REVIEW ARTICLE

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Curcumin Effects on the Wnt Signaling Pathway in Colorectal Cancer Stem Cells

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ABSTRACT

Colorectal cancer (CRC) is a leading cause of death worldwide. Despite improved treatment procedures, the disease can rarely be cured completely mainly due to its recurrence. It has been proved that cancer recurrence is caused by cancer stem cells (CSCs); rare and immortal cells which have the ability to initiate and develop tumors and protect them against anticancer agents. CSCs are generated as a result of failures in intracellular signaling pathways, in which Wnt/β-catenin plays a major role in colorectal cancer. The Wnt/β-catenin signaling pathway is thought to be the major pathway in the maintenance of homeostasis of intestinal stem cells. The proliferation and upward migration of colony crypt daughter cells, and their differentiation into different epithelial cell types is regulated in part by Wnt/β-catenin signaling, suggesting its essential role in intestinal development and homeostasis. However, continuous activation of this signaling pathway in intestinal stem cells due to somatic mutations is a hallmark of most CRCs. Hence, targeting Wnt/β-catenin signaling in CSCs can be a focus of new treatment strategies.

Curcumin, the effective compound of the plant Curcuma longa, has been studied as an anticancer agent. Recently, it has been shown that curcumin and its analogues can decrease the risk of tumor recurrence by targeting CSCs via various cell signaling pathways, in particular the Wnt/β-catenin pathway. In this review, we shall describe the relationship between Wnt-regulated CSCs and progression of CRC, and the efficacy of curcumin and its analogues in targeting colorectal CSCs and the Wnt/β-catenin molecular pathway involved.

Keywords: curcumin, Wnt/β-catenin pathway, colorectal cancer, cancer stem cells.

INTRODUCTION:

Colorectal cancer (CRC) is the third common cancer in developed countries[1]. It is predicted that the 5 year survival rate for patients with advanced colon cancer is only 8%. Various factors including a high fat diet, chronic inflammation, and genomic instability are strongly associated with CRC occurrence[2]. Despite conventional treatments include aggressive surgical resection of tumors and chemotherapy, almost 50% of CRC patients experience recurrence[3].

Intestinal stem cells (ISCs) have a major role in CRC progression[4]. Recent evidence suggests that factors including aging and the total number of stem cell divisions increase the frequency of gene mutations in ISCs[5] in addition to inherent germ line mutations[6]. While several biological pathways control ISC development, in most cases it is aberrant Wnt signals that drive the pathogenesis of CRCs[6]. It has been shown that 94% of CRCs are caused by mutations in one or more molecules involved in the Wnt signaling pathway[7] and these mutations are the first step in ISC-initiated gut malformations[8]. Due to continuous activation of the Wnt signaling pathway, normal ISCs can transform into cancer stem cells (CSCs) which have the capacity for tumor initiation with features such as self-renewal, differentiation and resistance to chemotherapeutic agents[9]. Hence, it seems that targeting active molecular mechanisms in these cells can play a significant role in cancer treatment.

In recent decades, intense efforts have been made to identify natural compound drugs. These products are of interest due to their low production costs, structural diversity, and multiple usage to treat various diseases[10]. Curcumin is a polyphenol compound, extracted from the plant curcuma longa, that has been shown to have anti-inflammatory, antimicrobial, and antioxidant activities[11]. In addition, increasing evidence shows that curcumin may have anti-cancer effects[12,13] and can inhibit molecular signaling pathways associated with CRC in different human cancer cell lines[14]. Recent advancements in identification of the details of the Wnt signaling pathway and its significant in CRC have induced scientists to test the effects of natural compound on this pathway[15]. It has been suggested that curcumin has the ability to target CSCs self-renewal pathways, one of the most important of which is the Wnt/β-catenin pathway[16]. What increases the importance of curcumin in cancer studies is that curcumin has asymmetrical effects on normal and cancer cells, an issue that has been a major obstacle to continuing chemotherapeutic regimes in many patients with CRC. Curcumin suppresses molecular abnormalities in the Wnt pathway in CSCs[17,18] but it has opposing effects on normal stem cells[19,20]. Also it has been predicted that curcumin has a much greater uptake by malignant cells compared to normal cells[21]. However, the clinical usage of curcumin is limited because of its low aqueous solubility, and poor pharmacokinetic profile. A good method for improving the poor biopharmaceutical characteristics of curcumin is to promote its aqueous solubility using nanocarriers. Nanocarriers are small compounds (typically 10-100 nm) and, in addition to improvement of solubility, they can be used for targeted drug delivery[22,23]. Nanocarriers can improve the circulation time of the loaded therapeutic agents. Besides, Nanocarriers can accumulate in much greater concentrations in tumor tissue than in normal tissues and create the 'enhanced permeation and retention' (EPR) effect[24,25].

