Regulatory Pathways of Colorectal Cancer and Their Synergistic Cross-talk Mechanism

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Abstract

Context: Cancer is a leading cause of death in the human population; it ensues from the accumulation of damage to genetic material and affects various organs. This review focused on the cell signaling cross-talk mechanisms of colorectal cancer (CRC) and their regulation. Genomic instability acts as the major driving force behind CRC. The major CRC cascade mechanisms such as the Wnt, Ras, TOPK, p53, and ubiquitin pathways are discussed in this review. These interlinked signals cross-talk with one another in various regulatory mechanisms, fulfilling a unique role in the development of CRC.
Evidence Acquisition: The major cross-talking signals of CRC comprise the most significant part of this review. Wnt is a resource situated at the center of the axis for cross-talk and interlinked signaling mechanisms. Wnt/β-catenin signaling is regulated by the frizzled receptor, co-factors, Ras, TOPK, and many other mechanisms; the studies pertaining to CRC were collected through a literature survey before being categorized using a number of keywords. The highly specific pathways interlinked with Wnt were identified as major targets in this review.
Results: The interlinked signaling pathways and gene networks were explained in terms of their specific roles in CRC. We highlighted the major regulatory signaling and interlinked pathways of CRC as novel therapeutic targets. Furthermore, we discussed the potential target genes, biomarkers, and therapies for CRC patients. By highlighting the gene cross-talking signaling cascade, we provided the source for gene network interaction and targeted therapy.
Conclusion: This study paves the way for multi-targeting of interlinked pathways, which would be perfect for suppressing CRC. The signaling pathways discussed in this review are not only focused on CRC but also act as potential targets and biomarkers for other cancers. Targeting multiple interlinked pathways could be useful for developing strong biomarkers for diagnostic and therapeutic purposes.

Keywords: Colorectal cancer, Cross-talking, Wnt/β-catenin, Ras, TOPK, p53, Ubiquitin, Targets, Biomarkers

Context

Cancer is lethal to the human population, ensues from the accumulation of damage to genetic material, and affects various parts of organs. This review is focused on the cell signaling cross-talk mechanism of colorectal cancer (CRC) and its regulations. Genomic instability acts as the major driving force behind CRC. The major CRC cascade mechanisms such as Wnt, Ras, TOPK, p53, and ubiquitin pathways were elaborately discussed in this review. These interlinked signals cross-talk with one another in various regulatory mechanisms, fulfilling a unique role in the development of CRC. This review topic is more interesting and useful for the researchers who carry out their work in colorectal cancer.

Evidence Acquisition

The most significant part of this review is comprised of cross-talking signals of CRC. The author's searched regarding, cross-talking signals of CRC from the articles published in the database, PUBMED (www.pubmed.com). Wnt acts as a resource for cross-talk and interlinked signaling mechanisms. Wnt/β-catenin signaling is regulated by the frizzled receptor, co-factors, Ras, TOPK, and many other mechanisms; the studies pertaining to CRC were collected through a literature survey before being categorized using the keywords. In this review, the pathways with high specificity interlinked with Wnt were identified as major targets.

  Cancer is a leading cause of mortality among humans. Although cancer may occur at any age, it is most commonly found in the elderly. Cancer can be defined as abnormal cell formation in tissues due to a loss of the normal cell cycle regulation, giving rise to a capacity of invasion into other parts of the body. Cancer is initiated or progresses due to factors like the use of tobacco and other chemicals, exposure to radiation, infection, changes in genetic materials, immune conditions, and mutations related to hormones and metabolism (1). Cancer exerts specific impacts on each part of the body secondary to mutations in genetic material (deoxyribonucleic acid [DNA]). Rapid mutations in DNA occur as oncogenes are switched on, resulting in detectable cancers. Polyps are small, non-cancerous clumps of cells that form inside the body; however, their development into larger sizes over a period of 10-20 years most certainly leads to cancer (2). Genomic instability is a driving force for most human cancers. Genomic instability tends to increase the mutation rate by altering the genome and giving rise to tumorigenesis. The colorectum (distal colon and rectum) is the final part of the gastrointestinal (GI) system, with a function apt for processing food materials to form energy and for flushing solid waste (fecal matter) out of the body. Abnormal and uncontrolled cell growth initiated in the colorectum leads to CRC. The CRC-related genes are mostly involved in cell growth, division, survival, angiogenesis, invasion, and adhesion processes. The CRC illness state is strongly influenced by sex and gender; the mortality rate of CRC is significantly higher among males (3). This cancer results from the continuous accumulation of genetic and epigenetic alterations that lead to the transformation of the normal colonic epithelium to an adenocarcinoma. (4). Genetic alterations in the Wnt and Ras-related signaling pathways are predominantly used as the targeting genes for CRC (5). The adenomatous polyposis coli (APC) mutation is the key to early tumorigenesis in CRC. The APC genes are responsible for both sporadic and familial CRC (6). According to studies on the related cross-talk and interconnected pathways, CRC arises from a combination of three different mechanisms including chromosomal instability (CIN), the CpG island methylator phenotype (CIMP), and microsatellite instability (MSI) (7). Genomic instability has its tendency to mutate pathways like Wnt/β-catenin, MAPK/PI3K, TP53, and TGF-β. Owing to these mutations, various genes like Ras, BRaf, PIK3CA, c-Myc, PTEN, SMAD2, and SMAD4 differentially initiate their functions in cell proliferation and survival (8). In most CRC cases, initiation leads to hyper-activation of the Wnt/β-catenin pathway. Due to these mutations, deactivation occurs in APC/β-catenin (9).

Results

Finally, we conclude that CRC is a disease entity with distinct biological characteristics. The interlinked signaling pathways and gene networks were explained with their specific role in CRC. The Wnt/β-catenin pathway, as a resource for the center of the axis for cross-talk and interlinked signaling mechanisms, is considered as the most significant targeted pathway for CRC. Rather than the significant targets, we discussed about RNF146 as the new emerging target for CRC. By highlighting the gene cross-talking signaling cascade, we provided the source for gene network interaction and targeted therapy. Furthermore, we discussed the potent target genes, biomarkers, and therapies for CRC patients. We also proposed that targeting multiple interlinked pathways could be useful in developing new potential biomarkers for diagnostic and therapeutic purposes. This study paves the way for multi-targeting of interlinked pathways, suggesting that these would be perfect for suppressing CRC. The signaling pathways discussed in this review are not only limited to CRC but also may be potent targets and biomarkers for other types of cancers.

