hence a 10 years interval was selected as the screening interval for colonoscopies in people with normal colonic mucosa at initial colonoscopy[3]. The second CRC model is based on "microsatellite instability" that causes mutations in DNA mismatch repair genes leading to accumulation of uncorrected replication errors resulting in hyper proliferation and eventually carcinoma[3].

Surgery was seen as curative and hence became the mainstay of therapy. However, recurrence was seen in as many as 50% of patients after curative surgery[6]. Later, various therapeutic approaches that aimed at targeting one or many steps in the above models surfaced by using chemotherapeutic regimens like FOLFOX, consisting of 5FU + oxaliplatin + leucovorin also known as mainstay for CRC chemotherapy[3], FOLFIRI, consisting of 5FU+ leucovorin+ irinotecan mainly for patients with liver metastasis, neoadjuvant chemotherapy with Bevacizumab, a recombinant monoclonal antibody that targets vascular endothelial growth factor and hence antiangiogenic and Cetuximab, an antibody that inhibits epidermal growth factor receptor[5]. In spite of these combinations of multiple chemotherapeutic agents, recurrence is seen frequently in CRC, especially in patients with metastasis.

CLINICAL SIGNS AND SYMPTOMS THAT PREDICT POOR PROGNOSIS AND RECURRENCE

Certain clinical signs and symptoms like bowel perforation, obstruction and change in bowel habits at presentation were hypothesized to predict poor prognosis as they are signs of advanced disease. Similarly, these signs were also said to be predictors of recurrence as per the prospective study of Aghili *et al*[4]. As per this study, 130 colorectal cancer patients with recurrence diagnosed during 1999-2005 were followed for a period of 20 mo with physical examination, serum carcinoembryonic antigen levels, chest X-ray and abdominopelvic sonography done every 2 mo in the first year, every 3 mo the second year, every 4-6 mo for the next 2 years and annually thereafter until the completion of the study. Patients were divided into "early recurrence group", those exhibiting recurrence within 2 years after surgery and "late recurrence group", patients with recurrence after 2 years. Bowel obstruction and change in bowel habits were seen more frequently in patients with early recurrence (10% and 20%, respectively) than in patients with late recurrence (1% and 4%, respectively). A plausible explanation for this could be that patients with early recurrence might have had more advanced disease at initial presentation; thus causing them to develop tumor related complications like luminal obstruction leading to change in bowel habits, perforation of bowel, earlier in the course of disease. On the other hand, patients with late recurrence

might not have had advanced disease at initial presentation, such as spread to serosa, adjacent organ involvement and lymph node involvement, that can potentially cause complications like bowel obstruction, perforation later on.

AGE, BMI AND PHYSICAL ACTIVITY

The effect of age was also studied by Aghili *et al*[4] and in their sample, they found that patients with early recurrence were younger, 48 ± 16 years than those with late recurrence, 54 ± 13 years. The reason was felt to be that younger patients at diagnosis had a more advanced cancer stage than the patients diagnosed with CRC at an older age. In addition, Agili *et al*[4] conducted their study in the Middle East and observed that in that geographic area, CRC is seen at a higher frequency in younger subjects when compared to the rest of the world and that most of these younger patients tend to have familial disease rather than sporadic CRC[4]. This could explain the earlier recurrence in younger patients; patients with hereditary forms of CRC such as familial adenomatous polyposis, hereditary nonpolyposis CRC, are known to have disease incidence and progression early on in their lives, e.g., in their twenties, compared to sporadic CRC that tends to occur later on. Hence, screening also begins early for people with hereditary types of CRC in their family history. In the case of sporadic CRC, studies from our lab showed that over-expression of epidermal growth factor receptors (EGFR) plays a role in causing colon cancer as age progresses[7-10]. Over-expression of EGFR was also seen to increase in CRC stem cells resistant to FOLFOX therapy[10-12]. These findings suggest that advanced age certainly influences not only the occurrence but also the recurrence of CRC.