The Wnt Signaling Pathway:

Wnt signaling is an evolutionarily conserved pathway and plays an important role in stem cell maintenance, embryogenesis, cell proliferation, differentiation, and apoptosis[26]. Wnts are glycoproteins which are full of

cysteine residue[27]. In normal situations, Wnt signaling is silent in a non-cycling cell[28]. The Wnt signaling cascade is typically initiated when the Wnt ligand binds to seven transmembrane receptor complexes which are a member of the Frizzled family and either of the single transmembrane low-density lipoprotein receptor related proteins 5 or 6 (LRP5/6)[29,30] which leads to accumulation of β-catenin in the nucleus[29].

β-catenin, a 781-amino acid protein encoded by the CTNNB1 gene, has two different roles in cells, as a scaffolding protein which is involved in cell adhesion and a transcriptional regulator factors. It seems that in normal gastrointestinal epithelial cells, β-catenin on the one hand is related to E-cadherin and therefore mediates adherent junctions, and on the other hand is related to α-catenin and therefore participates in the intracellular actin cytoskeleton[31]. The portion of β-catenin which is involved in the cell cytoskeleton has a long half-life in comparison with the free β-catenin in the cytoplasm and nucleus which is responsible for regulation of Wnt signaling[31].

In the absence of extracellular Wnt ligand binding to the Frizzled and LRP5/6 coreceptors, ubiquitous kinases such as Casein kinase 1α (CK1α) and glycogen synthase kinase 3 beta (GSK3β) phosphorylate β-catenin at distinct N-terminal serine and threonine residues. The phosphorylated β-catenin is recognized by F-box containing E3-ligase protein β-TrCP that forms a complex with Skp1/Cullin machinery to attach ubiquitin to β-catenin[32,33] and which is then rapidly degraded via the 26S proteasome[33,34]. The phosphorylation of β-catenin takes place in a dynamic multiprotein complex that has been termed the "destruction complex'', its components comprising two scaffolding proteins, the adenomatous polyposis coli (APC) and Axin in addition to ubiquitous kinases and Protein phosphatase 2A (PP2A)[35,36]. Axin can increase the phosphorylation rate of β-catenin by bringing both substrates (β-catenin and the enzymatic activities of GSK3β and CK1α) in close vicinity, enhancing effective concentrations 2400 fold[37]. Wnt signal transduction is initiated when Wnt ligand interacts with both FZD and LRP5/6 receptors and makes a bridge complex between their extracellular domains via various sites of the Wnt molecule and forms the WNT–FZD–LRP5/6 complex. The LRP5/6 receptor is then phosphorylated by the membrane-associated form of GSK3β and casein kinase I γ (CKIγ(in five repeated PPP(S/T)P motifs[38,39]. One or more of the phosphorylated LRP5/6 motifs bind with Axin, Axin sequester in the plasma membrane, and the content of Axin is reduced in the destruction complex[40]. Also, this inhibits the role of Axin in mediating β-catenin's nuclear export[41] (**Figure 1**).

In order for Axin to remain in the plasma membrane, Disheveled proteins are also required. Wnt signals are relayed from receptors to effectors with the help of Disheveled[42]. Upon Wnt-receptor binding, Disheveled rapidly becomes phosphorylated[43]. There is an amino-terminal DIX domain in Disheveled which is very similar to the DIX domain in the Axin molecule. Disheveled and Axin proteins interact with each other via the DIX domain. Also, this complex may be responsible for interaction between the FZD and LRP5/6 receptors[44]. Translocation of β-catenin from the cell membrane into the nucleus creates an increased interaction with proteins of the T-cell factor/lymphoid enhancer factor (TCF/LEF) DNA binding protein family[45] and seems to decrease their affinity to binding the family of transcriptional co-repressor proteins that include transducin-like enhancer of split/groucho related gene (TLE/GRG) family and possibly the CtBP and TCF/LEF proteins[46,47].