Wnt Regulation

  Wingless/Wnt signals are the major regulators of benign and malignant colorectal tumors. Wnt controls β-catenin/CTNNB1, the key modulator
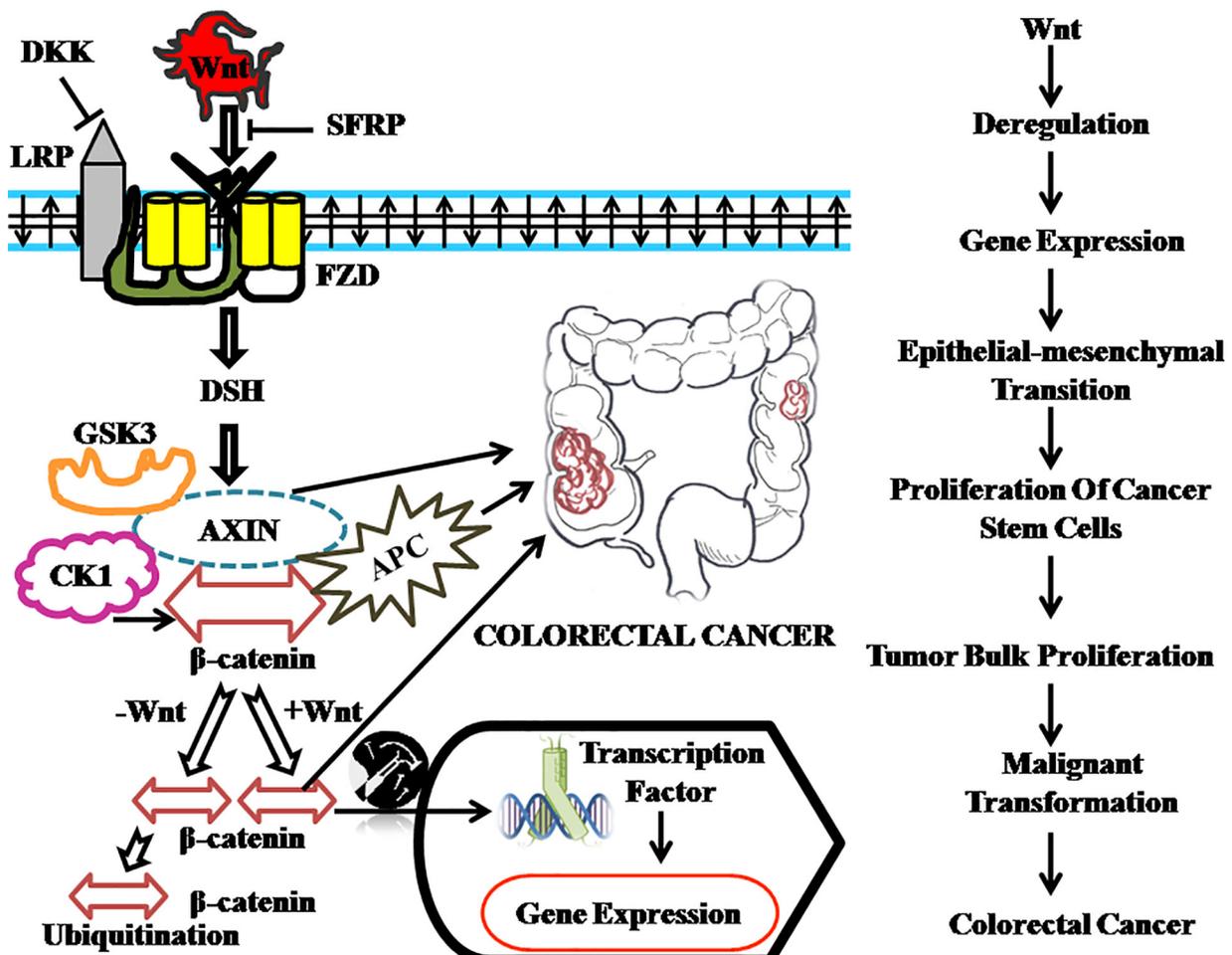
Figure 1: Deregulation of Wnt leads to colorectal cancer

for signal transduction application involving in phosphorylation and ubiquitin-mediated degradation. The Wnt signaling pathway fulfills a vital role in controlling many biological processes, including stem cell maintenance, cell fate determination, and cell proliferation. Deregulation of Wnt signaling stimulates CRC (Figure 1) (10). Genetic mutations occur in the Wnt components like APC, GSK-3β, CK1, and Axin genes as the destructive complexes of β-catenin that prompt deviant signals in most CRC cases (11). *APC* is a tumor suppressor gene that acts as a key regulator of *β-catenin*. *The* APC gene is known to be a central hub for the early stage of CRC and it negatively regulates the rapid degradation of β-catenin (12). The mutant type APC blocks the Growth arrest-specific (Gas) protein, induces the activation of β-catenin for the maintenance of homeostasis, and prevents tumor formation. In another aspect, the absence of the APC gene continuously activates Gas to bind with the differential extracellular agonist that leads to the activation and nuclear accumulation of β-catenin. The Gas activated β–catenin acts as the by-role pathway tumor formation in CRC. The Prostaglandin E2 (PGE2) hormone-like compound is a downstream mediator of COX-2 that stimulates random tumor cell proliferation via G-protein-coupled receptors (GPCRs). EP2 is the receptor of PGE2 in DLD1 cells, which inactivates mutations in the APC gene

and this results in the activation of β-catenin. The β-catenin protein is a multifunctional protein that plays a physiological role in homeostasis. It acts as a co-regulator transcriptional factor and as an adaptor protein in intracellular binding (13). Overexpression of β-catenin at the nuclear level plays a pivotal role in the worsened prognosis of CRC and acts as a marker for the late phase (14). Survivin is the stem cell marker that belongs to the Inhibitor of Apoptosis (IAP) family; it is overexpressed in CRC and is used as a direct target for β-catenin (15). The β-catenin associated with the T-cell factor/lymphoid enhancing factor (TCF/LEF), modulates the expression of target genes such as cyclin D1 and myc. Because these targeted genes are capable of affecting cell proliferation and differentiation, they lead to cancer progression (16). The overexpression of Wnt with β-catenin leads to transcriptional regulation in the c-myc and cyclin D1 gene. The c-myc gene is a gene regulator and proto-oncogene that plays a vital role in genome instability, sustenance of tumor growth, and tumorigenesis. The c-myc gene regulates gene expression in the epithelial stem cells of CRC tissues. At 70% of CRC upregulatory points, the c-myc protein exerts downstream effects in both the Wnt and Ras-dependent signal pathways. The c-myc gene acts as an attractive therapeutic target for CRC (17). The c-myc gene and Cancer Stem Cells (CSCs) are being considered as tumor initiators and

proliferators. These linked combos are capable of self-renewing and differentiation with resistance to therapies like chemotherapy and radiotherapy, and are associated with cancer metastasis. The Wnt/β-catenin pathway is implicated by playing a major functional role in CSCs (18). Cyclin D1 is a cell-cycle regulator protein that plays a vital role in cancer cell cycle progression for CRC. The cyclin D1 gene is related to sex hormones in the development of CRC. In tumors, the expression of cyclin D1 associated with CRC is higher in men compared with women. Hence, cyclin D1 should be assessed separately as a marker for men and women (19). The expression of cyclin D1 in CRC is downregulated by 3,3-Diindolylmethane (DIM), an antiproliferative compound. The downregulation of DIM via cyclin D1 triggers Endoplasmic Reticulum (ER) stress in CRC. DIM does not directly affect cyclin D1 but rather modulates its expression at the translational level (20). Phosphoinositide-dependent kinase-1 (PDK1) is a serine/threonine kinase family member that is phosphorylated by the activation of protein kinase B (PKB)/AKT and Serum/Glucocorticoid Regulated Kinase-1 (SGK). The *PDK1* gene serves as a direct downstream target for *Wnt/*β-catenin signaling. The knockdown of *PDK1* decreases the growth rate of *CRC in in-vivo conditions*. The PKB and SGK have similar structures that regulate the development of CRC. The PKB gene controls cell survival and apoptosis in CRC. The SGK1 gene regulates β-catenin in the development of CRC (21). Glycogen Synthase Kinase 3β (GSK-3β) belongs to the serine/threonine kinase family, a deregulator of the oncogenic Wnt/β-catenin pathway in the pathogenesis and oncogenesis in CRC. GSK-3β expression occurs chiefly during cell proliferation, differentiation, motility, cycle progression, and apoptosis. GSK-3β occurrence is not only involved in the Wnt/β-catenin pathway but also is a key regulator of the PI3K/AKT pathway. Hence, GSK-3β targeting is specific and attractive for chemotherapy (22). Casein kinase 1 (*CK1) has recently* emerged as a novel drug target for cancer therapy. Pyrvinium is a molecule that inhibits the growth of tumor cells and activates CK1 for β-catenin degradation (23). Protein phosphatase 2 (PP2A), with its B56 subunit, is required for the upstream Dishevelled protein (Dsh/Dvl) and downstream Wnt ligands. PP2A:B56 is a component that is responsibly involved in both canonical and non-canonical Wnt and is also used for targeting Wnt-related genes (24). The reported negative regulators of Wnt signaling inhibitors are Secreted Frizzled-Related Protein (*SFRP*) with its domains, Dickkopf Wnt Signaling Pathways Inhibitor 1 and 3 (DKK1 and DKK3), and Wnt inhibitory factor (WIF1) (25). SFRP belongs to the Cysteine-Rich Domain (CRD) family, and Frizzled-8 (FZD8) plays an important role in binding to Wnt and inhibiting its signaling. The WIF1 gene is an N-terminal signaling sequence that binds to