Obesity is a well known risk factor for CRC. Meyerhardt *et al*[13] conducted a prospective observational study of 1053 stage Ⅲ CRC patients enrolled in a trial of adjuvant chemotherapy (National Cancer Institute sponsored The Cancer and Leukemia Group B Trial). Height and weight reports were taken during 4 mo after surgery and 6 mo after chemotherapy. They found that patients with class Ⅰ, Ⅱ and Ⅲ obesity did not show any significant increase in CRC recurrence after chemotherapy when compared to normal weight patients with *P* trend = 0.54. The multivariate hazard ratio for cancer recurrence or death termed as "disease free survival" was found to be 1.00 and 1.24 for patients with class Ⅰ, Ⅱ and Ⅲ obesity, respectively after factors like age, sex, physical activity, tumor-related prognostic factors, tobacco history and performance status were adjusted. Similarly, both weight loss and gain after adjuvant chemotherapy to 6 mo post chemotherapy did not show any effect on recurrence[13] after the data were controlled for BMI and after exclusion of recurrence and/or death within 90 d of reporting weight at 6 mo post chemotherapy. When the exclusion of recurrence/death within 90 d was taken into account, patient weight loss of ⩾ 5 kg during and 6 mo post chemotherapy was associated with a poorer outcome than patients who lost < 2 kg. The mechanism is thought to be due to increased levels of C-peptide in obese patients that inhibits the insulin receptor responsible for decreased production of inflammatory cytokines like interleukin-6 (IL-6), tumor necrosis factor-alpha (TNF-α), C-reactive protein (CRP), *etc.,* that decrease P53 action, thereby leading to tumorigenesis[13].

Many researchers[14] have studied physical activity in association to CRC. Numerous case control and cohort studies showed that physical activity on a regular basis is associated with decreased incidence of CRC. Similarly, Harriss *et al*[14] reviewed studies to identify the existence of any relationship between physical activity and CRC recurrence. They found that in a prospective cohort study done by Haydon *et al*[15] on 526 CRC patients over a period of 5.3 years, an age adjusted disease specific survival of 12% and 39% in stage Ⅱ, Ⅲ respectively, in patients reported to be performing some sort of physical activity daily when compared to patients not involved in physical activity. Prospective studies of Allgayer *et al*[16] in CRC patients following primary therapy for CRC, showed that there is decreased urinary excretion of 8 oxo-dG indicative of DNA damage and thereby tumor formation/recurrence in patients (n = 19) undertaking moderate intensity exercise for 30-40 min for 2 wk. However, in the same study, similar findings were not seen in patients undertaking high intensity exercise defined as 50%-60% of maximal capacity for the same time period. The above results may be due to the existence of some unknown dose-response-like mechanism that plateaus after a certain dose of physical activity, giving the same or better results at moderate intensity rather than at high intensity. Another group of researchers, Meyerhardt *et al*[17] did a prospective study of recreational physical activity on 816 stage Ⅲ CRC patients 6 mo post adjuvant chemotherapy. They found a significant difference of hazard ratio = 0.51 in patients who reported putting in 18-27 h per week of physical activity over 2 mo compared to patients who were inactive during the same time period. Various mechanisms like cyclooxigenase-2 expression reduction, a marker of anti-inflammatory response, physical activity induced decrease in IL-6, TNF-α, CRP, stimulation of toll-like receptor 4 that causes production of inflammatory cytokines and innate immunity activation were thought to be the mechanisms responsible[14].

HISTOPATHOLOGICAL FEATURES

Numerous investigators have studied the role of histopathological features of tumors such as: number of lymph nodes, stage and grade of the primary tumor, adjacent organ involvement, the presence of inflammatory cells in the tumor, pathological type and gross pathological view of the tumor, in terms of causing recurrence. Among these Aghili *et al*[4] found that patients with Dukes C stage and adjacent organ involvement at initial