Accumulation of β-catenin in the nucleus leads to fundamental changes in the process of gene expression

regulation. A lot of genes are involved in this process, such as pro oncogene c-myc[48], Cyclin D1[49], several pro invasive factors including matrix metalloprotease 7 (MMP-7)/Matrilysin[50], membrane-type 1 MMP (MT1-MMP)[51], laminin-5 γ2chain[52], and CD44[8]. Also, β-catenin controls the expression of EphB2/EphB3 receptors and their ligand Ephrin B1, which plays a role in stem cell proliferation, differentiation, migration and also plays a role in the sorting of stem cells along the crypt-villus axis into their correct position[53].

Feedback control in the Wnt signaling pathway is important in order to limit the signal leading to tumor development[54,55]. Various negative and positive feedback loops have been described. Ectopic activation of the special promoter, lymphoid enhancer-binding factor 1 (LEF1) (an isoform of full-length LEF which possesses a binding site for β-catenin) creates a positive feedback[56]. However, it is important to note that this promoter differs from the intrinsic promoter that activates the negative isoform. Besides, activation of most abundant transcription factor Tcf1 isoforms that encode a protein which lacks the β-catenin interaction domain

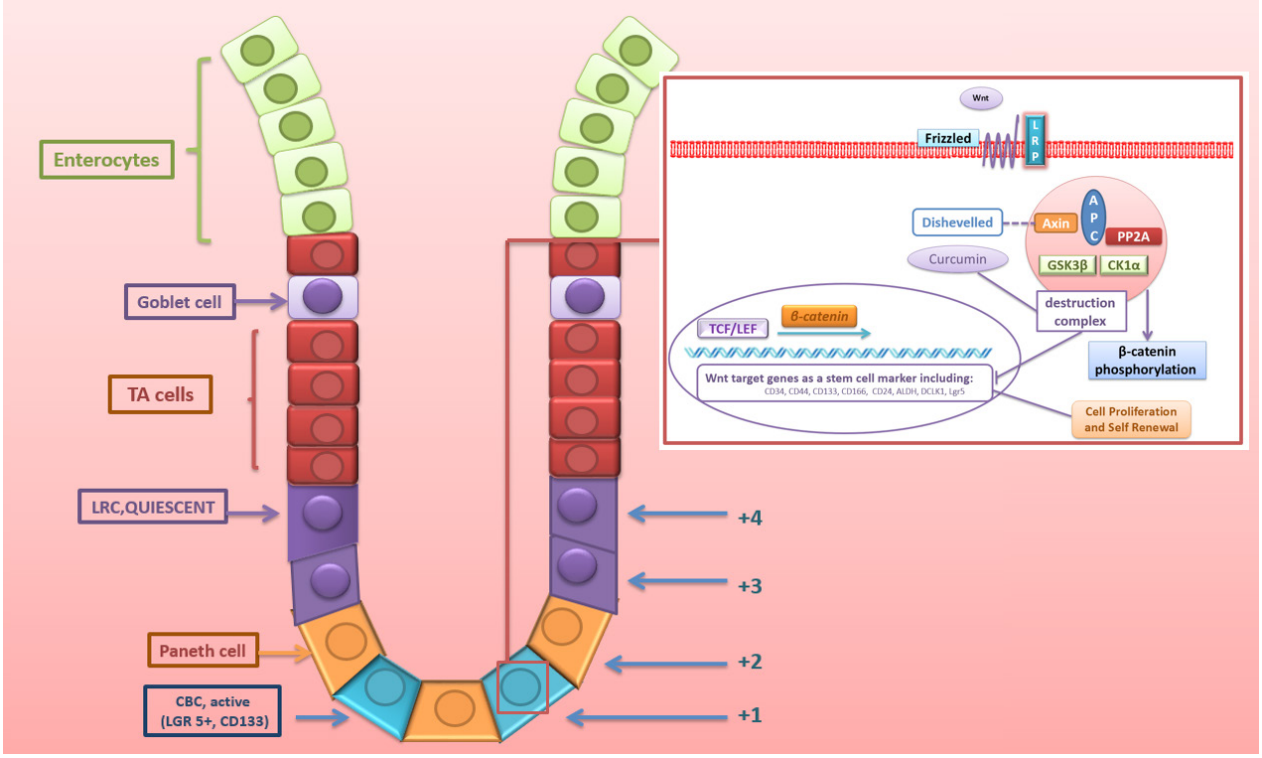

Figure 1. Illustration of the intestinal stem cells (ISCs) and Wnt signaling pathway. Active ISCs (blue) are located at the firth position bellow the Paneth cells, as measured from the crypt base. CRC is associated with aberrant activation of the Wnt signaling pathway in active ISCs. Curcumin suppresses the Wnt pathway in ISCs and prevents cell proliferation and self-renewal. TA: Transit-Amplifying; LRC: Label-Retaining Cells; CBC: Crypt Base Columnar; LRC 5+ (Leucine Rich Repeat Containing G Protein-Coupled Receptor 5); APC: Adenomatous Polyposis Coli; CK1α: Casein kinase 1α; GSK3β: Glycogen Synthase Kinase 3 Beta; PP2A: Protein phosphatase 2A; TCF/LEF: T-cell factor/lymphoid enhancer factor.

generally act as negative feedback loops[57]. Similar to Axin, Axin2/Conductin which expresses in a restricted manner can promote negative feedback of the Wnt pathway by phosphorylation and degradation of β-catenin[58-60]. Naked cuticle (Nkd) seems to exert negative feedback inhibition via binding to the PDZ domain of Disheveled[61]. Furthermore, EphB2/3 and ITF2 which are Wnt target genes repress tumor progression, and their inappropriate expression correlates with stage of malignancy[54,55].