Wnt-3a by strengthening the interaction between Wnt and Wnt ligands. DKK1 is a protein-coding gene that interacts with the Wnt co-receptor LRP-6 by downregulating β-catenin expression and upregulating Oct4 expression. DKK3 is a tumor suppressor gene known to potentiate as well as inhibit Wnt signaling. DKK3 inhibits LRP 5/6 interactions with Wnt and forms a temporary complex with KREMEN, promoting internalization of LRP 5/6 (26). In the early stage of CRC carcinogenesis, the Wnt responsible pathways are re-organized and negatively regulated in diverse forms (27).

Frizzled Receptors

Frizzled (FZD) proteins are seven transmembrane receptors that belong to the GPCR family, implicit in Wnt signaling leading to oncogenesis in CRC. CRD is one of the crucial members of the FZD receptor, occurring at the start of the pathway and being most necessary for Wnt ligand binding. The response of FZD receptors to Wnt proteins is determined by their co-receptors, low-density lipoprotein receptor-related proteins 5 or 6 (LRP5 or LRP6), which are involved in the activation of the canonical β-catenin pathway. As a receptor of Wnt, LRP6 acts as an inhibitor of GSK3β in response to Wnt stimulation. The members of FZD receptors interact with Wnt to activate canonical and non-canonical Wnt signaling pathways. The *FZD3*, FZD6, FZD7, and FZD10 receptors play key roles in *CRC* tumorigenesis *(28, 29)*. The FZD3 protein sets a hallmark for CRC metastasis and early-stage CRC carcinogenesis. The Naked Cuticle Homologue-1 (NKD1) factor inhibits Wnt signaling by binding with the Dsh/Dvl family, which upregulates the β-catenin independent FZD3 receptor in coronary artery disease (CAD) and CRC. The expression of the FZD3 protein serves as a potent prognostic marker in colorectal tumorigenesis and progression (30). FZD3 and FZD6 expression has been identified in the early stage of tumorigenesis in adenomas; the expression of FZD6 is higher in CRC (27). The FZD6 with its ligand Wnt4 and microRNAs-199a-5p is upregulated in the development of CRC and could be a potent target (31). The FZD7 receptor is abundantly expressed in mutated APC or CTNNB1 in CRC. The FZD7 receptor activates different branches of Wnt signaling like the canonical Wnt pathway and plays an important role in the development and metastasis of CRC. The overexpression of FZD7 with mRNA occurs in most CRC cell lines in the II, III, or IV stage of tumors. Reports state that the overall survival rate of patients with FZD7 expression is terse and this overexpression is significantly associated with the tumor state (32). The FZD7 receptor that binds with small interfering RNA (siRNA) may act as a potent therapeutic reagent for sporadic CRC (33). MET expression is regulated by Wnt/β-catenin signaling; by blocking this expression, it significantly reduces the growth

of CRC cells *in-vivo*. This receptor is also suggested to be an important invention for new therapeutic targets. In CRC, Epithelial-Mesenchymal Transition (EMT) contributes to invasion and metastasis, while also being implicated in chemotherapy resistance. The expression of EMT transcription factors is induced by the interaction of Wnt signaling with EMT (34, 35). FZD7 binds to the dynamic MET and EMT, reversibly underscoring progression in CRC morphogenesis. Through Wnt–FZD receptor complexes, the EMT and MET process in CRC could be therapeutically targeted (34). FZD10, which is encoded in the region of the 12q24.33 chromosome in humans, is a positive regulator for the Wnt/β-catenin pathway and upregulates the primary stage of CRC (29). The FZD10 receptor plays a unique role in mutating CRC. The upregulation of the FZD10 gene is carried out after the progression of polyps to CRC (36). Wnt signaling with FZD interaction is the onset for tumor suppressors in CRC and is targeted for various stages of tumors like morphogenesis, invasion, and metastasis. The Wnt–FZD interaction is an additional modulation in Wnt upstream to the components of both β-catenin dependent and β-catenin independent pathways. In the canonical Wnt pathway, the dynamic regulations of CRC have a major impact, meaning that this regulation could be targeted by Wnt-FZD signaling (34).

