Potential Role of Traditional Chinese Medicines by Wnt/β-Catenin Pathway Compared With Targeted Small Molecules in Colorectal Cancer Therapy

Jinrong Chang¹, Hoileong Wong Xavier², Dongfeng Chen¹, Yamei Liu¹, Hui Li¹* and Zhaoxiang Bian²*

¹School of Basic Medical Sciences, Guangzhou University of Chinese Medicine, Guangzhou, China, ²School of Chinese Medicine, Hong Kong Baptist University, Hong Kong, China

Colorectal cancer (CRC) has become a global public health problem because of its high incidence and mortality rate worldwide. The previous clinical treatment for CRC mainly involves conventional surgery, chemotherapy, and radiotherapy. With the development of tumor molecular targeted therapy, small molecule inhibitors present a great advantage in improving the survival of patients with advanced CRC. However, various side effects and drug resistance induced by chemotherapy are still the major obstacles to improve the clinical benefit. Thus, it is crucial to find new and alternative drugs for CRC treatment. Traditional Chinese medicines (TCMs) have been proved to have low toxicity and multi-target characteristics. In the last few decades, an increasing number of studies have demonstrated that TCMs exhibit strong anticancer effects in both experimental and clinical models and may serve as alternative chemotherapy agents for CRC treatment. Notably, Wnt/β-catenin signaling pathway plays a vital role in the initiation and progression of CRC by modulating the stability of β-catenin in the cytoplasm. Targeting Wnt/β-catenin pathway is a novel direction for developing therapies for CRC. In this review, we outlined the anti-tumor effects of small molecular inhibitors on CRC through Wnt/β-catenin pathway. More importantly, we focused on the potential role of TCMs against tumors by targeting Wnt/β-catenin signaling at different stages of CRC, including precancerous lesions, early stage of CRC and advanced CRC. Furthermore, we also discussed perspectives to develop potential new drugs from TCMs via Wnt/β-catenin pathway for the treatment of CRC.

Keywords: traditional Chinese medicines, colorectal cancer, Wnt/β-catenin, potential role, small molecules, therapeutic mechanism

INTRODUCTION

Colorectal cancer (CRC) is the third cause of cancer-related death worldwide according to the latest statistics of the International Agency for Research on Cancer (IARC) of the World Health Organization (WHO) (Authors Anonymous, 2021a). It estimated that there are 1.8 million new CRC cases and 880,792 CRC-related deaths in 2018 (Yang et al., 2020). Moreover, the incidence of CRC in some countries is on the rise gradually. Approximately 70% CRC cases are sporadic and
develop through the adenoma–carcinoma sequence (De Filippo et al., 2002; Fodde, 2002). Tumorigenesis is usually driven by multiple genetic and molecular alterations in the different stages. The mutations of adenomatous polyposis coli (APC) gene, were first discovered as the underlying cause of the hereditary colon cancer syndrome termed familial adenomatous polyposis (FAP); in 1991 (Kinzler et al., 1991; Nishisho et al., 1991). Then some researchers found that APC gene could interact with β-catenin and loss of APC function results in overactive T-cell factor 4 (TCF4)/β-catenin signaling. These findings establish a direct link between Wnt/β-catenin signaling pathway and human CRC. Furthermore, more than 90% of sporadic CRCs has been identified to carry mutations of one or more components of the Wnt/β-catenin signaling pathway including APC based on the genome-scale analysis (Network, 2012). Therefore, the canonical Wnt pathway plays an pivotal role in the development of CRC and may be a significant potential target for CRC treatment.

In clinical practice, standard conventional treatments for CRC are surgery, chemo-therapy and radiotherapy. Currently, with the development of tumor molecular targeted therapy, small molecule inhibitors present a great advantage in improving the survival of patients with advanced CRC. Moreover, long-term application of these therapies can lead to various side effects and toxicities, consisting of nausea, vomiting, mucositis, peripheral neuropathy, and diarrhea (Mcquade et al., 2017). Thus, it is urgent to identify new and more effective drugs for CRC treatment. TCMs have been used for more than 2000 years in China. Owing to the low toxicity and the multi-target capacity (So et al., 2019), TCMs are attracting increasing attention and acceptance for the treatment of CRC as it can alleviate chemotherapy-induced side effects and improve the quality of life of patients with CRC. Previous studies have shown that diverse TCMs exhibit excellent anti-tumor activities in both experimental models and clinical cases. In this review, we focused on ongoing strategies of TCMs used to target aberrant Wnt/β-catenin pathway compared with targeted small molecules as a novel therapeutic intervention in different stages of CRC. Taken together, TCMs will become promising alternative drugs to treat cancer with less toxicity and also be used as an adjunctive treatment together with classic drugs for improving therapeutic outcomes in CRC patients.