Moreover, in relation to the molecular mechanism of R-spondin, it has been shown that cell surface transmembrane E3 ubiquitin ligase Zinc and Ring Finger 3(ZNRF3) and its functional homolog Ring finger protein 43 (RNF43) are negative feedback regulators of Wnt signaling[62,63]. ZNRF3 and RNF43 which express by β-catenin binding to TCF transcription factors degrade Wnt receptors Frizzled and LRP6 via the Dishevelled protein[64]. Also the R-spondin protein family (RSPO1-4) create a positive feedback by sensitizing cells to low doses of Wnt proteins[65,66]. R-spondin recognizes LGR4/5/6 (Leucine Rich Repeat Containing G Protein-Coupled Receptor) as a receptor, and binds with ZNRF3/RNF43, inducing ubiquitination and degradation of ZNRF3/RNF43[67-69]. In general, R-spondin-ZNRF3/RNF43 signaling plays a critical regulatory mechanism in the Wnt pathway, therefore mutations that decrease RNF43/ZNRF3 or lead to overexpression of R-spondin can enhance the risk of cancer[70].

Intestinal stem cells

The gastrointestinal epithelium is a dynamic system which is continuously renewed every 4–5 days. The renewal process is controlled by multipotent stem cells which reside at the base of the intestinal crypts. These stem cells are fast-cycling crypt base columnar (CBC) cells which are dispersed between paneth cells at the crypt base[71]. CBCs produce the transit-amplifying (TA) progenitors cells, divide 4-5 times and migrate from the crypt bottom to the top of the villus and eventually differentiate into four primary epithelial cell types including absorptive, goblet, endocrine, and paneth cells, which, unlike the other cells move toward the bottom of the crypt[72]. CBCs are accountable for maintaining epithelial homeostasis in healthy conditions. It is assumed that Lgr5 which is a G-protein-coupled receptor and a target gene of Wnt β-catenin signaling is a marker of CBC cells[73]. Each crypt has 14-16 Lgr5 positive cells[74]. The division period in these cells lasts 21.5h and the mitosis process is symmetric with accidental distribution of DNA to daughter cells[75]. Accumulated evidence points to several additional markers of CBC stem cells such as Ascl2[76], sox9[77], and Olfactomedin4 (Olfm4) which are Wnt target genes[76]. Some other markers of CBC stem cells are Smoc2[75], Rnf43[63], Znrf3[78], Cd24[79], Cd44[80], Cd133[81], Cd166[82]and Musashi-1[83] (**Figure 1**). Another specified population of intestinal stem cells exists at the 4+ position of the crypts, which are inactive under intact conditions with slow cycling. There are 4-6 ISCs in this position, which divide every 24 hours(84). These cells are independent of the Wnt/β-catenin signaling pathway[85] and are termed label-retaining cells (LRC)[85], which create a stock of ISCs for regeneration after injury[86]. Relevant factors used to identify these cells are marker genes: Bmi1, HopX, mTERT and Lrig1[87-89]. Cell division in LRCs is asymmetric, and parental DNA strands were appropriated to larger offspring cells, while the newly synthesized strands were allocated to the smaller offspring cell. Hence, the larger cells possess stem cell properties and the smaller cells become TA progenitors for replacing injured cells in the epithelium[90]. The Lgr5 positive and Bmi1positive stem cells can convert into each other in order to maintain the quantity of stem

