Prospects

Therapeutic potential of targeting Wnt/β-catenin pathway in treatment of colorectal cancer: rational and progress†

Afsane Bahrami[1,*], Forouzan Amerizadeh[1,*], Soodabeh ShahidSales[4,*], Majid Khazaei[5,*], Majid Ghayour-Mobarhan[4], Hamid Reza Sadeghnia[1], Mina Maftouh[3], Seyed Mahdi Hassanian[2,3,#], Amir Avan[3,4,#]

1) Molecular Medicine group, Department of Modern Sciences and Technologies; Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran.
2) Department of Medical Biochemistry, School of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran.
3) Metabolic syndrome Research center, School of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran
4) Cancer Research Center, School of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran.
5) Neurogenic Inflammatory Research Center and Department of Physiology, Mashhad University of Medical Sciences, Mashhad, Iran

 # Corresponding Authors:
Amir Avan, Ph.D. Metabolic syndrome Research Center, Mashhad University of Medical Sciences, Mashhad, Iran. Tel:+9851138002298, Fax: +985118002287; E-mail: avana@mums.ac.ir & amir_avan@yahoo.com
Seyed Mahdi Hassanian, PhD, Department of Medical Biochemistry, School of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran. Tel:+9851138002298, Fax: +985118002287; E-mail: Hasanianmehrm@mums.ac.ir

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* Equally contributed as first author

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Abstract:

Wnt/β-catenin pathway is one of the main/frequent dysregulated pathways in several tumor types, including colon cancer. Aberrant activation of this pathway is associated with cell proliferation, invasive behaviors and cell resistance, suggesting its potential value as a therapeutic target in treatment of CRC. Several agents have been developed for targeting of this pathway (e.g, natural agents: curcumin, 3,3-diindolylmethane, phytoestrogen; Synthetic/small Wnt inhibitors: Rofecoxib; PRI-724, CWP232291; and monoclonal antibody against frizzled receptors, Vanituctumab. This review summarizes the current knowledge about the therapeutic potential of targeting Wnt pathway with particular emphasis on preclinical/clinical studies in treatment of colorectal cancer.

Key word: Colon cancer, Wnt/β-catenin, inhibitor, therapeutic target

Introduction

Colorectal cancer (CRC) is third leading cause of cancer mortality, which reported over 1.2 million new patients and 600,000 mortalities every year (Hosseini, 2016; Xiao et al., 2016). Progress in combination chemotherapy such as FOLFIRI, XELOX/ CAPOX, FOLFOX, and supportive consumption of therapeutic antibodies against epidermal growth factor receptor (EGFR) and vascular endothelial growth factor (VEGF) have been shown to increase survival time (Watanabe et al., 2015). Although, it is still remained as one of the leading casue of death, supporting the need for detection of novel agents in treatment of CRC.

Recently Wnt/β-catenin pathway is emerged as promising therapeutic target in CRC, , since it most of the patients with CRC have mutations in at the least one Wnt signaling cascade gene, such as the β-catenin (CTNNB1) and adenomatous polyposis coli (APC) genes. The APC gene was firstly identified as being main causative for the familial adenomatous polyposis (FAP) syndrome (Preisinger et al., 1991) .But later found to be commonly (~80%) mutated in sporadic colorectal cancers and about half of colon cancers with wild-type APC carry mutations in β-catenin (Morin et al., 1997). In this review, we provide an overview on targeting Wnt/β-catenin signaling pathway as a potential therapeutic approach in treatment of CRC.

Wnt signaling pathway

Wnt pathway is categorized into canonical and non-canonical (β-catenin independent) signaling pathway. In the absence of wnt ligands, β-catenin situates in cell membrane and communicates with E-cadherin, and mediates intercellular adhesion. Usually, free cytoplasm β-catenin is destabilized and damaged by a destruction complex including APC, Axin, casein kinase 1α (CK1α) and glycogen synthase kinase-3b (GSK3b). When Wnt cooperates with Frizzled and LRP, Wnt/ β-catenin signaling is activated. Both Dishevelled (Dvl) and Axin are undergo to cell membrane recruitment, followed by GSK3b suppression and β-catenin releasing of destruction complex. Wnt permits β-catenin to accumulate and transformed to the nucleus where it binds to various

transcription factors, such as T-cell factor (TCF) and lymphoid enhancer factor1 (LEF-1). In the involvement of transcriptional co-activators of the β-catenin or TCF4 complex, cAMP response element-binding protein (CREB)-binding protein (CBP). CBP encompass proteins associated with cell differentiation, proliferation, propagation, apoptosis, invasion and angiogenesis(Barker, 2008; Zhan et al., 2016).