Wnt/β-Catenin Pathway and CRC

Wnt signaling pathway is a highly conserved signaling pathway in eukaryotes and commonly divided into canonical (β-catenin dependent) and non-canonical (β-catenin independent) pathways (Polakis, 2012). Originally, many components of the Wnt signaling were identified as key mediators of patterning decisions during embryonic development by genetic screening (Mazzotta et al., 2016). In the last decade, aberrant Wnt/β-catenin pathway activation in carcinogenesis has most prominently been described for CRC. Data from the Cancer Genome Atlas (TCGA) suggests that Wnt/β-catenin pathway is activated in 93% of nonhypermutated CRC and 97% of hypermutated CRC (Li et al., 2012; Sebio et al., 2014; Voorneveld et al., 2015). The status of Wnt/β-catenin pathway is mainly related to the stability of β-catenin controlled by the β-catenin destruction complex that is comprised of scaffolding proteins APC, Axin and the kinases casein kinase 1 (CK1) and Glycose synthase kinase 3β (GSK3β). Absence of Wnt ligands stimuli, the cytosolic β-catenin is phosphorylated by GSK3β, ubiquitinated by β-TrCP200 and targeted for proteosomal degradation. The ligand Wnt binds to the cell surface receptor Frizzled and low-density lipoprotein receptor-related protein 5/6 (LRP5/6) to form a trimer, which recruits the Dishevelled (Dvl) protein to the plasma membrane, leading to dissociation of the destruction complex followed by cytosolic accumulation of β-catenin. Consequently, the β-catenin translocates to the nucleus where nuclear β-catenin cooperates with TCF/LEF family transcription factors to active target genes such as c-myc, MMP-7, SNAIL and EGF (Zhan et al., 2017). The activation of Wnt/β-catenin signaling is indispensable for the progression of CRC (Figure 1).

The best-known mutation of APC is the major driver of Wnt pathway in colorectal tumorigenesis which functions as a negative regulator and its importance was further highlighted by several recent studies (Hankey et al., 2018). By using the CRISPR/Cas9 technique to introduce APC mutation into human intestinal organoids, the tumorigenesis of CRC could be modeled in vivo (Drost et al., 2015; Matano et al., 2015). Moreover, these studies in human and mouse models indicated that the genotypes of APC mutations are consistent with the distinct levels of canonical Wnt pathway and these alterations are associated with characteristic tumor locations within the large intestine (Buchert et al., 2010; Christie et al., 2013). Besides APC, ring finger protein 43 (RNF43) mutations and R-spondin translocations are noted in over 18 and 9% patients with CRC respectively by preventing removal of Wnt receptor. Both RNF43 and R-spondin fusion are completely opposite to APC mutations (Schatoff et al., 2017). In addition to the well-established function of Wnt/β-catenin in CRC, there is accumulating evidence indicating that the KRAS is also an important and frequently mutant gene during colorectal carcinogenesis. Up to 40% of KRAS mutations occur in patients with CRC (Arrington et al., 2012). The discovery of small-molecule RAS inhibitors or a siRNA targeting RAS displayed anti-proliferative activity on xenografts of human CRC cell line SW480 (Song et al., 2020). The mutations of KRAS result in the hyper-activation of RAS-extracellular signal-regulated kinase (ERK) pathway involving transformation of cells and tumorigenesis. Series of studies confirmed the regulation of the RAS-ERK pathway by Wnt/β-catenin signaling and its roles, such as Axin, APC, and GSK3β, and so on (Vincan and Barker, 2008). The crosstalk of Ras and Wnt/β-catenin pathways relies on the phosphorylation of RAS mediated by GSK3β. GSK3β, a key component of the β-catenin destruction complex, is identified as a kinase inducing phosphorylations of β-catenin and RAS at the different sites of the threonine, and subsequently recruits the β-TrCP E3 linker for the proteosomal degradation. Inactivation of GSK3β caused by Wnt stimuli or APC loss further leads to high concentration of cytoplasmic β-catenin and KRAS (Lee et al., 2018a). Therefore, both mutations of APC and KRAS have a positive connection with the Wnt/β-catenin pathway in colorectal tumorigenesis (Figure 1).
Metastasis is a hallmark of advanced cancer and a major challenge to clinic treatment. Epithelial-mesenchymal transition (EMT) is a crucial process by which epithelial cells lose cell polarity and cell-cell adhesion, and closely associate with invasion and metastasis in many types of malignancies including CRC (Spaderna et al., 2006; Vu and Datta, 2017). There is a complicated network involved in the regulation of EMT, containing different signaling pathways. Many investigations indicated that aberrant activation of the canonical Wnt pathway promotes EMT-associated dedifferentiation located at the invasive front of colorectal tumors. Enhanced Wnt/β-catenin signaling in CRC cells induces the action of E-cadherin repressors SNAIL and upregulation of matrix metalloproteinases (MMP) involving CRC invasion and metastasis (Gu et al., 2016). However, inactivating mutations of APC and AXIN2 can up-regulate the canonical Wnt pathway, thereby promoting EMT. Furthermore, in vitro and in vivo experiments showed that WNT3a overexpression induces SNAIL expression and promotes invasion (Qi et al., 2014).

In addition, increasing evidences suggest that cancer stem cells (CSCs) theory underlies tumor proliferation, differentiation and metastasis. Although there is still no consensus on the concept of cancer stemness, the vital role of the Wnt pathway for the function of normal and cancer stem cells is commonly accepted (Reya and Clevers, 2005). In the intestinal crypt, Wnt/β-catenin signaling in CRC cells induces the action of E-cadherin repressors SNAIL and upregulation of matrix metalloproteinases (MMP) involving CRC invasion and metastasis (Gu et al., 2016). However, inactivating mutations of APC and AXIN2 can up-regulate the canonical Wnt pathway, thereby promoting EMT. Furthermore, in vitro and in vivo experiments showed that WNT3a overexpression induces SNAIL expression and promotes invasion (Qi et al., 2014).