cells[91].

Stem cells lie on a microenvironment comprising niche cells. There are two important cell lineages in niche microenvironment; paneth cells and pericryptal myofibroblasts. Wnt signals are secreted from niche cells for ISCs and there is a gradient of Wnt signaling along the crypt-villus axis[92]. It is reported that Paneth cells secrete Wnt3 -which is a ligand for LRP5/6 and Frizzled receptors on the surface of ISCs- which leads to upregulation of c-Myc expression[93,94]. It has also been reported that myofibroblasts are capable of secreting R-spondin1 which couples to receptors Lgr4/5, therefore increasing LRP6 on the cell surface and sensitizing ISCs to Wnt3[95,96]. Increasing evidence suggests that Wnt signals are not only important for ISCs proliferation, but also for their differentiation into mature Paneth cells. In addition it has been predicted that the Wnt pathway has a regulatory transcriptional role in Eph/Ephrin signaling[97]. Moreover, other factors such as bone morphogenetic proteins (BMP), antagonists gremlin1 and gremlin2 and Notch signaling pathways can affect ISCs behavior[98,99].

Curcumin and Cancer Stem Cells and the Wnt Pathway Involved

CSCs play a vigorous potential role in tumor initiation, recurrence and metastasis[100]. They are not only more resistant to traditional anticancer therapies in comparison with differentiated cells, but they also secure themselves by producing different ingredient, leading to a significant increase in their numbers following chemotherapeutic treatment[101]. Hence, targeting these cells and the cell signaling involved in their proliferation and growth can introduce promising approaches in cancer treatment.

Recently it has been reported that curcumin has the convincing potency to target CSCs in CRC by influencing the CSC self-renewal pathways, and in particular, the Wnt/β-catenin signaling pathway[102,103]. Also, it has been suggested that a combination of curcumin and traditional anti-cancer regimes may be capable of yielding better results in cancer treatment and in conquering tumor resistance[104,105]. Common cell surface markers used to identify cancer stem cells(CSCs) are CD34, CD44[106], CD133[107], CD24, aldehyde dehydrogenase activity(ALDH)[108], DCLK1 and Lgr5[109], some of which are Wnt target genes. Other hallmarks of CSCs are their capacity to form spheroids and holoclones in culture[110]. It has been reported that curcumin has the capability to inhibit sphere-forming ability in cultured stem cells that possess CSC properties in a conditioned medium[111-114].

Incubation of CRC cell lines with 5-fiuorouracil and oxaliplatin which are standard colon cancer therapies in the form of monotherapy or combination therapy, resulted in an increased expression of Wnt regulated cell surface markers CD133, CD44 and CD166, suggesting an increase in cancer stem cells. Addition of curcumin to these drugs decreased their ability to form a sphere and therefore the expression of cancer stem cell markers. Hence, combination therapy alongside curcumin can be more effective in order to eliminate cancer stem cells[113]. In another study, ALDH1 which is regulated by 4e-Catenin/TCF activity[108] was used as a marker for stem cells. Exposure of HCT116 to oxaliplatin increased ALDH1 activity in resistant HCT116 cells. Combination of curcumin with oxaliplatin inhibited the activity of ALDH[115]. It has also been reported that combination of curcumin with dasatinib (SRC inhibitor anti-cancer drug)[116] to treat FOLFOX resistant HCT-116 and HT-29 cell lines decreased the expression of ALDH1, CD44, CD133 and CD166 by

about 80-90% and inhibited colony and colonsphere formation[112]. The authors suggest that curcumin may be utilized to enhance the effectiveness of targeted cancer drugs. Also, this study indicates that curcumin can lessen the dasatinib dosage and therefore reduce its toxicity[112]. Also, curcumin downregulates DCLK1, CD44, ALDH1, Lgr5 and Nanog expression in colon cancer stem cells and halts spheroid culture and tumor xenograft growth[117]. In addition Kakarala et al showed that curcumin decreases the percentage of ALDH1 positive cells that were capable of mammosphere formation. In addition, the wnt signaling involved was assessed by utilizing the TCF-Lef Topflash reporter system and a potent inhibitory effect on Wnt signaling by curcumin was seen[111].