Non-canonical Wnt signaling is distinguished by β-catenin-independent mechanisms of signal transduction. Within Wnt/PCP signaling, Wnt ligands bind to ROR-Frizzled receptor and activate Dvl. Then, Dvl binds to the small GTPase Rho by disinhibition of the protein DAAM1 (Dvl associated activator of morphogenesis 1) in cytoplasm. The complex of small GTPase Rac1 and Rho trigger ROCK (Rho kinase) and JNK. Hence, this leads to rearrangements of the cytoskeleton and transcriptional responses. Later, Vangl, a major member of Wnt/PCP signaling cascade is activated via phosphorylation in a Wnt5a-dependent way. Wnt/Ca2+ signaling started by G-protein stimulated phospholipase C activity resulting to intracellular calcium effluxes and downstream calcium dependent cytoskeletal and transcriptional responses (Zhan et al., 2016). The non-canonical Wnt signaling pathway coordinates cell adhesion, cell polarity and tissue motility.(Vincan and Barker, 2008)

Colorectal cancer stem cells (CSCs) and Wnt signaling

There is growing body of evidence showing the a potential link between Wnt pathway and modulation of CSCs (Fodde and Brabletz, 2007). An active Wnt signaling cascade is related with CSC phenotype. Epithelial cellular adhesion molecule (EpCAM), aldehyde dehydrogenase 1-A1 (ALDH1A1), CD24, CD26 (dipeptidyl peptidase IV: DPPIV), CD44,CD133, CD166, Lgr5, and Musashi-1 have been identified as colorectal CSC markers (Lin et al., 2011; Todaro et al., 2010). In particular, Lgr5/orphan G-protein-coupled receptor and CD44 are the well-known TCF/ LEF target genes(Yamamoto et al., 2003). Moreover due to the up regulation of ATP-binding cassette (ABC) family transporters involved in drug-efflux pumps, CSCs often show resistance to conventional

chemotherapeutic regimens. Therefore, it is believed that CSCs are associated with increased risk for cancer relapse post-chemotherapy. ABCB1 is one of the TCF/LEF target genes and have been reported to be involved with intestinal tumorigenesis(Yamamoto et al., 2003).

β-catenin as a therapeutic target for colon cancer

Several agents have been developed for targeting this pathway. It has been shown that retinoic acid could inhibit Wnt signaling by direct interaction with β -catenin/competition for TCF binding (Xiao et al., 2003). Also, the active form of vitamin D ($1,25(OH)_2D_3$), encourages the β -catenin binding to the vitamin D receptor, consequently decreasing the amount of β-catenin. In addition, $1,25(OH)_2D_3$ modulate E-cadherin, which conserves β-catenin in the membrane, preventing its nucleus translocation (Pálmer et al., 2001). The polyphenols curcumin, quercetin, resveratrol and the green tea polyphenol epigallocathechin-3-gallate have been reported to suppress Wnt inhibiting activity (Sarkar et al., 2010). differentiation-inducing factors (DIF) have a phenol unit thus can inhibit β-catenin via activating GSK3-b (Takahashi-Yanaga et al., 2003).

Phytoestrogen and Genistein have suggested to inactivate Wnt signaling by over expression of E-cadherin and GSK3-b. Genistein, a soy-derived isoflavone, plays a role in reversing resistance to platinum and fluoropyrimidines compounds (Su and Simmen, 2009). This agent has been tested in a phase I-II clinical trial in combination with bevacizumab /FOLFOX for metastatic cancer patients. Another natural compounds may have Wnt inhibitor features include 3,3-diindolylmethane ,magnolol, indole-3-carbinol, and lycopene (Sarkar et al., 2010).

Synthetic compounds: NSAIDs

In colon cancer, sulindac and celecoxib have reported to decrease adenomas in FAP patients and celecoxib is FDA approved COX-2 selective inhibitor agent for the treatment of FAP. Celecoxib inhibit Wnt/β-catenin signaling via inducing the TCFs degradation, independent of COX-2(Sebio et al., 2014). However, the emergence of several COX-2 inhibitors, such as Celecoxib, Valdecoxib and

Rofecoxib (Vioxx) has provided the potential in combinational COX-2 inhibitor chemotherapy (Arun and Goss, 2004; Natale, 2003; Sweeney, 2003). Lew and colleagues evaluated the effects of Rofecoxib (Vioxx) and a liposome-mediated APC gene therapy in intestinal neoplasia (Lew et al., 2002). After Vioxx and APC treatment, 70% and 54% reduction were reported in total number of intestinal polyps, respectively, while the combination therapy reduced polyp formation up to 87% (Lew et al., 2002).