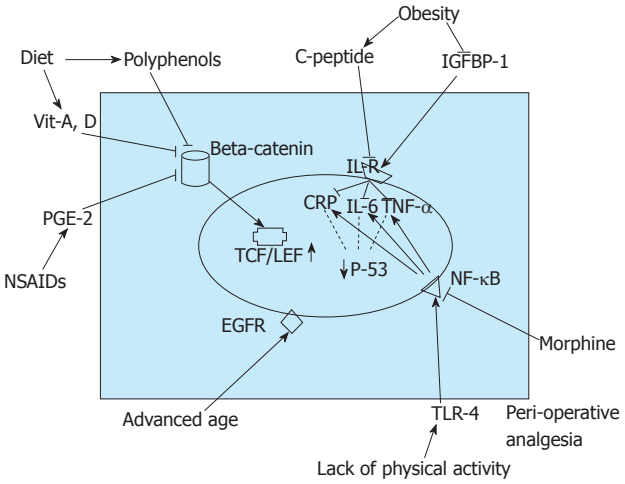

Figure 1 The role of various factors that are implicated in the recurrence of colon cancer. EGFR: Epidermal growth factor receptor; CRP: C-reactive protein; IL: Interleukin; TNF: Tumor necrosis factor; NF: Nuclear factor; TLR: Toll-like receptor; NSAIDs: Nonsteroidal anti-inflammatory drugs.

diagnosis had more early recurrence. In the same study, ulcerative tumors and non-mucinous adenocarcinomas seemed to recur more often within 2 years of surgery (48% and 62% respectively). On the other hand, number of lymph nodes involved and the site of primary tumor did not predict early or late recurrence. This finding is interesting as one would expect the number of lymph nodes involved to predict the rate of recurrence or prognosis. In fact, the number of lymph nodes involved by the primary or node positive primary at initial presentation was found to be an independent predictor of poor survival; and hence is part of a preoperative scoring system known as "basingstoke predictive index" in patients who were considered to have surgery for liver metastasis[18]. However, the prognostic potential of number of lymph nodes involved at presentation might vary with the stage of CRC at initial presentation, as the Basingstoke Predictive Index is applied for patients with liver metastasis, leaving the possibility that the number of lymph nodes involved at initial presentation might not have a similar prognostic value for patients with stage Ⅰ, Ⅱ and Ⅲ of the disease.

PERIOPERATIVE PERIOD

Recently, Gottschalk *et al*[19] reviewed the perioperative period factors that can potentially affect recurrence after cancer surgery. They found that the perioperative period is a period of stress: a known cause of cancer progression. Opioids such as Morphine were said to have both tumor progressing effects through angiogenesis and also tumor regressing effects through improved T cell mediated immunity, nuclear factor kappa B inhibition and by activating mu receptor splice variants[19]. COX inhibitors were found to suppress tumor growth by increasing apoptosis, suppressing angiogenesis and by decreasing the density of tumor microvasculature[19]. Hence, the authors felt that co-administration of opioids and COX inhibitors for management of pain during the perioperative period might contribute to decreased tumor recurrence. Similarly, certain alpha and beta blockers were also found to decrease the rate of recurrence through increased apoptosis, decreased tumor cell proliferation and by inhibiting Signal transducer and activator of transcription 3 activity[19]. Addition of regional anesthesia was also thought to decrease the stress response and thereby reduce recurrence. However, volatile anesthetics like Thiopental and Halothane were known to reduce the natural killer cell activity by decreasing their density in circulation thereby increasing the chances of recurrence and metastasis in animal models[20]. Other factors like anemia and perioperative hypothermia were found to be associated with a poorer outcome; whereas statins showed promising effects in reducing recurrence in animal models. These statin effects occurred *via*: antiangiogenic effect, anti-inflammatory and immunomodulatory effects through 3 hydroxy-3 methylglutaryl coenzyme A reductase dependant or independent pathways that alter the function of Lymphocyte function associated antigen, T helper 1, 2 cytokines and CRP, in turn inducing apoptosis and effecting signaling pathways of tumor progression[19]. The role of statins seems to be a promising area of investigation since CRC patients with co-morbid cardiovascular disease conditions can benefit from statin therapy for both their CRC and cardiovascular disease states. Figure 1 summarizes the role of various factors that are implicated in the recurrence of colon cancer and the various drugs currently under clinical investigation for the treatment of recurrent colon cancer are presented in Table 1.