Small Molecules Targeting Wnt/β-Catenin Pathway for CRC Treatment

Due to the importance of canonical Wnt/β-catenin signaling in human carcinogenic development, small molecule inhibitors targeting Wnt signaling have been developed for the treatment of CRC (Table 1). Activation of Wnt signaling through β-catenin is a critical event in CRC progression. Porcupine (PORCN) is a membrane-bound O-acyltransferase protein which regulates Wnt ligands secretion outside the cell membrane through palmitoylation. In recent years, PORCN has emerged as a molecular target for treating Wnt-driven cancers. ETC-159, WNT974 (LGK974) and Rxc004 has been identified as potent inhibitors of Wnt signaling.
inhibitors of Wnt secretion inhibiting β-catenin activity in preclinical studies. ETC-159 has been proven to be remarkably efficacious in treating CRCs with R-spondin translocation in vivo and in vitro experiments (Soo and Keller, 2015). During in vitro studies in RNF43 mutant and R-spondin fusion CRC cell lines, Rxc004 could potentially repress the cell proliferation by arresting cell cycle at G1/S and G2/M phase (Shah et al., 2021). IWP-2 is another inhibitor of PORCN. Experiments on organoid derived from CRC patients unveiled that IWP-2 is sensitive to the cancers and has greater stability and displays favorable pharmacokinetic properties to inhibit Wnt/β-catenin pathway for CRC treatment. PRI-724, the second generation specific CBP/catenin antagonist for oncology, has been proved to have an acceptable safety profile in early clinical trials and is now under further clinical investigation (Bahrami et al., 2017). Windorphen (WD) is an inhibitor of Wnt/β-catenin signaling by directly targeting p300 to disrupt the association of β-catenin with p300. These findings suggest that WD can selectively kill cancer cells with aberrant activation of Wnt signaling (Hao et al., 2013). Other small molecules, such as NSC668036 and Pen-N3, block the Wnt signaling pathway through binding to the Dishevelled (Dvl) PDZ domain and interrupting the receptor Frizzled (Fz)-Dvl interaction in colon cells (Shan et al., 2005; Zhang et al., 2009).

Some studies indicate that tankyrases (TNKS) are novel targets for Wnt inhibition by regulating stabilization of Axin and hence leading to increased β-catenin degradation. XAV939 and JW55 have been shown to target Wnt/β-catenin pathway through inhibiting the poly-ADP-ribose polymerase (PARP) domains of TNKS in DLD-1 and SW480 cell lines in vitro (Huang et al., 2009). JW55 also reduces the growth of tumor in conditional APC mutation mice (Waaler et al., 2012). G007-LK and G244-LM are two other types of small-molecule tankyrase inhibitors (Lau et al., 2013). In particular, G007-LK has greater stability and displays favorable pharmacokinetic properties to inhibit Wnt/β-catenin signaling in APC-mutant CRC xenograft tumors (Tanaka et al., 2017; Katoh, 2018). IWR-1 is another tankyrase inhibitor which interacts with PARP enzyme (Mashima et al., 2017).

β-catenin is a key mediator of Wnt signaling, regulating the stabilization of the destruction complex and consequently intracellular β-catenin levels. Ewan K et al. revealed that three small molecule inhibitors including CCT031374, CCT036477, and CCT070535 can block the Wnt/β-catenin signaling through reducing the level of β-catenin without altering its stability, which

| Small molecules          | Mechanism of action                                   | Preclinical vs. clinical trial (phase) vs. FDA approved | Reference               |
|--------------------------|-------------------------------------------------------|--------------------------------------------------------|-------------------------|
| ETC-159                  | Porcupine inhibitor                                    | Phase 1                                                | Soo and Keller (2015)   |
| WNT7874 (LGK974)         | Porcupine inhibitor                                    | Phase 1                                                | Shah et al. (2021)      |
| Rxc004                   | Porcupine inhibitor                                    | Phase 1/2                                              | Masaru (2017)           |
| IWP-2                    | Porcupine inhibitor                                    | Preclinical                                            | Monttazi-Borojeni et al. (2018) |
| Pyrvinium                | Binding to CK1a                                         | FDA approved                                           | Bahrami et al. (2017)   |
| ICG-001                  | Binding to CBP                                         | Preclinical                                            |                         |
| PRI-724                  | CBP/β-catenin inhibitor                                 | Phase 1b                                               | Hao et al. (2013)       |
| Windorphen               | P300/β-catenin inhibitor                                | Preclinical                                            | Shan et al. (2005)      |
| NOSC668036               | Binding to Dishevelled                                  | Preclinical                                            | Zhang et al. (2009)     |
| Pen-N3                   | Binding to Dishevelled                                  | Preclinical                                            | Huang et al. (2009)     |
| XAV939                   | Tankyrase inhibitor                                    | Preclinical                                            | Waaler et al. (2012)    |
| JW55                     | Tankyrase inhibitor                                    | Preclinical                                            | Lau et al. (2013)       |
| G007-LK                  | Tankyrase inhibitor                                    | Preclinical                                            | Narwal et al. (2012)    |
| G244-LM                  | Tankyrase inhibitor                                    | Preclinical                                            |                         |
| IWR-1                    | Tankyrase inhibitor                                    | Preclinical                                            | Mashima et al. (2017)   |
| CCT031374                | β-catenin inhibitor                                    | Preclinical                                            | Ewan et al. (2010)      |
| CCT036477                | β-catenin inhibitor                                    | Preclinical                                            |                         |
| CCT070535                | β-catenin inhibitor                                    | Preclinical                                            |                         |
| ICRT3                    | β-catenin/Tcf                                          | Preclinical                                            | Gonsalves et al. (2011) |
| ICRT5                    | β-catenin/Tcf                                          | Preclinical                                            |                         |
| ICRT14                   | β-catenin/Tcf                                          | Preclinical                                            |                         |
| PKF115-584               | β-catenin/Tcf                                          | Preclinical                                            | Yan M. et al. (2017)    |
| PKF222-815               | β-catenin/Tcf                                          | Preclinical                                            | Tian et al. (2012)      |
| CGP049090                | β-catenin/Tcf                                          | Preclinical                                            |                         |
| BC21                     | β-catenin/Tcf                                          | Preclinical                                            | He et al. (2017)        |
| NC403                    | β-catenin/Tcf                                          | Preclinical                                            | Lee et al. (2018b)      |
| KYA1797k                 | GSK3β activator                                        | Preclinical                                            | Cho et al. (2016)       |
| KY1022                   | GSK3β activator                                        | Preclinical                                            |                         |

| Reference                |                                                      |                                                       |                         |