Kim et al assessed novel data about the effects of curcumin on Lgr5 positive stem cells in azoxymethane (AOM) induced tumor. Their evidence suggests that curcumin reduces AOM-induced nuclear β/catenin levels in aberrant crypt foci and promotes apoptosis in damaged Lgr5 positive stem cells. DNA-damaged Lgr5 positive stem cells were more sensitive to curcumin in comparison with DNA-damaged differentiated cells. As a result they suggest that elimination of damaged Lgr5 positive stem cells by dietary factors can be a therapeutic strategy to reduce the risk of colon cancer[118].

Novel formulations of curcumin have better bioavailability than free curcumin[119]. Wang et al studied encapsulated curcumin (CSO-SA micelles) in comparison with free curcumin and empty CSO-SA. Curcumin-loaded CSO-SA micelles possessed more stability and efficiency. The expression rate of CSCs marker CD44+ and CD24+ markers were inhibited and spheroid formation was suppressed in vitro and in vivo[120]. Difluorinated-curcumin (CDF) -another new formulation of curcumin- was used to treat FOLFOX-resistant colorectal cancer stem cells. CSCs which were chemo-resistant were treated with a combination of CDF and FOLFOX, which led to significant inhibition of CSCs in comparison with free curcumin and FOLFOX[121].

Curcumin, CRC and Clinical Trials

The effects of curcumin in colorectal cancer have been studied in clinical trials during the past 25 years (**Table 1**). These studies address the pharmacokinetics, pharmacodynamics, safety and effective dose of this component. A few of them will be mentioned here. A pharmacodynamics and pharmacokinetic study of oral curcuma extract was performed in 15 patients with colorectal cancer. Curcuma extract was administered safely at doses of 440 to 2200 mg once a day for a period of 4 months which included 36-180 mg of curcumin. No toxicity was observed and all doses were tolerated by patients. Curcumin and its metabolites were not found in the plasma or urine. Levels of leukocytic M1G remained constant and were not affected by treatment, but a 59% decrease in lymphocytic glutathione S-transferase (GST) activity was seen following consumption of 440 mg for 29 days, which was not observed at higher doses. After 2 months of treatment, the venous blood CEA levels of one patient who had consumed 440 mg of extract had decreased by about 40%[122]. A dose escalation study of curcumin at doses of 0.5-12g in patients with preneoplastic lesions for a period of 3 months did not show any toxic effect of curcumin up to 8g per day. Patients did not accept doses of 12 g/day due to high volume pills. The serum concentration of curcumin was undetectable in doses of less than 4 g. At higher doses serum peak curcumin concentrations were seen 1 to 2 hours after ingestion

Table 1: Current Clinical Trials Investigating the Use of Curcumin in Colorectal Cancer