MOLECULAR BASIS OF CHEMO-RESISTANCE: STEM CELL REGULATION GONE AWRY

Researchers who studied extensively to find a cause/s for recurrence came up with the concept of cancer stem cell theory and colonel evolution model to explain recurrence. There is an emerging body of evidence suggesting that tumor cells resistant to chemotherapy represent a subpopulation of cells from the original tumor. These chemo-resistant cells are molecularly and phenotypically distinct and are also referred to as tumor-initiating, tumor-promoting or more commonly, cancer stem cells or cancer stem-like cells[21]. Cancer stem cell theory states that the process of carcinogenesis is brought about by mutations in a normal stem cell/progenitor cell that leads to hyper proliferation and malignant transformation resulting in the formation of a host of cancer cells that have the same genome[5,21-23]. These cancer stem cells, through their capacity to self-renew, are said to promote tumor growth and hence are difficult to kill by chemotherapy as current chemotherapeutic drugs are able to kill other cancer cells; the cancer stem cells,

Table 1 Drugs currently under clinical investigation for the treatment of recurrent colon cancer[1]

Trail	Drug(s)/agent(s)	Target	Mechanism	Prior experiments
Phase Ⅱ	R935788/fostamatanib disodium	Kinase inhibitor	Interference with cell communication thereby inhibiting tumor growth	On NCI-60 cell lines
Phase Ⅱ	Anti ESO-TCR engineered lymphocytes + IL-2 + ALVAC ESO-1 vaccine		Tumor regression secondary to secretion of IFN-gamma by the anti-ESO-TCR induced cells + development of immunity from ALVAC ESO-1 vaccine	On HLA-A2 and ESO double positive tumors
Phase Ⅱ	Bay 43-9006 + cetuximab	Kinase inhibitor	Inhibition of Raf pathway and VEGFR-2 by Bay 43-9006 + Ras and EGFR inhibition by cetuximab	With cetuximab
Phase Ⅱ	MSLS + CRS + HIPEC		CRC + HIPEC for overall improvement in survival and MSLS to detect peritoneal carcinomatosis early on	Study results with increased survival of 48-63 mo from CRC + HIPEC when compared to 5-16 mo from chemotherapy alone in metastatic CRC patients
Phase Ⅱ	Autologous CD8+ enriched young TIL + IL-2		TIL mediated regression of tumor bulk	Evidence of response rates in Metastatic melanoma upon infusion of young CD8+ enriched TIL + IL-2 after a lymphocyte depleting chemo preparative therapy.
Phase Ⅱ	Echinomycin	Intercalating agent	Anti tumor properties from inhibition of stem cell RNA and DNA synthesis by intercalating into both strands of DNA thereby preventing synthesis	Evidence from trials of southwest oncology group for compounds with ability to modify fluropyrimidine activity
Phase Ⅲ	Optimized chemotherapy + avastin strategy + tarceva			
Phase Ⅱ	Bortezomib			

[1]From www.cancer.gov. MSLS: Mandatory second look surgery; IL: Interleukin; CRS: Cytoreductive surgery; HIPEC: Hyperthermic intraperitoneal chemotherapy; TIL: Tumor infiltrating lymphocytes; CRC: Colorectal cancer.

whose survival leads to recurrence from self renewal, thus cause tumor formation[5,21-23]. According to the clonal evolution model or classical theory, any normal cell can be affected by mutations, transforming it into a malignant cell that undergoes unrestricted cell divisions leading to the formation of a mass of cancer cells with genetic variability, resulting in tumor formation and progression[5,21-23]. According to this theory, all the daughter cells originate from one parent cell and hence are homogenous. However, the trait of "homogeneity" cannot explain the concept of recurrence as current chemotherapeutic drugs can eliminate a specific cell type and, going by this model, should be able to eliminate all the cancer cells due to homogenity. Todaro *et al*[5] came up with a combination of both theories to explain recurrence, stating that CRC starts as a cancer stem cell disease with production and maintainance of cancer stem cells but progresses by clonal evolution thereby forming clones of cancer stem cells[5] that are currently not, or minimally, targeted by existing chemotherapeutic drugs.

Recent findings clearly indicate crypt stem cells as the cells-of-origin of intestinal cancer[24] as cancer stem cells have the capacity for unlimited self-renewal as well as the ability to initiate and drive tumor progression in an animal model[23,25]. Thus, they would seem the most probable candidate responsible for tumor chemo-resistance and recurrence. Moreover, gene expression studies have revealed a higher expression of multidrug resistance genes and DNA mismatch repair genes as well as genes that inhibit apoptosis in the cells displaying higher expression of cancer stem cells surface antigens[26].