Axin

Axin may act as both a positive and a negative

effector in the Wnt signaling pathway. It has its associated β-catenin protein, found to be interacting with its components including GSK-3β and APC. Axin also acts as an inhibitor of the Wnt signaling pathway that interacts with β-catenin to activate CRC (9). We have reported that Axin regulates the ON/OFF mechanism in the Wnt pathway. The interactions of the Axin protein by its ubiquitinated RNF146 and TNKS regulate the Wnt signaling pathway for the degradation of β-catenin (11). The somatic mutations in Axin2 are found to be associated with defective mismatch repairs in CRC. In the Axin family, the Axin2 gene is majorly involved in the Wnt signaling pathway for upregulating CRC. (37). *TCF*/LEF is a transcription factor member that acts as a major progenitor for CRC. TCF-related target genes like TCF4 and TCF7L1 along with components of Axin play a unique and crucial role for CRC. The mutated expression of *Axin2* in CRC occurs with defects in mismatch repair and thereby activates *TCF* signaling in the development of CRC. Thus, in CRC, *TCF*-related genes are used to target Axin2 (38). Micro RNA-103/107 (miR-103/107) is a highly involved binding gene that is dependent on Axin2 to attain a negative regulation of the Wnt signaling pathway. The overexpression of miR-103/miR-107 is associated with metastasis of CRC in cell lines. MiR-103/107 targets Death-Associated Protein Kinase (DAPK), a metastasis suppressor gene, and Kruppel Like Factor-4 (KLF), a zinc finger transcription factor that potentiates cell growth, adhesion, inhibition of
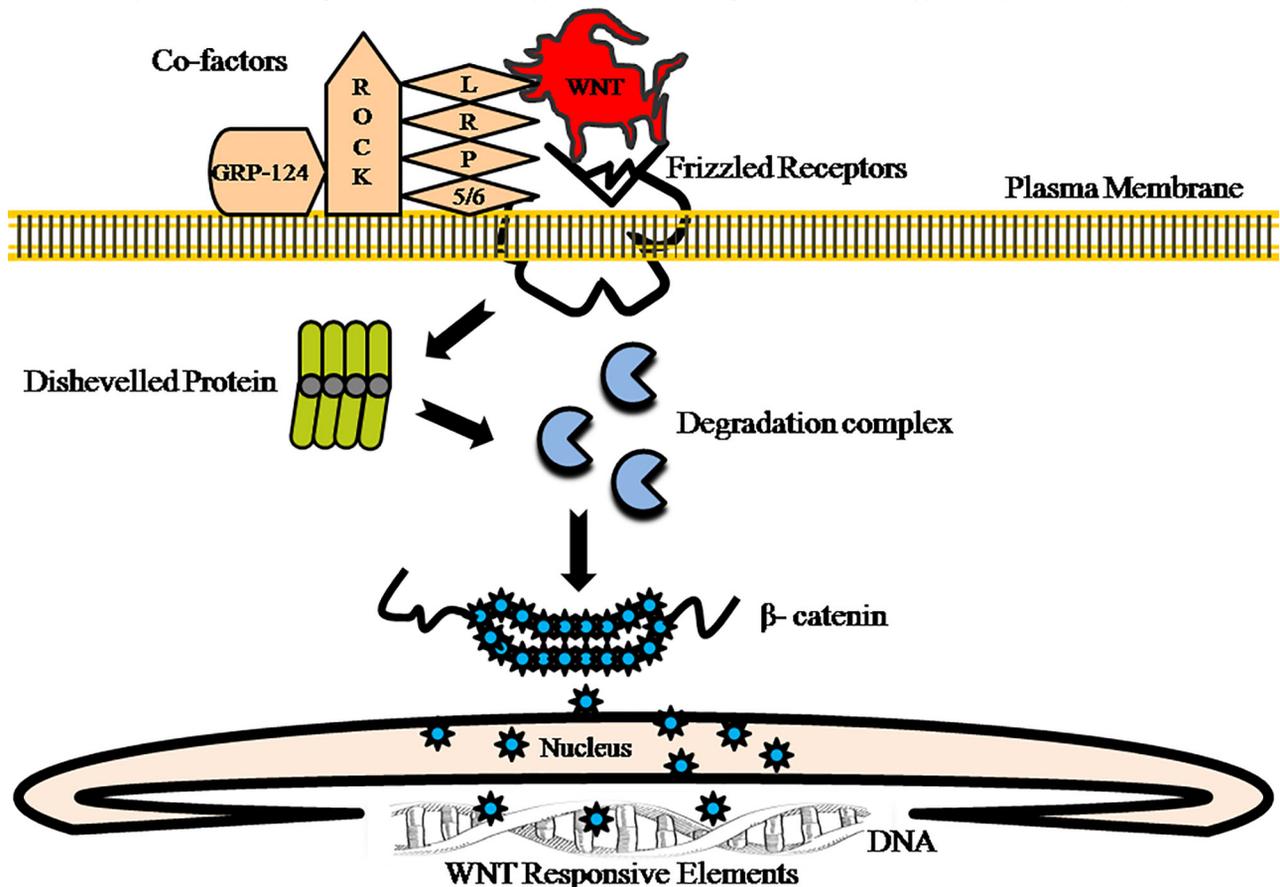

Figure 2: Wnt/ β- catenin signaling regulation by frizzled and co-factors

cell adhesion, motility, and invasion in CRC (39). Axin contains a Regulator of G-protein Signaling (*RGS*) domain, which is a negative regulator for the activation of the Wnt signaling pathway with their respective signaling proteins. RGS2 is the domain of RGS which downregulates CRC tissues, suggesting that these genes may be responsible for metastasis. Downregulation in the RGS2 gene leads to a poor prognosis rate and may act as a potent marker for evaluating patients in the second and third stages of CRC (40). Phosphoprotein Phosphatase 2A (PP2A) is a tumor suppressor protein that binds to Axin and APC. The PP2A in binding with Axin regulates the Wnt pathway in both a positive and negative manner. PP2A expression shows a poor outcome in alterations related to metastasis of CRC and acts as a novel therapeutic target for CRC (41). An overview of the Wnt/β-catenin signaling regulation by FZD proteins and their co-factors is depicted in Figure 2.

Ras Regulation

Ras is an oncogene that plays a vital role in controlling the growth of normal and transformed cells by altering their genetic transformation. Ras is a small guanosine triphosphate (GTP) binding protein that is metabolically active and acts as an important regulator of cell growth, whereas it becomes less active in the guanosine diphosphate (GDP) state. The exchange of GDP to GTP in the Ras mechanism mainly depends on the *Growth Factor Receptor-Bound Protein* (Grb2) and Son of Sevenless (SOS). Ras stimulates Raf-1 via GTP binding activation through mitogenic effectors. The Raf protein is identified as the downstream effector kinase of Ras found with its isoforms A-Raf, B-Raf, and C-Raf or Raf-1. The Raf-1 protein is a proto-oncogene serine/threonine kinase that activates MEK followed by MAPK, leading to mitogenesis (42). The interaction of Grb2 protein with Shc protein activates Ras by initiating its role in the activation of the MAPK pathway (43). On average, 98% of Ras genes associated with cancers are found as residues of G12, G13, or Q61. G12 mutations are predominantly found in alternatively spliced Kirsten murine sarcoma virus/wild type Ras (K-Ras) and Harvey murine sarcoma virus type Ras (H-Ras), while G13 mutations are predominantly found in neuroblastoma cell line type Ras (N-Ras) as the most frequent reason for the activation of human cancers. Ras cross-talk with LKB1, or APC gene, or Tumor Protein P53 (TP53), activates tumor formation and urges toward a progressive state. In spite of early genesis in Ras mutation, the evidence suggests that the rapid mutant Ras expression is necessary for tumor maintenance. Similarly to the mouse model, research carried out on the inductive mutant Ras suggests that the exodus of Ras expression promotes tumor regression (8). Gankyrin is an oncogene that mediates Ras-induced tumorigenesis by suppressing the activity of Rho-Associated-*Protein*-Kinase (ROCK). In cancers, ROCK plays a particular role in cell motility, metastasis, and angiogenesis. Gankyrin is a key regulator of Ras (mediated through AKT), inhibiting the downstream ROCK pathway and Ras-induced tumorigenesis (44). K-Ras is most frequently mutated (86%) in Ras-related cancers, followed by N-Ras (11%) and H-Ras (3%) (45). The exon 1 of codon regions 12 and 13 in K-Ras mutations activates approximately 90% of tumor initiation and progression. In the CRC condition, K-Ras is predominantly mutated (86%), N-Ras mutations are frequent at a rate of 14%, and H-Ras mutations are not detected (8). Overexpression of MET is mostly found in CRC and occurs in both K-Ras wild type and K-Ras mutant type tumors. The interlined co-operation between K-Ras signaling and MET signaling promotes the growth factors of CRC (46). Activated K-Ras is the early component of CRC in development with advanced polyps and sporadic colorectal adenomas. Deregulation in K-Ras expression leads to the activation of mutations in major signaling pathways like Ras/Raf/MAPK and PI3K/AKT. The activated MAPK together with the expression of PI3K and K-Ras activates BRaf, which in turn activates MEK (8, 47). The exons 11 and 15 are hotspots of BRaf found in serrated and hyperplastic polyps. BRaf mutations fall on microsatellite instability (MSI) and CpG island methylator phenotype mechanisms, whereas K-Ras mutations are more common in CIMP at low and microsatellite at stable tumors. BRaf is a direct target of K-Ras, and both these activate the Mitogen-Activated Protein Kinase (MAPK) pathway. K-Ras and BRaf have distinct roles in the development and progression of CRC (48). In therapy, K-Ras and BRaf are used for the prediction of Epidermal Growth Factor Receptor (EGFR) treatment, while also being useful in the prediction of the patient's prognosis in CRC. Mutations in K-Ras and BRaf are most important for future prognostic studies of CRC (8). The Phosphatidylinositol-4,5-Bisphosphate 3 and 4 (PIP3 and PIP2) signals act as downregulators for activating PI3K/AKT pathways, leading to aggressive and metastatic transformation in CRC. These signals do not directly implement their role in K-Ras activation (49). The Ras inhibitors are approved as Ras oncoproteins and are widely used as undruggable cancer targets (50).