**TABLE 1 | List of small molecules targeting Wnt/β-catenin pathway for CRC treatment.**
### TABLE 2 | Effects of monomers, extracts, formula of TCMs on CRC by Wnt/β-catenin pathway.

| Herbal medicine | Stage | Cell | Animal | Cellular mechanism | Wnt related targets | References |
|-----------------|-------|------|--------|-------------------|---------------------|------------|
| Berberine       | Polyps| KM12C| Apc Min/+ mice | Proliferation | β-catenin, APC | Zhang J. et al. (2013) |
| Genistein       | EESB  | HT29 | SD Rat BALB/c nude mice | Differentiation | Wnt5a, Strp1,2,5 | Zhang et al. (2020) | Wei et al. (2017) |
| Bryonicin       | CRC   | DLD1, SW480, LoVe | Nude mice | Proliferation, Apoptosis | APC, 8-catenin, Dkk1 | Ren et al. (2019) |
| Luteolin        | CRC   | HCT15 | BALB/c mice | Proliferation | GSK-3β, 8-catenin, Aehokium and Sudhantran (2011) | Menval et al. (2015) |
| C. brachycephalum PAG | CRC | HCT116 | - | Proliferation, Apoptosis | GSK-3β, 8-catenin | Qiu et al. (2017) |
| Wogonin         | NG | HCT116, SW620 | - | Progesterone, Apoptosis, | GSK-3β, 8-catenin | Li et al. (2020) | Wen et al. (2019) |
| IBC             | CRC   | HCT116, HT29, SW480, LoVe, C57Bl-CoN-841 | BALB/c nude mice | Proliferation, Apoptosis | APC, β-catenin | Li et al. (2019) | Ye et al. (2019) |
| Rg3             | CRC   | HCT116, SW480 | Athymic nude mice | Proliferation | β-catenin | He B.-C. et al. (2011) |
| Isoquercitrin    | CRC   | HCT116, DLD-1, SW480 | Xenopus embryos c57Bl/6 mice | Cell cycle, Stemmness, EMT | β-catenin, YAP, LKB1 | Guo et al. (2020) |
| RTHF            | CRC   | SW620, HT29 | | Proliferation, EMT | β-catenin | Wu et al. (2018) |
| Teg              | CRC   | NICHT3, HT29, HCT116, SW480 | Female athymic nude mice | Proliferation, Apoptosis | β-catenin, YAP, LKB1 | Li et al. (2019) | He B.-C. et al. (2011) |
| Curcumin        | CRC   | SW620, HCT116 | - | Proliferation, Apoptosis | β-catenin, Wnt3a | Jiang X. et al. (2019) |
| Beta- elemene    | CRC   | HCT116, HT29 | - | Proliferation, Apoptosis | β-catenin, Wnt3a | - | |
| Celasostol      | CRC   | HCT116, HT29, SW480, LoVo, | APC Min/+ mice c57Bl/6 mice | Proliferation, Apoptosis | β-catenin, YAP, LKB1 | Wang et al. (2019) | Guo et al. (2020) |
| Quercetin        | CRC   | SW480, clone 26 | | Proliferation, Apoptosis | β-catenin, Tcf4 | Shan et al. (2019) | Lei et al. (2019) |
| Apigenin         | CRC   | SW480, HCT15 | - | Proliferation | β-catenin, Tcf4 | Xu et al. (2016) | Kaur et al. (2010) |
| Silbinin         | CRC   | SW480 | - | Proliferation | β-catenin, Tcf4 | |
| Lonchocarpin     | CRC   | HCT116, SW480 | Xenopus embryos | Progesterone | β-catenin, Tcf4 | Predes et al. (2019) | Li et al. (2013) | Krishnarnachary et al. (2019) | |
| Hennyn          | CRC   | HCT116, SW480, HT29, | Nude mice | Proliferation, Apoptosis | β-catenin, Tcf4 | Zhang T et al. (2013) | Ji et al. (2013) |
| Resveratrol      | CRC   | T1,T2 | | Stemness | β-catenin, Tcf4 | |
| IPM711           | CRC   | HCT116, SW480 | | | β-catenin, Fzd | |
| TKP             | CRC   | DLD1, HCT116 | - | MMP2, MMP9 | GSK-3β | Sun et al. (2020) |
| Cinnamaldehyde   | CRC   | HCT116, SW480 | BALB/c nude mice | EMT, Stemness | β-catenin, GSK-3β | Wu et al. (2019) |
| ZJW             | CRC   | SW403 | | Proliferation, MMPs | β-catenin, Axin1, Dvl2,3, GSK-3β, Le1,Tcf4 | Pan et al. (2017) |
| WCA             | CRC   | HCT116 | - | MMPs, EMT | β-catenin | Tao et al. (2019) | Chan et al. (2020) |
| AP              | CRC   | HT29 | ICR mice | Proliferation | β-catenin | Li et al. (2020) |
is different from drugs involving inhibition of TCF-dependent transcription in SW480 cells (Ewan et al., 2010). Interaction of β-catenin with TCF binding proteins is a crucial step in the activation of target genes in response to the activation of Wnt/β-catenin pathway. A cohort of Wnt antagonists including iCRT3, iCRT5, iCRT14, PKF115-584, PKF222-815, CGP049090, and BC21 have been demonstrated to suppress the Wnt/β-catenin signaling by breaking the association between Tcf4 and β-catenin (Gonsalves et al., 2011; Tian et al., 2012; Yan M. et al., 2017). NC043 is an inhibitor of β-catenin/Tcf4, which decreases β-catenin/Tcf4 association without affecting the cytosol-nuclear distribution of soluble β-catenin in vivo and in vitro (He et al., 2017).