Cancer stem cells can be responsible for recurrence due to the following reasons. One, the concept of existence of cancer stem cells and their clonal proliferation can explain the failure of current regimens that target the rest of the tumor cells, leading to recurrence. Secondly, there is evidence of cancer stem cells being accumulated; the presence of certain markers of cancer stem cells being found in chemotherapy resistant/recurrent tumors. Thus, the theory of colon cancer stem cells has opened up a new area of research to find chemotherapeutic or other drugs that can target these cells to prevent their recurrence.

INAPPROPRIATE EXPRESSION OF STEM CELL PATHWAYS

The human adult colonic epithelium undergoes perpetual regeneration fueled by intestinal epithelial stem and progenitor cells located at the colon crypt base. Perturbation of the pathways regulating stem cell renewal contributes significantly to neoplastic transformation. The link between genes important for normal stem cell development and colon cancer has been established[23]. Since both normal stem cells and cancer stem cells share basic characteristics of self renewal, it seems reasonable to propose that newly arising cancer cells possess the machinery for self-renewal which is normally expressed in stem cells. Evidence shows that many pathways that are classically associated with oncogenesis may also regulate normal stem cell development that includes: Notch,

Sonic hedgehog and Wnt signaling pathways[11,27,28].

One particularly interesting pathway that has also been shown to regulate both self-renewal and oncogenesis in different organs is the Wnt signaling pathway[29-31]. Further, among these pathways, canonical Wnt signaling plays a major role in maintaining the fate of intestinal stem cells and progenitor cell proliferation[21]. Cell fate decisions in the intestine have been shown to involve Notch signaling, which specifically directs cells toward a secretory lineage in the gut[32]. All of the evidence suggests there is a close interaction of several key pathways in directing intestinal epithelial stem cell renewal and differentiation. Yet how these different pathways coordinate in the specific anatomical compartment of the intestine remains mostly unknown. Since colon cancer is one of the most common cancer types, understanding the proliferation program governing the stem/progenitor cell compartment and the differentiation program of colon epithelial cells is of particular importance.

TARGETING WNT/BETA-CATENIN SIGNALING

Recent studies have reported the pivotal role of Wnt/beta-catenin signaling pathway in the regulation of colonic epithelial stem cell self renewal in addition to its vital role in cellular proliferation, cellular movement and establishment of cell polarity[33-35]. In contrast, deregulation of Wnt/beta-catenin signaling has been implicated in colon carcinogenesis[36,37]. Wnt signaling has been defined as occurring either through the canonical or non-canonical pathways. Canonical Wnt signaling is characterized by the stabilization and cytoplasmic accumulation of beta-catenin, which then translocates to the nucleus to facilitate the activation of a variety of Wnt target genes.

In human cancers, including colon cancer, the frequent occurrences of mutations within this highly conserved signaling pathway have been reported[38]. Because Wnt signaling plays such a critical role in the regulation of stem cell proliferation, targeting this pathway may yield important clinical benefits[39]. Studies suggest that Wnt signaling is essential for maintaining colonic crypts and for the regulation of cellular differentiation[40]. However, the regulatory role of Wnt/beta-catenin signaling in the maintenance and growth of CSCs still remains elusive. In a recently reported study the formation of epithelial tumors in mice has been linked with the activation of Wnt/beta-catenin signaling in epidermal stem cells[41]. In agreement with these results, we have shown that the Wnt/beta–catenin pathway is majorly responsible for the regulation of growth and maintanace of CSCs enriched colonies derived from various human colon cancer cells termed as colonospheres[28]. It is worth mentioning that colonospheres conatin multi-potent cancer stem cells that are hypothesised to be causing recurrence. They are generated in vitro under sreum free conditions and in the presence of special stem cell growth factors and are considered to be surrogate tumors. The colonospheres were found to express: LGR5, CD44, CD166, Musashi-1 and EpCAM, that are markers of colon cancer stem cells[11,28]. Apart from this, the colonospheres were also found to to have elevated levels of total beta-catenin leading to transcriptional activation of the *TCF/LEF* gene responsible for the progression of cancer stem cells[28]. In 2009, Fang *et al*[6] did a prospective study on 620 CRC patients in various stages of the disease, post curative surgery (resection), for a median duration of 52 mo, using high density tissue microarray technology and immunohistochemical analysis, to determine the molecular markers that can predict recurrence. They found that certain target genes of the Wnt/Beta Catenin pathway like *Survivin*, *Cyclin D1*, *TCF 4*, (important components of Wnt pathway) and a cancer stem cell surface receptor TROP 2 were elevated in patients with disease recurrence[6].