TOPK Regulation

Lymphokine-Activated Killer T (T-LAK) cells stimulate the cytotoxic effect to kill cancer cells. T-LAK Originated Protein Kinase (TOPK) is known to be a PDZ-binding kinase (PBK), a serine-threonine kinase member identified as a human homolog of Drosophila disk large (hDlg) interacting protein and a mitotic kinase with a motif at the C-terminal. TOPK regulation of mitosis and DNA repair is intricate in cancer development and evolution. Immense expression of TOPK is tumor-specific

and leads to poor clinical outcomes in cancers, including CRC. Expression of TOPK potentiates DNA damage, p53 induction, HRas-induced cell transformation, UVB-induced c-Jun N-terminal kinase (JNK) activity, and activation of extracellular signal-regulated kinase (ERK) and PI3K/AKT (51). LRP-1 manages malignant cells in the adhesive state, which favors invasion and thereby controls the ERK and JNK dependent pathways. The specific targets of TOPK include JNK1 and ERK2, which are the positively attained loops that may contribute to tumorigenesis in CRC formation. TOPK and ERK with their combined pathways are used as potential therapeutic targets for CRC (52).

MAPKK

OPK/PBK belongs to the Mitogen-Activated Protein Kinase Kinase (MAPKK) family and regulates cellular functioning, tumor development, apoptosis, and inflammation. MAPKK is a kinase enzyme that phosphorylates the Mitogen-Activated Protein Kinase (MAPK) signaling pathway with its major components (Ras, Raf, MEK, and ERK) implicated in cell injury, disease, and cancer. The activation and targeting of few MAPK signals with LRP-1 maintain an adhesive state that favors invasion. The ERK pathway is the most important signaling cassette of the MAPK signaling pathway. ERK1 and ERK2 are responsible for the occurrence of deregulation in transcription factors. The interlinked MAPK and ERK (MEK) are modules of the Raf-Ras family that onset its role on cancer. ERK-Ras activates the essential oncogenic signals that promote cell growth and development in cancer (53). MEK suppresses the proliferated ERK1/2 signals and activates Forkhead Box O3 (FOXO3). Activation of FOXO3 interacts with Murine Double Minute 2 (MDM2), giving rise to cell multiplication and tumorigenicity (54). In another aspect, ERK1/2 signals regulate the activity level of B-cell lymphoma 2 (Bcl-2) family-related proteins such as pro-apoptotic protein BIM and anti-apoptotic protein MCL-1, thereby promoting the survival of cancer cells (53, 55). In a recent study, the interlinked Ras/Raf/MEK/ERK and PI3K/AKT/mTOR signaling pathways were identified as promising therapeutic targets for cancer therapy (56).

Interleukin 8 (IL8)

Interleukin 8 (IL8) belongs to the Chemokine Receptors (CXC) family, an autocrine factor otherwise known as CXCL8. CXCL8 is used as an independent prognostic marker for colon cancer. CXCL8 robustly associates with CRC by promoting tumor growth and invasion and inducing tumor cell proliferation and migration. In CRC, the macrophages and monocytes secrete CXCL8 to regulate the angiogenesis process. CXCL8 negatively interacts with anoikis in CRC cells by potentiating cross-talk multiple signaling pathways like PI3K or AKT and Raf or MEK or ERK. The combination

of CXCL8 and TOPK provokes higher expression for trounce prognosis (57). CXCL8 links up with CXCR1 and CXCR2 receptors, initiating its role in the pathogenesis of inflammatory bowel disease (IBD) and tumor genesis (58). With the utterance of CXCL8 via its correlated genes like CXCL1-3 and CCL20 (Chemokine Ligand 20), the physiological ligands are specifically found to be higher in the metastasis stage of CRCs. Targeting the CXCL1, CXCL7, CXCL12, RELA, DARC, IL1B, IL1A, ARRB1, GRK6, VASP, GTF3A (AP2), PP2A, MMP9, EGFR, JUN and FOS genes via small drug molecules could be a coherent way for manipulating the CXCL8, CXCR1, or CXCR2 signaling pathways (59). CCL20 is the sole receptor of CCR6; both mutually upregulate the colorectal malignancy expression in CRC. *In-vitro* studies have reported CCL20 to be the stimulant that specifically activates ERK-MAP kinase to promote cell proliferation and migration in CRC. The co-expression of CXCL8 and CCL20 strengthens the activation of PI3K or AKT and Raf or MEK or ERK1/2 pathways to promote cell proliferation, cell migration, and cell invasion in CRC tissues (60). CCL20 and CXCL8 signals discretely have diverse roles in initiating tumorigenesis in CRC; co-expression of these signals induces EMT, which promotes CRC metastasis and progression (61).

PI3K/AKT/mTOR

Phosphatidylinositol-3-kinase (PI3K) signaling is the most important intracellular pathway that regulates the effector pathway of the EGFR and many other cellular functions. PI3K is a key regulator of cell growth, cell metabolism, and angiogenesis; it is considered as the master regulator of cancer. The PI3K catalytic subunit p110α is most frequently mutated in CRC. The PIK3CA (Phosphatidylinositol-4,5-Bisphosphate 3-Kinase Catalytic Subunit Alpha) gene encodes the p110α subunit of PI3Kα and belongs to class IA of the PI3Ks. PIK3CA is a protein-coding gene that increases the risk of CRC mortality. The PI3K aliases p110α, p110β, and p110δ are found to be Ras-binding domains that affect the Ras-related molecules differently and lead to mutagenesis. The p110δ subunit has functions in cell regulation, homeostasis, B and T cell activation, and development (62). PIP2 and PIP3 act as signal transducers for both intracellular and extracellular components that are downregulated by the activation of PI3K/AKT signals. This downregulation in PI3K/AKT signaling happens strongly due to p38 and c-myc. Both these pathways are involved independently in cell death and cell survival, resulting in apoptosis (63). AKT activation leads to an increase in β-catenin expression and plays a key role in tumorigenesis. GSK-3β occurrence is not only involved in the Wnt/β-catenin pathway but also is a key regulator and acts as a downstream substrate for PI3K/AKT. Activation of PI3K is identified as

a potential mechanism for resistance to therapies like radiation, chemo, hormonal, and various other targeted therapies. In AKT, Neuregulin 1 (NRG1)-mediated ERBB3 activates anti-apoptotic signals in CRC cells. ERBB3 expression is structurally related to EGFR and is described as the primary or metastatic CRC (22, 64). The long non-coding RNA region interacts with PI3K signaling in CRC upregulation at AB073614, Linc00659, Linc01296, PlncRNA-1, and RNA-422; downregulation takes place at RP4, GAS5, and RNA-422. PIK3CA mutation in CRC is less compared to its two main hotspots helicase domain exon 9 and kinase domain exon 20. In comparison with these PIK3CA subunits, exon 9 has the most signal frequency and plays a more important role than exon 20 in CRC mutation. The PI3K/AKT/mTOR pathway is dysregulated in almost all human cancers, including CRC, which emphasizes the value for targeting this pathway as a potential therapeutic in the treatment of cancer (64).