Clinical Trials. gov Identifier	status	Study title	Intervention	Condition
			Curcumin 500 mg twice per day for 2 Weeks.	
NCT02724202	Recruiting	Curcumin In Combination With 5FU For Colon Cancer	Curcumin At Same Dose For An Additional 6 Weeks plus 3 Cycles Of 5FU. p	Metastatic Colon Cancer
			Curcumin 3.6 Gram Daily for 7 days	
NCT01294072	Active, Not Recruiting	Study Investigating The Ability Of Plant Exosomes To Deliver Curcumin To Normal And Colon Cancer Tissue	Curcumin Conjugated With Plant Exosomes for 7 days No Intervention	Colon Cancer
NCT00295035	Unknown	Phase III Trial Of Gemcitabine, Curcumin And Celebrex In Patients With Metastatic Colon Cancer	Celecoxib Curcumin	Colon Neoplasm
NCT00027495	Complete	Curcumin for the Prevention of Colon Cancer	Dietary Supplement: Curcumin	Colorectal Cancer
NCT00973869	Unknown	Curcumin In Preventing Colorectal Cancer In Patients Undergoing Colorectal Endoscopy Or Colorectal Surgery	Dietary Supplement: Curcumin	Colorectal Cancer
NCT00365209	Completed Has Results	Phase II A Trial Of Curcumin Among Patients With Prevalent Subclinical Neoplastic Lesions (Aberrant Crypt Foci)	Laboratory Biomarker Analysis Pharmacological Study Drug: Curcumin	Healthy No Evidence Of Disease Tobacco Use Disorder
NCT01490996	Ongoing	Combining Curcumin With FOLFOX Chemotherapy In Patients With Inoperable Colorectal Cancer (CUFOX)	Drug: Oral Complex C3 Curcumin + Chemotherapy Drug: Chemotherapy Only	Colon Cancer Metastasis
NCT01948661	Recruiting	Anthocyanin Extract And Phospholipid Curcumin In Colorectal Adenoma (MIRACOL)	Dietary Supplement: Mirtoselect® + Meriva®	Colorectal Adenoma Risk Reduction
NCT01859858	Active, Not Recruiting	Effect Of Curcumin On Dose Limiting Toxicity And Pharmacokinetics Of Irinotecan In Patients With Solid Tumors	Dietary Supplement: Curcumin Drug: Irinotecan	Advanced Colorectal Cancer

Table 1: Continue...

Clinical Trials. gov Identifier	status	Study title	Intervention	Condition
NCT00745134	Active, Not Recruiting	Curcumin With Pre-Operative Capecitabine And Radiation Therapy Followed By Surgery For Rectal Cancer	Drug: Curcumin Drug: Placebo Radiation: Radiotherapy Drug: Capecitabine	Rectal Cancer
NCT00176618	Terminated	The Effects Of Curcuminoids On Aberrant Crypt Foci In The Human Cancer	Drug: Sulindac (150 Mg Po Bid) Drug: Curcumin (250 Mg Po Bid)	Aberrant Crypt Foci
NCT00118989	Terminated	Curcumin For The Chemoprevention Of Colorectal Cancer	Dietary Supplement: Curcuminoids	Adenomatous Polyps
NCT00248053	Withdrawn	Use Of Curcumin In The Lower Gastrointestinal Tract In Familial Adenomatous Polyposis Patients	Drug: Curcumin	Familial Adenomatous Polyposis
NCT02439385	Enrolling By Invitation	Avastin/FOLFIRI In Combination With Curcumin In Colorectal Cancer Patients With Unresectable Metastasis	Drug: Avastin/FOLFIRI Dietary Supplement: Curcumin	Colorectal Cancer
NCT01333917	Completed	Curcumin Biomarkers	Curcumin C3 Tablet	Colorectal Cancer
NCT00641147	Completed	Curcumin In Treating Patients With Familial Adenomatous Polyposis	Drug: Curcumin Other: Laboratory Biomarker Analysis Other: Placebo	Familial Adenomatous Polyposis
NCT03061591	Not Yet Recruiting	Turmeric Supplementation On Polyp Number And Size In Patients With Familial Adenomatous Polyposis	Wholistic Turmeric capsules Other: Placebo	Familial Adenomatous Polyposis FAP Gene Mutation
NCT03122613	Not Yet Recruiting	Curcumin Versus Placebo For Prevention Of Relapse In Patients With Ulcerative Colitis	Dietary Supplement: Curcumin Drug: Placebo	Familial Adenomatous Polyposis FAP Gene Mutation