Targeted therapies

Several studies have performed for targeting the Wnt cascade components as reported in Table1.

Small molecules

Porcupine is a membrane bound O-acyltransferase that mediates the palmitoylation of Wnt ligands. Suppression of Porcupine by its inhibitor leads to block of Wnt/β-catenin signaling activities (Bartscherer and Boutros, 2008). Inhibitors of Wnt production (IWPs) is a novel type of Porcupine-targeted Wnt antagonists. Initially, Chen *et al* discovered IWPs through screening about 200,000 small molecules in L-Wnt-STF cell line using a cell-based Wnt/β-catenin signal reporter assay. IWPs competitively bound to Porcupine and inhibited Wnt production, while Porcupine overexpressing restored Wnt signaling in cells treated with IWP. Further biochemical researches confirmed that IWPs bind to Porcupine and prevent Wnt palmitoylation, which is substantial for Porcupine -mediated lipidation essential for Wnt secretion (Chen et al., 2009).

LGK974 is another compound which binds and block the porcupine enzyme. LGK974 impedes the growth of murine tumor xenografts model through mouse mammary tumor virus (MMTV)-originated ectopic Wnt1 expression. A phase I open-label clinical trial of LGK974 is ongoing (Liu et al., 2013).

ICG-001 is as antagonist of the protein –protein interaction of β-catenin with CBP(Emami et al., 2004). Suppression of the β-catenin/CBP interaction eradicated tumor-initiating cells and cell differentiation advancement. ICG-001 decrease the growth of colorectal cancer cells in vitro. Antitumor effects of ICG-001 was found in mouse having mutation in one allele of the APC or nude mouse SW620 of colorectal cancer (Eguchi et al., 2005).

PRI-724, the second-generation of CBP/catenin antagonist, was shown to increase p300/β-catenin binding and stem-cell differentiation (Ma et al., 2005). PRI-724, showed acceptable toxicity profile in a phase I clinical trial (Lenz and Kahn, 2014). A phase II trial of combination chemotherapy consisting of bevacizumab alone and with PRI-724 is now being planned for patients with metastatic colorectal cancer.

CWP232291, is another small molecule that inhibited Wnt-mediated transcriptional activity. Active form of CWP232204 binds to Src-Associated substrate in Mitosis of 68 kDa (Sam68) and promotes apoptosis. This agent is being investigated in a phase I trial for hematological cancers (Sebio et al., 2014).

Several small molecules that antagonized the interaction between TCF/LEF1 and β -catenin, have been recognized such as CGP 049090, PNU74654, 2,4 diamino-quinazoline, or PKF115-584(Sebio et al., 2014). Interestingly, common core chemical structure of these compounds indicated that they may have similar binding structure on β-catenin or Tcf and interrupt the Tcf/β-catenin complex in a similar way(Lepourcelet et al., 2004).

Pyrvinium is an anti-pinworm drug, that is reported to activate the CK1 α kinase and prevent Wnt signaling (Saraswati et al., 2010; Thorne et al., 2010). Other effective small-molecule compounds FJ9(Fujii et al., 2007), NSC668036(Shan et al., 2005), and 3289 –8625(Grandy et al., 2009) target Dvl PDZ domain (PSD95/Disclarge/Zonula occludens-1) which is necessary for its frizzled interaction (Wong et al., 2003).

Grandy and colleagues identified 3289–8625 (Grandy et al., 2009). They showed that this agent can interact with PDZ domain of Dvl effectively and thereby suppress Wnt3A-induced signaling. (Metcalfe et al., 2010). Recently, salinomycin, an anti-coccidial drug, is detected to target CSCs via suppression of Wnt signaling by LRP6 degradation(Lu and Li, 2014).

Small-molecule XAV939 is a Wnt inhibitor targeting tankyrases 1 and 2 (TNKS1/2)(Huang et al., 2009). TNKS1/2, poly-ADP ribosylate axins and poly-ADP ribosylated axins, are exposing ubiquitination and degradation. In the other words, inhibition of poly-ADP ribosylation consequently leads to stabilization of axins and antagonist Wnt signaling. XAV939 potentially inhibit the proliferation of APC-depletion colorectal cancer cells. Recently, a selective TNKS inhibitor, NVP- TNKS656 was identified through structure-based optimization of XAV939 (Huang et al., 2009).

BBI608 is a small molecule inhibits signal transducer and activator of transcription-3 (Stat3) and targets CSCs (Ciombor et al., 2015a). BBI608 can also suppress β-catenin signaling. A recent phase III trial of BBI608 against metastatic colon cancer was terminated due to loss of expected efficacy in the short-term analysis. At present, BBI608 is under clinical survey in combination with chemotherapeutic drugs such as cisplatin, gemcitabine, paclitaxel, temozolomide, sorafenib, and pemetrexed(Ciombor et al., 2015b).