Recent studies[22,42] have revealed that several multidrug resistance genes, including *MDR-1*, *ABCG2*, *ABCA3*, and *BRCP1* are also intrinsically expressed in stem and/or progenitor cells and may contribute to the side population phenotype of malignant cells. Wnt/β-catenin signaling seems to play an important role in *ABCB1/MDR-1* transcription[43,44]. Putative TCF binding elements were also identified in the *ABCB1* promoter (-1813 to -275 bp)[44]. Canonical Wnt signaling is believed to play an important role in the maintenance of hematopoietic progenitors and also in the lineage commitment of progenitors during hematopoiesis. Interestingly, many of the cell surface markers (including LGR5/GPR49, CD44, CD24 and EpCAM) that have been used to identify and isolate putative tumor stem cell populations in a variety of tissues including colon are direct Wnt targets. Since colon cancer stem cells are believed to be dependent on beta-catenin signaling to maintain their properties, targeting wnt/beta-catenin pathway may yield promising clinical benefits by inhibiting the proliferative capacity of CSCs or possibly force terminal differentiation.

PREVENTING RECURRENCE OF COLON CANCER WITH NATURAL COMPOUNDS

All cancers are thought to be preventable[45-47]. The two most important ways to reduce cancer risk are the avoidance of cancer-causing biologic, chemical, and physical agents and the habitual consumption of diets high in foods that protect against cancer. It has been estimated that approximately, 30% to 40% of cancer incidents are preventable by consuming a healthy diet, regular physical activity, maintenance of optimum body weight and consumption of fruits and vegetables[48]. Because of their safety, low toxicity, antioxidant properties, and general acceptance as dietary supplements, fruits, vegetables, and other dietary elements (phytochemicals and minerals) are being investigated for the prevention of cancer. Numerous recently published reports have indicated the usefulness of natural agents in reversing the recurrence of various cancers including colon cancer.

A thorough online search on the website: clinicaltri-

Table 2 Major natural compounds targeting Wnt/β-catenin pathway undergoing clinical trials for the treatment of colon cancer

Category	Compound	Target	Mechanism	Clinical trials identification No.
Vitamins	Retinoids/vitamin-A	Beta-catenin	Compete with TCFs, production of inhibitory protein-disabled-2	NCT00270647, NCT00712647
	Vitamin-D	Beta-catenin	Production of inhibitory proteins-dickkopf-1 and 4	NCT00208793, NCT00870961, NCT01198548, NCT00399607, NCT01150877, NCT00585637
	Vitamin-E	Unknown	Unknown	NCT00706121, NCT00270647
	Pyridoxine	Unknown	Unknown	NCT00559858
Polyphenols	Quercetin	Wnt/beta-catenin	Unknown	NCT00003365
	Epigallocatechin-3-gallate from green tea	Wnt/beta-catenin	Plieotropic	NCT01239095, NCT00718094
	Curcumin	Wnt/beta-catenin	Plieotropic	NCT00365209, NCT00295035, NCT00027495, NCT00973869, NCT00745134, NCT00118989, NCT00927485, NCT00641147
	Resveratrol	Wnt/beta-catenin	Unknown	NCT00256334, NCT00578396, NCT00433576, NCT00920803
	Dictyostelium discoidem	Wnt/beta-catenin	Activation of GSK-3beta by differentiation inducing factors-1,3	No current trails
Lipids	Omega 3 fatty acids and n-3 polyunsaturated fatty acids	Mitochondrial membrane of tumor cells	Enhanced reactive oxygen species generation and decreased cell anti-oxidant capacity leading to apoptosis in tumor cells	NCT01127867, NCT00145015, NCT01218841, NCT00432913, NCT00488904, NCT00168987, NCT00510692, NCT01070355, NCT01048463

ID numbers source: clinicaltrails.gov.