Regulation of p53

Protein 53 (p53) is a phospho-protein of the cellular Simian Virus 40 (SV40) that acts as a large T-antigen binding protein and stress inducer for transcription factors. The p53 or tumor suppressor gene (TP53) encodes a transcription factor that regulates the expression of genes involved in apoptosis, angiogenesis, cell cycling, and genome maintenance in cancers. The TP53 gene located on chromosome 17p consists of 10 introns and 11 exons. Mutations in the p53 gene are found in approximately 50% of CRCs, while most mutations occur in exons 5–8. Regulated p53 genes are responsible for cell cycle arrest, which involves Murine Double Minute 2 (MDM2), potent cyclin-dependent kinases inhibitor (P21), cyclin G, and Growth Arrest and DNA Damage Factor (GADD-45). The deregulation of TP53 is the most frequent feature in the aggressive and metastatic transformation of CRC. This dysfunction of p53 mutation occurs in approximately 40% to 50% of cases related to progression and sporadic CRC. In fact, p53 plays a potent role in tumor suppression and apoptosis, also acting as a prognostic marker. The remaining 50% of human cancers hold the p53 wild type (WT) of oncoprotein MDM2, a negative regulator of p53 that augments cell metastasis and accelerates cancer progression. When p53 is ubiquitinated by MDM2, E3 ubiquitin-protein ligase leads to proteasomal degradation by inhibiting tumor suppressor activity. MI-43 is an MDM2 inhibitor that induces cell cycle arrest and cell death/apoptosis in p53 targeted genes including p21, NOXA, and PUMA. The p21 gene is encoded with the WAF1 gene (that belongs to the CDK inhibitor family) and leads to progression in the cell cycle. The p21 protein is crucial in promoting cancer cell motility and tumor formation. The expression of p21 with p53, activates the wild type p53, relating its response with DNA damage. In another

scenario, inactivation of the p53 mutation leads to the rapid triggering of the p21 gene, activating cell proliferation in an uncontrolled manner. This p21 triggering is important for the activation of the PI3K/AKT and c-myc signaling pathways. The alteration in the p21 gene induces cancer vulnerability to therapies like targeted therapy, radiotherapy, and chemotherapy (65). TOPK interacts with p53 and promotes tumorigenesis by inhibiting p53 functions (52). Leukemia Inhibitory Factor (LIF) is one of the important targeted genes related to the p53 protein; LIF initiates its role in downregulating p53 functions in CRC and suppresses the tumor rate. LIF negatively regulates p53 by activating the Signal Transducer and Activator of Transcription 3 (Stat3) and inducing the *Inhibitor of DNA Binding 1* (ID1). ID1 is a regulator of cellular growth, cell senescence, cell differentiation, apoptosis, angiogenesis, and neoplastic transformation. The ID1 gene upregulates MDM2 (a negative regulator of p53) and promotes protein degradation (66). BAX, an apoptosis regulator gene, interacts with p53 and plays a crucial role in CRC progression and chemotherapy response. Dysregulation of these gene expressions was found to be significant in CRC pathogenesis. The BAX gene has its pro-apoptotic protein Bcl2-related Ovarian Killer (BOK), which is used in CRC as a prognostic marker. NOXA, known in Latin as "damage", is the only family member of BH3 that binds to MCL1 and BFL1, being involved in NOXA-dependent p53 induces apoptosis and tumorigenesis. The interacting of another Bcl2 domain as a stimulator resistant to drugs with the NOXA gene prevents NOXA-mediated cell death/apoptosis. The *p53 Upregulated Modulator of Apoptosis* (PUMA) is a novel gene identified from the expression of p53 and used as a biomarker for measuring the tumor response in CRC. The PUMA protein promotes mitochondrial translocation in interaction with BclXL and Bcl2 through the BH3 family, promoting DNA damage and leading to rapid expression of apoptosis in CRC (55, 67). Cyclin B1 is a key regulator for the genesis of mitosis found in most malignancies; it regulates the invasion mechanism and is used as a novel target against metastasis of CRC. The interaction of cyclin B1 and E-cadherin suppresses the rate of invasion and metastasis in CRC cells. Deregulation of cyclin B1 potentiates its function in CRC progression (68, 69). The p53-inducible ribonucleotide reductase small subunit-2 (p53R2) is a direct target for the p53 gene and is the tumor suppressor located at the 8q23.1 chromosome region. The p53R2 expression in response to DNA repair induces G2 arrest while DNA damage occurs and provides a DNA precursor. Reprimo is another gene that induces p53 and is present at chromosome region 2q23. Reprimo also arrests the G2/M phase by inhibiting cyclin B1 and CDK1 activity. Deregulated Reprimo expression increases cell proliferation and DNA damage as the repair mechanism becomes blocked. As discussed

above, both the p53R2 and Reprimo genes serve as p53-inducible targets for monitoring the cell cycle and DNA repair (70). Mammary serpin (Maspin) is a tumor suppressor gene that belongs to the serine protease inhibitor (serpin) family in the domain of the SERPINB5-*gene*. Maspin, a unit of the p53 anti-oncogenic pathway, plays roles in apoptosis, angiogenesis, and hypoxia. In CRC, Maspin acts as a prognostic marker for the initial occurrence of stages 3 and 4 (71). Plasminogen Activator-Inhibitor (PAI) belongs to the serine proteinase family located on chromosome 7 and is associated with the suppression of the p53 gene. PAI-1 is a key inducer for invasion and metastasis of cancer and is a potent target for malignancy of CRC, also acting as a marker for senescence (72).

MDM2 Regulation

The MDM2 gene codes the E3 ubiquitin ligase protein that binds to p53 in the amino-terminal and ubiquitinates p53 in proteasomal degradation. Proteasomal degradation in p53 suppresses cancer cells. Gankyrin is the anti-apoptotic inhibitor that binds to MDM2, increasing ubiquitylation and degradation in p53. The expression of MDM2 in relation to the p53 gene leads to the development of colorectal adenocarcinoma. Spiro oxindole is the derivative of MI-43 (the inhibitor of the MDM2-p53 interaction) and induces the target genes p21, NOXA, and PUMA. MI-43 in cooperation with p53 induces cell cycle arrest and cell death/apoptosis in CRC. Another inhibitor of MDM2 involved in CRC is MI-219, which was shown to induce apoptosis in colon cancer cell line HCT-116. MI-219 in activity with Oxaliplatin, a chemotherapeutic drug that acts on p53, provides a high-profile mechanism for mediating the apoptotic response (65). Activation of Forkhead/-Winged-Helix-Box-Class-O3 (FOXO3) gives rise to cell multiplication and tumorigenicity in interaction with MDM2. In CRC, the FOXO3 gene acts as a tumor suppressor and a promoter for metastasis. The FOXO3 gene is a potential biomarker for both diagnostic and prognostic purposes in CRC. The Ras–ERK pathway is known to upregulate protein expression in MDM2. The strong inhibition with tumorigenicity and cell proliferation is determined by the FOXO3 gene, which interacts with MDM2 degradation. The Ras with ERK and MDM2 with FOXO3 interactions initiate negative regulation in the process and act as a novel targeting pathway (54). Ribosomal Proteins (RPs) are tumor suppressor genes that bind to MDM2, leading to stabilization and activation of P53. In CRC, the *RPs gene is* associated and involved in various stages of differentiation, progression, and metastasis. The expression of the RPS15A gene is high in human CRC. The knock-down of RPS15A expression induces suppression of cellular growth and cell cycle arrest via upregulation in p21 and downregulation in CDK1. RPS15A is identified as a potential therapeutic target against

the action of CRC (73). The p14ARF gene is a tumor suppressor gene that binds by inhibiting the functions of MDM2 and promotes ubiquitination in the p53-gene. Gene p14ARF can modify the levels of p53. The expression of *ARF* increases p53 activity mainly due to the interaction of *MDM2*-protein. The alterations in the interlinked pathways of p14ARF with MDM2 and p53 occur during the progression of CRC. Meta-analysis indicates that gene methylation in p14ARF may significantly associate with MSI of CRC (74). MDMX is the homology of MDM2 that enhances and induces P53 degradation. *MDMX* has its target gene *FL118,* which inhibits the expression of multiple anti-apoptotic proteins like MDMX and survivin in CRC (75).

