Invited Review

New perspectives on β-catenin control of cell fate and proliferation in colon cancer

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2 ABSTRACT

Colorectal cancer is a major health problem worldwide. Aberrant activation of the Wingless-type mouse mammary tumor virus integration site family (Wnt)/β-catenin signaling pathway is the most common and initial alteration in sporadic colorectal tumors. Numerous experimental studies have indicated that β-catenin is a key regulator of colorectal cancer. Indeed, β-catenin activity was shown to designate colon cancer stem cells (CSC) and is, therefore, an attractive target for new therapeutic agents. Thus, it is necessary to further understand its biology and search for effective therapy. Here we review the current literature regarding the functions of β-catenin control of intestinal cell fate and proliferation. Further, we provide a brief commentary on our current understanding of the role that β-catenin plays in colorectal tumor. These results show that β-catenin may serve as a good diagnosis biomarker of early-stage tumor development and a novel potential therapeutic target for colon cancer.

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1. Introduction

Colorectal cancer (CRC) is the second most frequent malignancy and the second leading cause of death from cancer in the world (Walker et al., 2014). CRC is one of the best-studied malignancies, and despite recent advances in chemotherapies that have improved survival, patients with late-stage disease still have poor prognosis, and the overall mortality of the disease is around 40% (Luo et al., 2014). The carcinogenesis of colon cancer has been associated with both genetic and environmental factors (Bharati et al., 2014; Damaschke et al., 2013). Despite a prolonged latency phase, too few lesions are identified at a stage where they can be surgically excised.

It has been found that several signal pathways, including K-ras, Src/PI3K/Akt, β-catenin, TGF-β and p53 play critical roles in its pathogenesis (Chen and Huang, 2009). The corresponding signal pathways involved in colon cancer have also been characterized (Puglisi et al., 2013). Environmental factors, including obesity, diabetes and diet also play important roles in colon cancer (Li et al., 2014b). It has been recognized that the development of colon cancer proceeds in sequential stages from polyps to adenocarcinoma (Selgrad et al., 2014).

Thus, it is necessary to further understand the cancer biology and develop effective treatment. Colon cancer has been studied in many colon cancer cell lines (Findlay et al., 2014; Huber et al., 2014). More recent insights from the cancer stem cell (CSC) field have reshaped our view of malignancies (Masuko et al., 2012). The CRC stem cell model poses an interesting framework in which tumors are hierarchically organized tissues with CSCs at the top of the hierarchy driving tumor growth and progression (Mathonnet et al., 2014; Zheng et al., 2010). The CSC theory has dramatic consequences for the way in which we perceive cancer initiation and progression and currently serves as a basis for targeted therapies (Allen and El-Deiry, 2013; Deng et al., 2014). However, the development and clinical use of effective therapies will depend on a good understanding of the molecular processes regulating CSCs (de Sousa et al., 2011).

In this review, we summarize the latest insights on molecular pathways regulating colon CSCs, with specific emphasis on the Wnt signaling cascade, and provide a functional view into colon cancer molecular pathogenesis and preventive intervention.

2. The pathogenesis of colon cancer

Understanding of the mechanism of colorectal carcinogenesis has been gaining momentum for some years on account of its high incidence and impact on the lives of individuals affected (Li and Lai, 2009). Different genetic abnormalities have been found in colorectal cancers from different sites (Mouradov et al., 2014). The mecha-
nisms of CRC pathogenesis have been widely investigated and current evidence indicates that genetic mutations, epigenetic changes, and aberrant immunologic signaling pathways are major contributors to CRC (Arends, 2013). Mutations in tumor suppressor genes or oncogenes of colonic epithelial cells lead to dysregulated signaling networks that control cellular metabolism, differentiation, proliferation, and survival, resulting in the transformation of normal cells into cancer cells (Grady and Pritchard, 2014). In addition to genetic mutations, epigenetic modulation of tumor suppressor genes, oncogenes, or proinflammatory mediators represent other important mechanisms whereby homeostatic balance is lost (Torres et al., 2013; Wang et al., 2014). Cancer development and progression is a complex process involving a myriad of host–tumor interactions and countless molecular and cellular elements in the tumor microenvironment (Tosolini et al., 2011).

From the anatomical and embryological standpoints, the proximal and distal colons are located within the peritoneal cavity, while the rectum lies within the pelvis, the location of which is relatively inaccessible (Grynhberg et al., 2009). Proximal colon embryologically originates from the midgut, while segments from the splenic flexure to the upper anal canal containing the distal colon and rectum arise from the hindgut (Ellina et al., 2014; Yee et al., 2014). In the normal colonic epithelial tissue, three connexin isoforms, connexin26 (Cx26), Cx32 and Cx43, have been shown to be expressed at the protein level (Sirnes et al., 2014). Colorectal cancer development is associated with loss of connexin expression or relocalization of connexins from the plasma membrane to intracellular compartments (Sirnes et al., 2012).