als.gov, will yield about 100 such trials that are ongoing with most promising natural compounds being investigated against various types of cancers[46]. These include, but are not limited to, the use of (-)-epigallocatechin gallate (EGCG) (green tea), curcumin, resveratrol, genistein, pomegrante, lycopene, n-3 poly unsaturated fatty acids, folic acid, ellagic acid, lupeol and betulinic acid for targeting many cancers including solid tumors and gastrointestinal (GI) cancers (Table 2)[46]. Almost all these natural compounds have also been found to synergistically increase the efficacy of other drugs in cell culture and animal models[3,46,49-52]. Interestingly, these agents have also been shown to prevent or delay the progression of cancer, which could in part be due to their ability to attack CSCs by attenuating the Wnt and Hedgehog signaling pathways. There is strong evidence that natural compounds such as EGCG, curcumin, resveratrol and n-3 poly unsaturated fatty acids can be used to resensitize chemotherapy-resistant colon cancer cells. Among these natural compounds, EGCG, curcumin and resveratrol have been extensively studied for their efficacies against colon cancer.

It has been known that in many malignancies, EGCG has concentration dependent inhibitory effects on Wnt signaling[53]. In colon cancer cells, treatment of EGCG led to a potent inhibition of GSK3-α and GSK-3β activity, the important regulatory molecules involved in Wnt signaling[54]. It was observed that the amount of phosphorylated β-catenin was diminished and the overall amount of β-catenin and the TCF/LEF-mediated luciferase expressions were decreased by EGCG treatment[54]. Further, it has also been reported that the canonical Wnt signaling could be inhibited at the level of TCF/LEF by EGCG in antler progenitor cells[55]. These studies strongly indicate that EGCG can be a promising agent in the management of colon cancer recurrence.

Similar to EGCG, curcumin and resveratrol have also been known for their plieotropic effects against many cancers with most promising effects exhibiting against colon and other GI cancers. Our own study[51] showed that that the combination therapy of curcumin and resveratrol is highly effective in inhibiting the growth of colon cancer cells *in vitro* and *in vivo*. The combination of 5FU and oxaliplatin (FOLFOX) forms the backbone of colorectal cancer chemotherapeutics. Our studies *in vitro* demonstrated that curcumin, either alone or in combination with FOLFOX (5FU + oxaliplatin), caused a greater growth inhibition of FOLFOX-resistant colon cancer cells than either agent alone[12]. Furthermore, in response to curcumin treatment caspase-3-mediated cleavage of β-catenin, E-cadherin, APC and decreased transactivation of β-catenin/TCF/LEF, DNA-binding activity of the β-catenin/TCF/LEF complex and the levels of c-Myc protein were observed[56]. These results suggested that curcumin could impair both Wnt signaling and cell-cell adhesion pathways, leading to the G2/M phase arrest and apoptosis of colon cancer cells suggesting a promising potential against recurrence of colon cancer. Indeed, our recent findings suggested that curcumin can inhibit the growth of chemo-surviving colon cancer cells that are highly enriched in CSCs[57]. Failure to eliminate these cells is thought to be one of the underlying causes of cancer recurrence. Our studies have shown that curcumin can not only synergize with FOLFOX but also with dasatinib to inhibit the growth of colon cancer cells[12,57,58]. On the basis of these recent observations and the fact that curcumin and/or other natural derivatives has plieotropic effects in down-regulating the survival signals seen in chemo-surviving cells, we have hypothesized that combination of non-toxic natural

agents like curcumin and FOLFOX can be a potential therapeutic strategy for colon cancer recurrence.

CONCLUSION

Drugs or natural compounds that target aberrant activation of the Wnt signaling cascade have enormous potential as novel cancer therapeutics. The constitutive activation of Wnt signaling primarily occurs during embryogenesis and tissue repair in the adult. Significant levels of toxicities are not expected with the use of antitumor agents that target Wnt signaling pathway. Various efforts are already underway to develop Wnt and/or beta-catenin antagonist that may specifically drive stem cells toward differentiation. As described above, there are a number of drugs and natural compounds that have already been identified to have therapeutic value against cancers associated with aberrant Wnt signaling. Because of the complex communication between cell signaling networks, cancer cells always show alterations in multiple cellular signaling pathways. Therefore, regulation of multiple cell signaling pathways controlling the behavior of cancer cells, such as inhibition of cell growth or induction of apoptosis, requires agents that could target multiple pathways. It is believed that many of natural products can perform these tasks. Identification of the target molecules and determination of the precise mechanism(s) by which different natural and/or synthetic agents exert their action is essential for the development of therapeutic strategies against various malignancies.

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