Tankyrase Regulation

Tankyrase is an enzyme encoded by the TNKS gene that catalyzes the degradation of Axin via poly-ADP-ribosylation. Tankyrase is involved in various processes like Wnt/β-catenin signaling, cellular homeostasis, and cell cycle progression. When inhibition of poly-ADP ribosylation takes place, Axin is stabilized and Wnt signals are antagonized. The two major TNKS isoforms expressed in CRC are TNKS-1 (ARTD5 or PARP5A) and TNKS-2 (ARTD6 or PARP5B). *The* TNKS inhibitor *IWR-1* inhibits the Wnt pathway by stabilizing the destruction complex AXIN2. In CRC, the *IWR-1* gene can inhibit mesenchymal transition. In relationship with EMT and the expression of survivin, *IWR-1* suppresses the tumor expansion rate in CRC metastasis. A research study (75) on tankyrase proteins explored the therapeutic target effect to identify potent inhibitors using known drug molecules such as IWR-1 and XAV939. IWR-1 could be considered as the therapeutic agent for treating CRC. Initially, they believed the dynamic interaction between TNKS-1 and TNKS-2 is similar to IWR1. The results they obtained showed similar structural and dynamic properties of TNKS-1/TNKS-2 with IWR1 complexes. Finally, the study concluded that TNKS1 & TNKS2 may be used as potent drug targets (76). *XAV939* is a small molecule that acts as a potent inhibitor of tankyrase and is used for targeting TNKS-1 & TNKS-2. XAV939 effectively inhibits proliferative APC in CRC cells and exploits the importance of TNKS as a promising target for drugs. With the inhibitor XAV939 as a lead, further molecular studies and quantum mechanics were carried out for identifying the potent inhibitor for TNKS. The outcome of their studies suggests that XAV939 and other identified hit compounds act as a promising target for TNKS proteins (77). The Phosphatase and Tensin Homolog (*PTEN)* is a novel *tankyrase*-binding protein that plays a major role in apoptosis. In CRC pathogenesis, the PTEN gene is involved via activating other signaling pathways like PI3K/AKT. The inhibition of CRC in cell proliferation and cellular cycle arrest

through PTEN is closely related to its interlinked PI3K-AKT-FOXO signals (78). The Angiomotin (AMOT) protein associated with TNKS promotes degradation through RNF146 in CRC. AMOT may act as an oncogene for the progression of CRC and activates yes-Associated *Protein*-1 (YAP) via ERK or activates PI3K via the AKT signaling pathway. Targeting AMOT with TNKS proteins may lead to better treatment outcomes for CRC patients (79).

RNF146 Regulation

Ring Finger Protein 146 (RNF146) is an E3 ligase that positively regulates Wnt signaling by ubiquitinating tankyrase proteins (TNKS-1 and TNKS-2) and mediates parsylated target proteins Axin1/Axin2 in CRC. When overexpressed, RNF146 acts as an oncogene that could be targeted as a promising therapy for CRC. In a previous study of ours, we used the structural-based virtual screening method to find the best potential inhibitors that suppress the ubiquitinated Axin and RNF146 proteins. We found two key compounds in the TCM and NCI database with good binding complexes of RNF146, namely RNF146-TNKS and RNF146-Axin (11). RNF146 is a positive regulator of Wnt signaling. By the action of RNF146 with TNKS, the low level of Axin production leads to β-catenin degradation. RNF146 (with RNAi) also plays a major role in inhibiting Wnt-3 and stimulating β-catenin stabilization; in its absence, Wnt promotes phosphorylation in β-catenin, leading to degradation (80). Thereby, RNF146 acts as a new emerging target for identifying the various domain regions and interlinked proteins that help to suppress the growth of CRC.

Interlinked Signaling Cascades of CRC

The Wnt signals play a major role in CRC by interlinking with upregulation and downregulation mechanisms. GSK-3β is the key regulator for the Wnt and PI3K/AKT pathway. In the Wnt pathway, GSK-3β is the deregulator for the oncogenic Wnt/β-catenin pathway for pathogenesis and oncogenesis in CRC. GSK-3β also acts as a downstream substrate for the PI3K/AKT pathway. Axin has an associated β-catenin protein and is found to interact with GSK-3β and APC as its components. Axin also acts as an inhibitor of Wnt signaling, interacting with β-catenin to activate CRC. Axin, via its ubiquitinated complexes with RNF146 and TNKS, regulates the Wnt signaling pathway for the degradation of β-catenin. Protein p53 is negatively regulated by MDM2 and this interlinking leads to increased cell metastasis and accelerated cancer progression. The p53 together with p21 activates the wild type p53 and relates its response with DNA damage. In another scenario, inactivation of p53 mutation leads to the rapid triggering of the p21 gene, activating cell proliferation in an uncontrolled manner. Notably, *p21* and cyclin D1 (Wnt-targeted gene) activate and promote cell proliferation via the PI3K/*AKT* pathway. The c-myc gene is targeted in signaling pathways directly because it switches the direct repression of p21. The c-myc gene plays downstream effects in both Wnt and Ras-dependent signal pathways. TOPK interacts with p53 and promotes tumorigenesis by inhibiting p53 functions. P53 has its inducible targets p53R2 and Reprimo, which are involved in monitoring the cell cycle and DNA repair. Reprimo arrests the cell at the G2/M phase by inhibiting cyclin
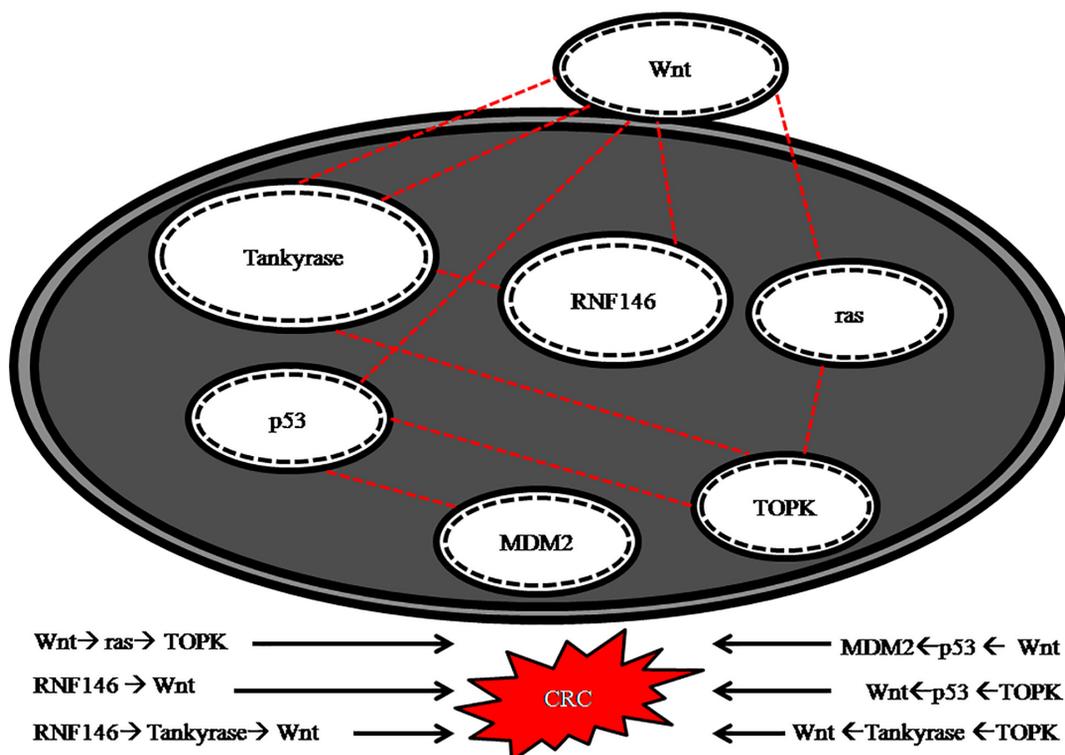

Figure 3: Interlinked signaling cascades of CRC

Table 1: List of biomarkers identified in different pathways associated with colorectal cancer.