Recent studies also provide information concerning the association between colorectal cancer incidence and a number of metabolic syndrome components, especially BMI, waist circumference (WC), lipid levels, plasma glucose and glycosylated hemoglobin (HbA1c) (Pais et al., 2009). There was a dose–response association between colorectal cancer incidence and the number of metabolic syndrome components present at baseline after multivariate adjustment (Muhidin et al., 2012). Vargas and co-workers have shown that expression levels of several genes related to metabolic syndrome and associated alterations were analyzed by real-time qPCR in two equivalent but independent sets of stage II colorectal cancer patients (Vargas et al., 2014). Apolipoprotein A-II (APOA2), apolipoprotein C1 (APOC1), apolipoprotein C2 (APOC2), apolipoprotein D (APOD), ATP-binding cassette sub-family A member 1 (ABCA1), and leptin receptor (LEPR) increased gene expression levels were associated with short disease-free survival (DFS) (Vargas et al., 2014). Moreover, among adipocytokine-secreted hormones, the most relevant to colorectal tumorigenesis are adiponectin, leptin, resistin and ghrelin. All these molecules have been involved in cell growth and proliferation, as well as tumor angiogenesis and it has been demonstrated that their expression changes from normal colonic mucosa to adenoma and adenocarcinoma (Riondino et al., 2014).

A progression from normal mucosa to adenoma to carcinoma was supported by the demonstration of accumulating mutations in genes of K-ras, adenomatous polyposis coli (APC), tumor protein P53 (TP53), and deleted in colorectal carcinoma (DCC), all of which are thought to be of significance, but are not able successfully to account for all CRCs (Savas and Younghusband, 2010; Walther et al., 2009). There is heterogeneity in the pathogenetic pathway leading to CRCs, and there are two major tumorigenic pathways (Kanthan et al., 2012). The first is driven by chromosomal instability (CIN), namely the model mentioned above, the progress of which involves both oncogenes and tumor-suppressor genes including chromosomes 5q, 17p, and 18q (Yagishita et al., 2008). Recently, epigenetic changes have been considered as an important mechanism of colorectal carcinogenesis, though it had long been relegated to an almost non-existent role in tumorigenesis (Vaiopoulos et al., 2014). However, in this review, we show that the Wnt/β-catenin signaling cascade plays a key role in colon cancer molecular pathogenesis. We summarize new perspectives on β-catenin control of cell fate and proliferation in colon cancer.

3. **The Wnt/β-catenin signaling pathway**

The Wnt signaling cascade is conserved throughout the animal kingdom and, depending on the context, plays various roles that encompass stem cell maintenance, cell proliferation, differentiation, and apoptosis (Guo et al., 2014; Xing et al., 2014). Secreted Wnt proteins may bind to a plethora of potential Wnt membrane receptors which include Frizzleds, low density lipoprotein receptor-related proteins (LRPs), RYK receptor-like tyrosine kinase (RYK)/Derailed, retinoid-related orphan receptor (Ror)-2, and FRL1/Cripto, and elicit different types of intracellular responses (Pendas-Franco et al., 2008; Wang et al., 2013). The canonical pathway is mainly regulated at the level of β-catenin, a protein kept under low cytoplasmic concentration by the destruction complex (Astudillo and Larrain, 2014). Wnt binding to Frizzled and LRPS/6 co-receptors induces β-catenin protein stabilization and entry into the nucleus where it modulates the transcription of target genes (Amado et al., 2012).

The canonical Wnt signaling pathway is driven by β-catenin, a scaffold protein, linking the cytoplasmic tail of classical cadherins in the endothelium via β-catenin to the actin cytoskeleton (DiRocco et al., 2013; Frode and Brabletz, 2007). Without Wnt stimulation, cytoplasmic β-catenin levels are kept low by a degradation complex, consisting of axin, adenomatous polyposis coli (APC), casein kinase 1α (CK1α), and glycogen synthase kinase 3-β (GSK3-β) (Caliceti et al., 2014). Binding of Wnt to its receptors Frizzled and LRP leads to inhibition of the degradation complex function, enabling β-catenin signaling (Bin-Nun et al., 2014). Wnt allows β-catenin to accumulate and translocate to the nucleus where it binds to several transcription factors, for example, T-cell factor (TCF) and LEF-1 (Rahmani et al., 2012).