and decreased gradually within the next12 hours[123]. In a phase I clinical trial, curcumin at doses of 0.45 to 3.6 g was administered to 15 patients with advanced CRC who were refractory to chemotherapy for a duration of 4 months. Curcumin treatment was well tolerated, and there was no toxicity at any doses. Although oral curcumin does not affect malignancy by changing tumor markers or serum cholesterol, but it does however decrease inducible prostaglandin E2 (PGE2) and inhibit cyclooxygenase-2 (COX-2) activity[124]. In another study, curcumin capsules (3600, 1800, or 450 mg daily) were ingested by patients with CRC daily for 7 days. Curcumin at a dose of 3600 mg decreased the level of M1G (pyrimido [1, 2-a] purin-10(3H)-one) significantly, but the level of COX2 was not affected in malignant colorectal tissue. The study showed that the ingestion of 3.6 g curcumin daily is pharmacologically efficacious. Trace levels of curcumin showed negligible distribution in peripheral circulation and outside of the gut[125]. Also in one study 126 patients with CRC were treated by 360 mg curcumin daily, and results were compared with a placebo group. Results in the group receiving curcumin treatment comprised increased body weight, diminished serum TNF-alpha level and improved apoptotic pathway in cancer cells. Moreover, the general health in patients had improved through the mechanism of enhanced expression of the p53 molecule in tumor tissue[126]. The effects of oral curcumin on aberrant crypt foci (ACF) was assessed in a phase IIa clinical trial. Curcumin at doses of 2 or 4 g per day for 30 days was administered to 44 eligible smokers with 8 or more ACF. Curcumin was well tolerated at both doses. Although curcumin did not have any effect on PGE2, 5-HETE and Ki-67 in ACF or normal mucosa, ACF numbers were significantly reduced in patients receiving the 4 g dose, accompanied by an increase in post treatment plasma curcumin/conjugate levels[127]. Moreover in a clinical pilot study curcumin C3 complex (2.35 g) was administered once daily for 14 days to 24 patients who had colorectal endoscopy or surgical resection in their treatment program. Curcuminoids were detected in all urine samples, 9 out of 24 plasma samples and 23 colon mucosa biopsies. Curcumin glucuronide which is the major conjugate form of curcumin was detected in 29 out of 35 biopsy samples. Active levels of topical curcumin were detected in colonic mucosa after multiple tissue washes. Also, the absence of systemic accumulation confirmed the long term safety of curcumin consumption[128]. Inflammatory bowel diseases such as ulcerative colitis and Crohn's disease can increase the risk of colorectal cancer among patients[129]. There is a lot of evidence that curcumin has anti-inflammatory effects[130]. The effects of curcumin in inflammatory bowel diseases has been investigated in clinical trials. In a placebo-controlled double-blind study of curcumin, 10 patients with either ulcerative colitis or Crohn's disease received 1.11 or 1.65 g daily for 2 or 3 months. The results suggest that curcumin in combination with conventional drugs can be used in the clinic for treatment of ulcerative colitis and Crohn's disease and can delay disease progression[131]. Similarly, the effects of curcumin on prevention of relapse of patients with quiescent ulcerative colitis were assessed in a double-blind, placebo-controlled trial. Ingestion of 2g curcumin twice daily in addition to sulfasalazine or mesalamine for 6 months showed significant improvement of the clinical activity and endoscopic indices compared to the placebo plus sulfasalazine or mesalamine group[132]. In a placebo-controlled pilot study 23 patients with distal ulcerative colitis received NCB-02 (standardized extract of Curcuma longa with 72% curcumin) enema plus oral

5-ASA and their results were compared with 22 patients who received placebo enema plus oral 5-ASA. After 8 weeks of treatment, the outcomes of clinical remission and improvement on endoscopy were significantly better in the NCB-02 arm[133]. These results hint that there is no need to worry about the safety of curcumin, but due to the small sample size in these studies, it is difficult to introduce curcumin as a chemopreventive agent in CRC.

CONCLUSION:

Wnt signaling is an important pathway in the regulation of ISCs homeostasis, and any disturbance in the regulation of the expression of the genes involved in this pathway may lead to adverse events. Its major role in homeostasis of ISCs and development of CRC has been a focus of CRC treatment studies, so that several agents have been developed to target this pathway. Recently the use of plant compounds in cancer studies has been considered. The anticancer effects of curcumin have been shown in various studies. This compound has now entered the clinical phases of evaluation (**Table 1**). Evidence suggests that curcumin can targets the Wnt pathway in intestinal CSCs and inhibit their proliferation and resistance to chemotherapy. The advantage of curcumin compared to chemotherapeutic agents is its asymmetrical effect on normal and cancerous cells. Therefore, although curcumin inhibits the proliferation of cancer cells, it can be used without harm, as has been confirmed by its use as a culinary spice for hundreds of years. However, the discovery of anticancer mechanisms of plant compounds can provide novel cancer therapeutic strategies.

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