S. No	Identified Bio-Markers	Relating Pathway	References
1.	β-catenin	Wnt	14
2.	Survivin	Wnt	16
3.	cyclin D1	Wnt	19
4.	FZD3	Wnt	30
5.	RGS2	Wnt	40
6.	CXCL8	TOPK	57
7.	BAX	p53	55
8.	PUMA	p53	67
9.	Maspin	p53	71
10.	PAI-1	p53	72
11.	FOXO3	MDM2	54

B1 and CDK1 activity. The interaction of cyclin B1 with E-cadherin suppresses the rate of invasion and metastasis in CRC cells. The interaction of Grb2 with Shc protein activates Ras by initiating its role in the activation of the MAPK pathway. The Ras protein cross-talk with LKB1 or APC gene or TP53 reaction activates tumor formation and pushes toward the progressive state. Gankyrin is a key regulator for Ras (mediated via AKT), inhibiting the downstream ROCK pathway and Ras-induced tumorigenesis. Overexpression of MET is mostly found in CRC, occurring in the K-Ras wild type and K-Ras mutant-type tumors. The interlined cooperation between K-Ras signaling and MET signaling promotes growth factors of CRC. Deregulation in K-Ras expression leads to the activation of mutations in major signaling pathways like Ras/Raf/MAPK and PI3K/AKT. BRaf is a direct target of K-Ras and both these activate the MAPK pathway. The activated MAPK signals with the expression of PI3K and K-Ras activate BRaf, which in turn activates MEK. MAPK signaling pathway is the major component of Ras, Raf, MEK, and ERK; this interlinking is known as the chain of proteins implicated in cell injury and cancers. The AMOT protein associated with TNKS promotes degradation through RNF146 in CRC. AMOT may act as an oncogene for the progression of CRC and activates YAP via ERK or PI3K via the AKT signaling pathway. Targeting AMOT with TNKS proteins might produce a better inhibitory effect for CRC. *PTEN* is a novel *tankyrase* binding protein that plays a major role in apoptosis. In CRC, the PTEN gene is involved in the pathogenesis via activating other signaling pathways like PI3K/AKT. The inhibition of CRC in cell proliferation and cellular cycle arrest through PTEN is closely related to its interlinked PI3K-AKT-FOXO signals. *The* TNKS inhibitor *IWR-1* inhibits the Wnt pathway and stabilizes the destruction complex Axin2. In CRC, the *IWR-1*gene can inhibit mesenchymal transition. In relationship with EMT and the expression of surviving, *IWR-1* suppresses the tumor expansion rate in CRC metastasis. PDK1 is a direct downstream target gene for Wnt and AKT, acting downstream of PI3K. MDMX is the homolog of MDM2 that enhances and induces P53 degradation. *MDMX* has its target

gene *FL118* that inhibits the expression of multiple anti-apoptotic proteins like MDMX and survivin in CRC. RNF146 is an E3 ligase that positively regulates Wnt signaling via ubiquitinating tankyrase proteins (TNKS-1 and TNKS-2), mediating parsylated target proteins Axin1/Axin2 in CRC. The summary of this cascade mechanism is diagrammatically illustrated in Figure 3.

Conclusion

We conclude that CRC is a disease entity with distinct biological characteristics. In this study, we have proposed that targeting multiple interlinked pathways is useful in developing new potent biomarkers for treatment and diagnosis purposes (Table 1). The Wnt/β-catenin pathway, as the major signaling mechanism, is considered as the most significant targeted pathway for CRC. Genetic mutations occur in Wnt components like APC, GSK-3β, CK1, and Axin genes, acting as a destructive complex for β-catenin. GSK-3β acts as a key regulator of the Wnt/β-catenin and PI3K/AKT pathways. Overexpression of β-catenin in CRC acts as a marker for the late phase. Axin and β-catenin play major roles in the regulation of the ON/OFF mechanism in Wnt signaling. Other negative regulators of Wnt signaling inhibitors like *SFRP*, DKK1, DKK3, and WIF1 were also reported. When interlinked, RNF146, TNKS, and Axin possess the major regulatory role in the Wnt signaling mechanism for the degradation of the β-catenin complex. The response of FZD receptors to Wnt proteins is determined by their LRP5 or LRP6 co-receptors, which are involved in the activation of the canonical β-catenin pathway. In the canonical Wnt pathway, the dynamic regulations of CRC have a major impact, meaning that this regulation could be targeted by Wnt-FZD signaling. The overview of the Wnt/β-catenin signaling regulation by FZD and its co-factors were explained. The Ras/Raf complex was identified as the downstream effector kinase. The Ras isoforms play important roles in both the upregulation and downregulation of the pathway. Raf activates MEK followed by MAPK, thereby activating transcription factors that lead to mitogenesis. The Ras inhibitors are approved as Ras oncoproteins and used as undruggable cancer targets. TOPK/PBK belongs

to the MAPKK family and is involved in a signaling pathway that includes Ras, Raf, MEK, and ERK as its major chain components. This pathway is implicated in cell injury and disease and is overactive in cancers. The Ras/Raf/MEK/ERK and PI3K/AKT/mTOR signaling pathways are identified as promising therapeutic targets for cancer therapy. Overexpression of TOPK not only has been associated with CRC but also plays a critical role in leukemia, myeloma tumors, and various other cancers. Interleukin 8 CXCs potentiate the cross-talk of multiple signaling pathways like PI3K or AKT and Raf or MEK or ERK. Targeting the genes of the CXC family via small drug molecules may act as a therapeutic target for CRC. The p53 interlinked relationship with MDM2 and p53 targeted genes p21, NOXA, and PUMA in CRC were discussed. Notably, p21 is important for the activation of the PI3K/AKT and c-myc signaling pathways. TOPK interacts with p53 and promotes tumorigenesis by inhibiting p53 functions, so targeting p53 via its genes acts as a better target for CRC. TNKS plays a vital role in the Wnt signaling and acts as a specific target for CRC and several other types of cancers. TNKS 1 and 2 act as promising and attractive therapeutic targets for developing anticancer drugs. MDM2, in relation to

the p53 gene, leads to the development of colorectal adenocarcinoma. The Ras with ERK and MDM2 by FOXO3 initiates the negative regulation process and acts as a novel targeting pathway for CRC. RNF146 is a positive regulator of Wnt signaling. By the action of RNF146 with TNKS, Axin production is reduced and leads to β-catenin degradation. RNF146 is a novel target and may act as a promising therapeutic target for CRC. By explaining the cross-talking signaling cascades, we have provided the source for gene network interaction and targeted therapy. This study paves the way for multi-targeting of interlinked pathways, suggesting that these would be perfect for suppressing CRC. The signaling pathways discussed in this review are not only limited to CRC but also may be potent targets and biomarkers for other types of cancers.

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