The interaction of Wnt with Fzd/LRP changes the state of things in ways for which a number of biochemical and cellular mechanisms have been proposed (Ring et al., 2014): it may trigger the formation of Dishevelled (Dvl)/Fzd complex, which in turn, sequesters axin to the plasma membrane thus blocking the formation of destruction complex (Grumolato et al., 2010); Wnt induced phosphorylation of LRPs at five conserved PPP(S/T)PX(S/T) (PPPSPPX) motifs by GSK3β and CK1γ may create docking sites for axin, facilitating its sequestration on the membrane and inactivation of the destruction complex (Tanneberger et al., 2011); Wnt induced phosphorylation of PPPSPPX motifs of LRPs may inactivate the catalytic pocket of GSK3β, thus directly blocking its activity against β-catenin (Paul et al., 2013); Wnt signaling may promote internalization of GSK3β by multivesicular bodies (MVBs), thus preventing it from phosphorylating β-catenin (Vinyoles et al., 2014), β-catenin is a direct target for alterations but is a central player in the Wnt/β-catenin signaling pathway (Fig. 1).

4. **β-catenin control of cell fate and proliferation in colon cancer**

Wnt/β-catenin signaling plays a crucial role in the regulation of colon tissue regeneration and the development of colon cancer (Schon et al., 2014). A recent article by Kim and co-workers suggested that ursolic acid (UA) and corosolic acid (CA) exert their anticancer activities against colon cancer cells by promoting the N-terminal phosphorylation and subsequent proteasomal degradation of β-catenin (Kim et al., 2014). This result has shown that β-catenin may serve as a good biomarker and a novel potential therapeutic target for colon cancer.

Under physiological conditions, β-catenin activity is tightly controlled. However, the molecular details of β-catenin dependent gene
transcription in cancer cells are still not comprehensively explored. Deregulation of Wnt/β-catenin signaling is a hallmark of the majority of sporadic forms of colorectal cancer and results in increased stability of the protein β-catenin (Herbst et al., 2014). β-catenin is then shuttled into the nucleus where it activates the transcription of its target genes (Li et al., 2014a). Berry and co-workers indicated that KDM4B downregulation resulted in reduced expression of the β-catenin/TCF4 target genes JUN, MYC and cyclin D1, all of which encode for oncoproteins (Berry et al., 2014). Furthermore, Keerthivasan and co-workers suggested that activation of Wnt/β-catenin signaling in effector T cells and/or Tregs is causatively linked with the imprinting of proinflammatory properties and the promotion of colon cancer (Keerthivasan et al., 2014). What’s more, To and co-workers also indicated that hypoxia stimulated co- upregulation of β-catenin and Nur77 in a number of human colorectal cancer (CRC) cell lines. Interestingly, expression of β-catenin and Nur77 by hypoxia formed a mutual feedback regulation circuits that conferred aggressive growth of CRC (To et al., 2014). In addition, Groulx and co-workers demonstrated that the α6A variant is a pro-proliferative form of the α6 integrin subunit in CRC cells and appears to mediate its effects through the Wnt/β-catenin pathway (Groulx et al., 2014). These findings show that β-catenin plays a key role in individual therapy in colon cancer. 

Apart from the role of β-catenin signaling in sporadic colon cancer, in colitis associated colon cancer, the role of β-catenin signaling is also critical. Inflammatory diseases are associated with increased risk of developing colitis-associated colon cancer (Piazzì et al., 2014). APC is a nucleo-cytoplasmic shuttling protein, best known for antagonizing Wnt signaling by forming a cytoplasmic complex that marks β-catenin for degradation (Zeineldin et al., 2014). Zeineldin and co-workers have shown that the azoxymethane (AOM) – dextran sodium sulfate (DSS)-induced colon adenoma histopathology, proliferation, apoptosis, stem cell number and β-catenin and Kras mutation spectra were similar in APC (mNLS/mNLS) and APC (+/+ ) mice. However, AOM-DSS-treated APC (mNLS/mNLS) mice showed more weight loss, more lymphoid follicles and edema, and increased colon shortening than treated APC (+/+ ) mice, indicating a colitis predisposition (Zeineldin et al., 2014). Moreover, Tsai and co-workers revealed that administration of garcinol significantly downregulated cyclooxygenase-2, cyclin D1, and vascular endothelial growth factor expression via inhibition of the Wnt/β-catenin signaling pathways (Tsai et al., 2014). This result suggests that garcinol may merit further clinical investigation as a chemoprotective food that helps prevent colitis-associated colon cancer. Furthermore, Morales and co-workers indicated that attenuation of colon cancer in these mice correlated strikingly with reduced mutation rates of β-catenin, increased efficiency of the DNA repair machinery, and reduced expression of proinflammatory cytokines, including interleukin (IL)-17 and IL-23 in the tumor microenvironment (Morales et al., 2014). In addition, Shenoy and co-workers demonstrated that early activation of Wnt/β-catenin signaling is critical for colitis-to-cancer transition and that high levels of Wnt/β-catenin signaling can further demarcate high-ALDH tumor-initiating cells in the non-dysplastic epithelium of ulcerative colitis patients (Shenoy et al., 2012). As such, these findings offer plausible diagnostic markers and therapeutic target in the Wnt/β-catenin signaling pathway for early intervention in colitis associated colon cancer.