In recent years, a small molecular KYA1797K has been identified to suppress the formation of CRCs along with the mutations of APC and KRAS via activating GSK3β and subsequently reducing the level of both β-catenin and Ras as showed both in vitro and in vivo studies. Moreover, KYA1797K can alleviate the resistant to the EGFR-targeting therapies because of KRAS mutations (Lee et al., 2018b). Whereas, KY1022 destabilizes both β-catenin and Ras by targeting the Wnt/β-catenin signaling in the process of metastasis involving EMT, which is different from the action of KY1797K (Cho et al., 2016). As indicated above, small molecule inhibitors targeting Wnt/β-catenin pathway exhibit promising therapeutic effects on CRC. However, to the best of our knowledge, few of these small molecules has gone into clinical trials. In the future, many scientists will make great efforts to identify more small molecules targeting Wnt/β-catenin and convert them into effective therapies.

Therapeutic Mechanism of TCMs Against CRC via Wnt/β-Catenin Pathway

It is well documented that uncontrolled cell proliferation is a typical feature in many types tumor development, especially in CRC. The complex balance between proliferation and apoptosis is intimately connected with tissue homeostasis (Diwanji and Bergmann, 2018) and in general, increased cell proliferation along with reduced apoptosis, drives tumor formation. It has been found that many compounds or extracts from TCMs could inhibit colorectal tumorigenesis by targeting different molecules in Wnt/β-catenin pathway. Therefore, we summarized the single-herb and formula of TCM against the different stages of CRC via Wnt/β-catenin pathway (Table 2).

EFFECT OF ACTIVE COMPOUNDS ON PRECANCEROUS CRC

The presence of adenoma (polyps), is a precursor and a major risk factor for CRC (Nguyen et al., 2020). Currently, endoscopic removal is the most effective therapeutic regimen for these patients. However, TCMs also have been reported to exhibit important therapeutic effects on colon adenomas. Alkaloid berberine, which is previously used as an anti-inflammatory drug, has proximately been demonstrated to possess anti-tumor activity by reducing Wnt activity and its mechanism of action may involve inhibition of β-catenin translocation to the nucleus by enhancing the expression of APC gene and stabilizing the complex of APC-β-catenin. Studies looking at berberine treatment in vivo have found that it gave rise to reduced formation of polyps accompanied with a decrease in cyclin D1 and c-myc expression in the intestinal adenoma model. Furthermore, oral administration of berberine has been confirmed to significantly reduce the size of polyps in patients with FAP (Zhang J. et al., 2013). In addition, the discovery of Aberrant crypt foci (ACF) in early colorectal adenomas provided new opportunities to explore the pathogenic mechanism of CRC. Genistein, a soya isoflavone, is capable of decreasing the number of total aberrant crypts in the colon cancer model with azoxymethane (AOM) injection by repressing the expressions of Wnt/β-catenin target genes, including Wnt5a, Sfrp1, Sfrp2, Sfrp5, and c-Myc. These results revealed a novel role for genistein as a suppressor of carcinogen-induced Wnt/β-catenin signaling and the prevention of early colon neoplasia (Zhang et al., 2020).

THERAPEUTIC MECHANISM OF ACTIVE COMPOUNDS AGAINST CRC IN SITU

Ninety-three percent of CRC cases has at least one mutation in Wnt/β-catenin pathway genes (Pearlman et al., 2017). The most frequently mutated gene in CRC is APC which may be a promising target for drug development in CRC. The ethanol extract of Scutellaria barbata D. Don (EESB), used for the treatment of various types of cancer clinically (Wei et al., 2017; Zhang et al., 2017; Liu et al., 2018), has been found to prevent the development of human CRC via increasing APC expression with a concomitant decrease in the expression of β-catenin, leading to inactivation of the Wnt/β-catenin pathway in a CRC xenografted mouse model and HT-29 cell line. Brucine and strychnine from nux vomica have remarkable effects in improving circulatory system and relieving arthritic and traumatic pains. Recently, Ren H et al. (2019) found both two compounds can suppress the growth significantly by inducing the apoptosis of CRCs in nude mice by enhancing the expression of APC and reducing that of β-catenin. Meanwhile, they can greatly promote DKK1 expression, which is proved to negatively regulate Wnt/β-catenin pathway. On the other hand, some monomers derived from traditional Chinese herbs such as Luteolin, C. brachycephalum, pterisolic acid G (PAG), wogonin, nerigoside (NG) and isobavachalcone (IBC), exhibit anticancer functions by affecting the phosphorylation state of GSK-3β and β-catenin in CRC. However, nerigoside has been found to destroy the balance of proliferation and apoptosis through the ERK/GSK3β/β-catenin signaling pathways, whereas isobavachalcone exerts its anticancer effect via the AKT/GSK-3β/β-catenin pathway in CRC (Ashokkumar and Sudhandiran, 2011; Mervai et al. (2015); Qiu et al., 2017; Li et al., 2019; Tan et al., 2019; Wen et al., 2019).