Monoclonal antibody against frizzled receptors

Vanituctumab (OMP-18R5) is a humanized monoclonal antibody that interact with frizzled receptors and block canonical Wnt signaling in a patient-derived colorectal cancer mouse xenograft model(Gurney et al., 2012).

Decoy receptor

OMP-54 F28 is a recombinant protein of the frizzled 8 cysteine-rich domain that fused to the Fc part of immunoglobulin. OMP-54 F28 competes with frizzled 8 for ligand binding as a decoy

receptor, and then antagonizes Wnt signaling.OMP-54 F28 suppresses tumor growth and inhibits cancer stem cell function (Le et al., 2015). A phase I study of OMP-54 F28 monotherapy is performed in 25 patients with solid tumors. Dysgeusia, fatigue, muscle spasms, decreased appetite, nausea, and vomiting of grades I/II were reported. But, no adverse event was detected (Masuda et al., 2015).

Conclusions and future perspectives

Wnt signaling pathway is one of the key dysregulated pathways in colorectal cancer, which is associated with increased cell proliferation and resistance of the tumor cells to chemotherapy, suggesting its value as a therapeutic target in treatment of CRC. Several agents have been developed to target this pathway and some them are now in clinical phases. However, several questions are still remained to be elucidated.

In aggregate future challenges should focus on the (1) optimization and evaluation of Wnt/b-catenin inhibitors, (2) selection of patient who might benefit from therapy, (3) detection of prognostic and predictive markers that can be used to monitor treatment response; (4) targeting of other key signaling pathways (e.g., AKT/PI3K, NOTCH, mTOR pathway) (Avan et al., 2017; Maftouh et al., 2014; Pashirzad et al., 2016), in parallel, to overcome cell resistance.

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Table 1. Summary of Wnt-β-catenin pathway inhibitors in clinical development						
Compound name	Molecular target	Tumor	Trial Phase	Arms	Status	Trial identifier
LGK974 (WNT974)	Inhibitor of Porcupine	BRAF mutant colorectal cancer	I/II	WNT974+ LGX818+Cetuximab	not yet recruiting	NCT02278133
LGK974 (WNT974)	Inhibitor of Porcupine	BRAF Mutant Colorectal Cancer	I	LGK974	suspended participant recruitment	NCT01351103
Foxy-5	Wnt5a mimetic	colorectal cancer	I	Foxy-5	recruiting participants	NCT02655952
Foxy-5	Wnt5a mimetic	Colorectal Cancer	I	Foxy-5	terminated	NCT02020291
Vanituctumab (OMP-18R5)	Frizzled -1, − 2, − 5, −7, − 8 receptors	Solid tumors	I	OMP-18R5	completed	NCT01345201
OMP-54 F28 (Decoy receptor)	Frizzled-8 receptor	Solid tumors	I	OMP-54 F28	completed	NCT01608867
Niclosamide	Frizzled receptor	Colon Cancer	I	Niclosamide	not yet recruitment	NCT02687009
OMP131R10	Anti-R-spondin3 antibody	RSPO3 biomarker-positive metastatic colorectal cancer	I	OMP-131R10 FOLFIRI	recruiting participants	NCT02482441
PRI-724	Interaction of β-catenin and CBP	Advanced solid tumors	I	PRI-724	terminated	NCT01302405
PRI-724	Interaction of β-catenin and CBP	Metastatic Colorectal Cancer	II	PRI-724 +mFOLFOX6+bevacizumab	not yet recruitment	NCT02413853
Genistein	GSK3- b	Metastatic Colorectal Cancer	I/II	Genistein	recruiting participants	NCT01985763
Aspirin	COX-2	Colorectal Adenoma	II	Aspirin Placebo	not yet recruitment	NCT02965703
BBI608	Unknown	Metastatic colon cancer	I	BBI608 +FOLFIRI+Bevacizumab	recruiting participants	NCT02641873
BBI608	Unknown	Metastatic colon cancer	Ib/II	BBI608 + Pembrolizumab	recruiting participants	NCT02851004
BBI608	Unknown	Metastatic colon cancer	III	BBI-608 + Leucovorin+ Irinotecan+Bevacizumab Fluorouracil +Leucovorin+ Irinotecan+Bevacizumab	recruiting participants	NCT02753127
Curcumin	Tcf/β-catenin complex	Colorectal Adenoma	II	Anthocyanins +Phospholipidic Curcumin Placebo	recruiting participants	NCT01948661
Resveratrol	PDE4	Colon cancer	I	Resveratrol	completed	NCT00256334