\section{5. β-catenin as a therapeutic target for colon cancer}

An incredible collection of natural and synthetic compounds form the basis of intense efforts in high-throughput drug-screening programs (Albin et al., 2014). The past decades have seen major advances in understanding the molecular framework of Wnt signaling, which provides an optimal platform for testing these libraries of compounds (Nishiyama et al., 2014; Robertson et al., 2014). The class regulates axin2 stability and, importantly, also targets β-catenin degradation in the presence of APC mutations (Metcalfe et al., 2010). Although of potential interest for various Wnt signaling-dependent malignancies, the benefit for CRC is questionable as the first class of inhibitors will, in theory, be inefficient when APC mutations render the tumor Wnt-ligand independent (Moon et al., 2014; Watanabe et al., 2014). However, as mentioned, APC mutations rarely represent complete null mutations. In agreement, Wnt ligands are expressed in various CRC cell lines, and blockade of Wnt1 with monoclonal antibodies can trigger apoptosis in cell lines bearing APC as well as β-catenin mutations (Fanali et al., 2014). Although of great potential, most Wnt inhibitors are still in preclinical testing or in the developmental stage (Wu et al., 2011). Additionally, given the fact that Wnt signaling is such an important pathway involved in regulation of tissue homeostasis, interference with crucial components of this cascade is predicted to be associated with serious adverse events (Shackelford et al., 2013).

Preclinical studies have shown a correlation between efficacy of chemoprevention and the Wnt modulatory effects of these compounds. Non-steroidal anti-inflammatory drugs (NSAIDs) have complex modes of action, and only part of them converge to an inhibition of the cyclooxygenase (COX) enzymes (Cerella et al., 2010). Sulindac significantly inhibited proliferation of HT-29 colon cancer cells in a dose- and time-dependent manner (Limoncelli et al., 2010). Sulindac was found to induce the apoptosis of HT-29 cells and inhibit the Wnt/β-catenin pathway (Tai et al., 2014). The inhibition was further confirmed by the decreased protein levels of β-catenin (Li et al., 2013). Moreover, the protein and mRNA expression of β-catenin are all decreased by resveratrol concentration – dependently in a human colon cancer cell line (Liu et al., 2014). Tussilagine suppressed the β-catenin activity and also decreased the expression of cyclin D1 and c-myc, representative target genes of the Wnt/β-catenin signaling pathway, and consequently inhibited the proliferation of colon cancer cells (Li et al., 2014a). Furthermore, berberine inhibits colon tumor formation through inhibition of Wnt/β-catenin signaling and berberine might be a promising drug for the prevention of colon cancer (Zhang et al., 2013). In addition, Ramírez and co-workers found that bozepinib inhibited both mamo- and colono-spheres for-
Cancer xenograft mouse model (invivotreatment with esculetin suppressed tumorgrowth in a colon antago nized the cellu lar effectsof β-catenin-dependent activity, and Bozepinib induced the down-regulation of c-myc, β-catenin and Sox2 siRNA (single-chain fragment variable antibody(scFv)-truncated protamine- for colon cancer: an epithelial cellular adhesion molecule (EpCAM) ingly, Hao and co-workers demonstrated that a new therapeutic drug pounds have been identified to have potential therapeutic value ical diagnosis biomarker believed to reflect early-stage colon cancer assuming to be constant in their biological behavior or relative risk factors. These findings suggest that β-catenin may be a good clin- ical events that drive cancer initiation, progression, and drug regimens require a thorough understanding of the basic bio- logical events that drive cancer initiation, progression, and maintainance. Moreover, therapeutic agents targeting β-catenin might result in innovative new therapies for colon cancer. In the near future, these new areas of drug development will tackle the various CSC regulatory axes and will hopefully yield efficient therapy regi- mens resulting in improved clinical outcome. All in all, we believe β-catenin may be very useful for the prevention and treatment of colon cancer.

6. Conclusions and future perspectives

Colorectal cancers from different anatomic sites should not be assumed to be constant in their biological behavior or relative risk factors. These findings suggest that β-catenin may be a good clinical diagnosis biomarker believed to reflect early-stage colon cancer (Manna et al., 2014). The discovery and generation of new cancer drug regimens require a thorough understanding of the basic biological events that drive cancer initiation, progression, and maintenance. Moreover, therapeutic agents targeting β-catenin might result in innovative new therapies for colon cancer. In the near future, these new areas of drug development will tackle the various CSC regulatory axes and will hopefully yield efficient therapy regimens resulting in improved clinical outcome. All in all, we believe β-catenin may be very useful for the prevention and treatment of colon cancer.

Conflict of interest

The authors declare that there are no conflicts of interest.

Transparency document

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