There are some compounds inhibiting CRC by mediating the core molecule of canonical Wnt pathway. Ye ZN et al. discovered that the anti-tumor effect of four β HWE is to promote the phosphorylation and degradation of β-catenin and the subsequent inhibition of its nuclear translocation in CRC cells.
(Ye et al., 2019). While, Ginsenoside Rg3 and isouqueretin were demonstrated to inhibit Wnt/β-catenin pathway by blocking nuclear translocation of the β-catenin protein and hence inhibiting β-catenin/Tcf transcriptional activity (He B. et al., 2011). Moreover, some experiments in vitro showed that Radix Tetrastigma hemsleyani flavone (RTHF), 1,4,6-Tri-O-galloyl-β-d-glucopyranose (TGG) as well as tetrandrine (TET) could suppress colorectal tumor growth and downregulate target genes expression (He B.-C. et al., 2011; Wu et al., 2018; Li et al., 2019). Curcumin is another inhibitor of β-catenin in many cancers (Deguchi, 2015). Previous studies illustrated caudal type homeobox-2 (CDX2) is a mediator of the Wnt signaling pathway, and curcumin can reduce cell proliferation and increase apoptosis by restoring CDX2 which inhibited the Wnt/β-catenin signaling pathway (Jiang X. et al., 2019). Besides, curcumin might exert anti-resistant effect of 5-FU on rHCT-116 cells by controlling WNT signal pathway to reverse the EMT progress (Lu et al., 2020). Beta-elemene, however, could elevate sensitivity to 5-FU through down-regulating miR-191 and preventing the Wnt/β-catenin pathway in CRC cells (Guo et al., 2018). Lately, accumulating evidence has strongly suggested Hippo signaling interacted with Wnt/β-catenin pathway. (Jiang Z. et al., 2019). found that celastrol, isolated from Triptererygium wilfordii plant, exerted antitumor effects by accelerating β-catenin degradation via the HSF1–LKB1–AMPKα–YAP pathway in CRC. In addition, miRNA microarray analysis suggested that black raspberry (BRB) anthocyanins can reduce the expression of miR-483–3p accompanied by an increased level of DKK3 expression, which is one negative regulator of Wnt pathway (Guo et al., 2020).

Some studies revealed that quercetin and columbamine (inhibitors of the Wnt/β-catenin pathway) could decrease nuclear catenin and downregulate the transcriptional activity of β-catenin/Tcf, leading to inhibition of cell proliferation in SW480 cell lines (Pahlke et al., 2006; Lei et al., 2019). Similar to quercetin and columbamine, apigenin can suppress CRC proliferation by inhibiting β-catenin nuclear entry and thereby prevented the expression of Wnt downstream target genes (Xu et al., 2016). Silibinin and lonchocarpin, also exert anticancer functions through the regulation of β-catenin/Tcf transcriptional activity in animal and cell models (Kaur et al., 2010; Predes et al., 2019). Yet silibinin exhibited selective growth inhibitory effects on SW480 cells (human CRC cells), but not HCT116 cells, by inhibition of Wnt signaling. Henryin, used to control pain for a long time, has been reported to be capable of impairing the inhibition of Wnt/β-catenin/Tcf transcriptional complex through direct blockade of β-catenin binding to TCF4, but not to affect the cytosol to nuclear distribution of soluble β-catenin (Li et al., 2013). In addition, γ-mangostin, found in Mangosteen fruit, can interact with the transcription factor TCF4 at the β-catenin binding domain, which results in the suppression of the expression of cyclin D1 and c-Myc. Furthermore, γ-mangostin treatment significantly decreased the levels of stem cell markers such as LGR5, Dclk1 and CD44 in HCT116, LS174T and DLD1 cells, which also confirmed in vivo models (Krishnamachary et al., 2019). In the last few decades, the existence of CSCs is central to chemo-resistance and recurrence of many tumors. Some studies identified Huaier aqueous extract can take action against CRC by eradicating CSCs and the Wnt pathway may be considered as a potential target of Huaier for the treatment of CRC (Zhang T. et al., 2013).

**EFFECT OF TCM FORMULAS ON CRC**

As well as the monomers and extracts derived from TCMs, an increasing body of evidence suggests that TCM formulas possess anticancer properties, too. Zuo Jin Wan (ZJW) has been used in the treatment of gastrointestinal and liver diseases in China for ages (Chao et al., 2011; Sun et al., 2019), which is composed of Rhizoma Coptidis and Evodia Rutaecarpa at a ratio of 6:1. Berberine and evodiamine are two key elements of ZJW extract and possess anti-tumorigenic activity, respectively (Ayati et al., 2017; Wang et al., 2019). Over the past few decades, many clinical studies had found that some subtypes of 5-HT receptors (5-HTRs) would enhance the proliferation of CRC cells. Recent studies showed that ZJW extracts can exert anti-tumorigenic effects by suppressing the canonical Wnt/β-catenin pathway in animal and cell experiments, similar to that seen with 5-HTR antagonists (Pan et al., 2017). Weichang' an (WCA) is a traditional Chinese medicinal formula used as an anticancer drug and the experimental data also showed the antimetastatic function by blunting the activation of Wnt/β-catenin pathway and reducing the expression of MMP9 and the EMT-related protein ZEB1 (Tao et al., 2019). Furthermore, TCM formulations could provide an adjunct for chemotherapy in cancer patients. Huanglian jiedu Decoction (HLJDD) has been revealed to significantly alleviate the diarrhea induced by chemotherapy in a mouse model. The experiments from the intestinal segments of 5-Fu/CPT-11-treated mice proved pre-treatment with HLJDD could activate the Wnt/β-catenin
pathway by inducing the expressions of Wnt signaling components, comprised of Wnt3, Fzd5, Axin2, and Pygo2 (Chan et al., 2020). These data suggest that HJJD could boost the regeneration of intestinal progenitor cells after chemotherapy, probably by activating Wnt/β-catenin.

In addition, TCMs can also prevent the development of colitis associated colorectal cancer (CACC) through canonical Wnt signaling. It is showed that apple polysaccharide (AP) from apple residues could affect the activation of Wnt/β-catenin signaling pathway in vivo, but not in vitro experiments (Li et al., 2020). Previous studies showed that AP treatment could effectively decrease the proliferation of Fusobacterium in AOM/DSS-induced intestinal tract. Therefore, AP may restrain the activation of Wnt/β-catenin signal pathway in CACC mice through controlling the imbalance of intestinal flora.

### Development of New Drugs in Clinic

CRC is often diagnosed at an advanced stage when tumor cell dissemination has already taken place and chemotherapy was one of the major methods for the treatment of CRC in the past few decades. In clinic, it is obviously clear that fluoropyrimidines, irinotecan, and oxaliplatin have been widely applied to chemotherapeutic regimens for tumors (Gustavsson et al., 2015). The recent introduction of small molecular target agents, such as anti-EGFR (cetuximab, panitumumab) and antiangiogenic molecules (bevacizumab) have led to profound improvements in the life expectancy of patients with advanced CRC (Franke et al., 2019), but with potential lethal adverse drug events and drug resistance. Therefore, it is necessary to develop new and neo-adjuvant therapies in combination with other chemotherapeutics. TCMs and their active compounds with multi-targets was reported to prevent and treat CRC patients as promising candidates, which is distinct from small molecular inhibitors that depend on single target (Yeh et al., 2020). In addition, because of relatively lower toxicity and cheaper price, TCMs can be more accepted by patients with CRC physically and psychologically.

On account of the significance of Wnt/β-catenin pathway in CRC development and metastasis, some native components of TCMs was developed as novel drugs specifically targeting this signaling pathway and are already in clinical trials (Table 3). Resveratrol is a naturally occurring polyphenol with antioxidant, which has been used in many diseases involving cancers. Recently, in vitro studies suggest that resveratrol exhibited preventative colon cancer effects and this was associated with Wnt signaling (93). In this clinical trial, patients with colon cancer were randomly provided a treatment with resveratrol, and relevant studies tested its effects directly on colon cancer and normal colonic mucosa. These results showed that resveratrol could inhibit Wnt/β-catenin signaling in the normal colonic mucosa, but not in colon cancer (Nguyen et al., 2009). Thus, resveratrol represented a potential colon cancer preventive strategy in this phase I study. Genistein is also identified to block Wnt/β-catenin signaling and has a cooperative effect with chemotherapeutic agents in lab. According to pre-clinical data, investigators found that combining genistein with standard chemotherapeutic regimens could reduce chemotherapy resistance and improve patient’s response rates (Authors Anonymous, 2021b).

Besides, small molecular weight Wnt 974, a potential inhibitor of Wnt/β-catenin signaling, has been used to assess its safety and antitumor activity in combination with chemotherapeutic agents in patients with BRAF-mutant metastatic CRC and Wnt pathway mutations (Authors Anonymous, 2021c). Nevertheless, so far, the study results have not been published. ABT-888 (veliparib) has also been used in combination with chemotherapeutic drugs to inhibit the growth of metastatic CRC in phaseI/II clinical trials (Authors Anonymous, 2021d). But it has not yet been approved.

| New drug       | Disease or condition | Combination with                | Aim or result                                                                 | Phase | Recruitment status |
|----------------|----------------------|--------------------------------|-------------------------------------------------------------------------------|-------|--------------------|
| Resveratrol    | Colon cancer         | -                              | Resveratrol represented a potential colon cancer preventive strategy in this phase I study | Phase I | completed          |
| Genistein      | Metastatic CRC       | FOLFOX or FOLFOX-Avastin       | Combining genistein with the standard of care chemotherapeutic regimens reduced chemotherapy resistance and improved response rates | Phase I and II | completed |
| Wnt 974 (LGK974) | BRAFV600-mutant Metastatic CRC | LGX818 and Cetuximab | The triple combination of WNT974, LGX818 and cetuximab could result in anti-cancer activity with the inhibition of Wnt and BRAF signals | Phase Ib/II | completed |
| ABT-888 (veliparib) | CRC that cannot been cured by surgery | Temozolomide | Combining veliparib and temozolomide was well-tolerated at doses up to 200 mg/m²/day of temozolomide | Phase II | completed |
| Foxy5          | Metastatic CRC       | -                              | The aim is to set up the recommended drug dose for use in the subsequent clinical phase 2 study and develop Foxy-5 as a first-line drug in anti-metastatic cancer | Phase I | completed          |
| Foxy5          | CRC with low Wnt-5a  | Surgery to remove the tumour and then giving treatment with FOLFOX about 6 months | In this trial the safety and tolerability of Foxy-5 will be built and early signs of anti-metastatic activity will be evaluated in subjects with resectable colon cancer | Phase II | Recruiting         |
| Niclosamide    | FAP                  | Placebo                        | Niclosamide has been indicated to have a inhibitory effect on tumorigenesis via inhibition of Wnt pathway with no significant safety issues | Phase II | Recruiting         |

### TABLE 3 | New drugs inhibiting Wnt/β-catenin pathway for treatment CRC in clinic.
CONCLUSIONS AND FUTURE PERSPECTIVES

CRC has become a global public health problem on account of its high incidence and mortality rate worldwide. The clinical treatments for CRC mainly involve surgery-based chemotherapy. In recent years, with the application of targeting small molecules against cancer, the quality of life for CRC patients has improved. Nevertheless, chemotherapy-induced side effects and drug resistance remain a major issue for clinical practice. Numerous studies have shown that TCMs can be used to exert potent anticancer activity and alleviate the side effects associated with chemotherapy. It is confirmed that various mutations in one or more members of the canonical Wnt signaling pathway take place in the progression of CRC. Therefore, in this review, we aimed to intensively explore molecular mechanisms of TCMs against cancer at the different stages in CRC processes, including precancerous lesions, early stage CRC and CRC invasion and migration based on the inhibition of the Wnt/β-catenin signaling pathway. Cell culture and animal experiments have found that TCMs play anticancer roles by regulating APC/β-catenin, GSK-3β/β-catenin, and β-catenin/TCF4 pathways which represent the main elements of the Wnt/β-catenin pathway involved in the treatment of CRC. Thus, understanding the molecular mechanisms of action of TCMs and how they target Wnt/β-catenin may shed light on future therapies for CRC. However, it needs multi-level and multi-link comprehensive action to anti-tumor because of the complex composition of traditional Chinese medicine. This suggests that we need to investigate the crosstalk between Wnt/β-catenin signal pathway and others. In addition, there remains very few new clinical treatments under development due to lack of strict evaluation system for effectiveness and safety of TCMs. Therefore, it will hopefully pave the way for the CRC clinical treatment and may also relieve the side effects related to chemotherapy if there is a breakthrough in the study of multi-target intervention of TCM in CRC.

AUTHOR CONTRIBUTIONS

Chang JC wrote the article; ZB and HW took part in the critical revision of this article; all of the authors had no objection to the final article.

FUNDING

Supported by the Guangdong Natural Science Foundation, No. 2017A030312009.

ACKNOWLEDGMENTS

I wish to thank all members of ZB’ research team in school of Chinese medicine, Hong Kong Baptist University, as well as Jianyong Xiao.

REFERENCES

Amado, N., Predes, D., Moreno, M., Carvalho, L., Mendes, F., and Abreu, J. (2014). Flavonoids and Wnt/β-Catenin Signaling: Potential Role in Colorectal Cancer Therapies. Int. J. Mol. Sci. 15 (7), 12094–12106. doi:10.3390/ijms150712094
Arrington, A. K., Heinrich, E. L., Lee, W., Duldulao, M., Patel, S., Sanchez, J., et al. (2012). Prognostic and Predictive Roles of KRAS Mutation in Colorectal Cancer. Int. J. Mol. Sci. 13 (10), 12153–12168. doi:10.3390/ijms131012153
Ashokkumar, P., and Sudhandiran, G. (2011). Luteolin Inhibits Cell Proliferation During Azaoyxymethane-Induced Experimental Colon Carcinogenesis via Wnt/Beta-Catenin Pathway. Invest New Drugs 29 (2), 273–284. doi:10.1007/s10637-009-9559-9
Authors Anonymous (2021a). Cancer Today. Available at: https://gco.iarc.fr/today/fact-sheets-cancers.. (accessed 6 9 2021)
Authors Anonymous (2021b). Genistein in Treatment of Metastatic Colorectal Cancer - Full Text View - ClinicalTrials.Gov. Available at: https://clinicaltrials.gov/ct2/show/NCT02519582?term=colorectal+cancer&draw=2&rank=1. (accessed 6 9 2021)
Authors Anonymous (2021c). Study of WNT974 in Combination with LGX818 and Cetuximab in Patients with BRAF-Mutant Metastatic Colorectal Cancer (mCRC) and Wnt Pathway Mutations-Full Text View-ClinicalTrials.Gov. Available at: https://clinicaltrials.gov/ct2/show/NCT02278133?term=wntr974&cond=colorectal+cancer&draw=2&rank=1. (accessed 6 9 2021)
Authors Anonymous (2021d). A Study of ABT-888 in Combination with Temozolomide for Colorectal Cancer - Full Text View-ClinicalTrials.Gov. Available at: https://clinicaltrials.gov/ct2/show/NCT01051986?term=ABT-888&cond=colorectal+cancer&draw=2&rank=1. (accessed 6 9 2021)
Authors Anonymous (2021e). Phase I Study to Evaluate Safety, Tolerability, Anti-tumor Activity and PK Profiles of Foxy-5 in Metastatic Breast, Colon or Prostate Cancer - Full Text View-ClinicalTrials.Gov. Available at: https://clinicaltrials.gov/ct2/show/NCT02020291?term=Foxy5&cond=colorectal+cancer&draw=2&rank=1. (accessed 6 9 2021)
Authors Anonymous (2021f). Drug Trial to Investigate the Safety and Efficacy of Niclosamide Tablets in Patients with Metastases of a Colorectal Cancer Progressing after Therapy - Full Text View - ClinicalTrials.Gov. Available at: https://clinicaltrials.gov/ct2/show/NCT02519582?term=niclosamide&cond = colorectal+cancer&draw=2&rank=1. (accessed 6 9 2021)
Ayati, S. H., Fazeli, B., Montazami-Boroujeni, A. A., Cicero, A. F. G., Pirro, M., and Sahebkar, A. (2017). Regulatory Effects of Berberine on microRNAome in Cancer and Other Conditions. Crit. Rev. Oncology/Hematology 116, 147–158. doi:10.1016/j.critrevonc.2017.05.008
Bahrami, A., Amerizadeh, F., Shahidsales, S., Khazaei, M., Ghayour-Mobarhan, M., Sadeghnia, H. R., et al. (2017). Therapeutic Potential of Targeting Wnt/β-Catenin Signaling.
β-Catenin Pathway in Treatment of Colorectal Cancer: Rational and Progress. J. Cell. Biochem. 118 (8), 1979–1983. doi:10.1002/jcb.25903

Barker, N., Ridgway, R. A., van Es, J. H., van de Wetering, M., Bögthel, H., van den Born, M., et al. (2009). Crypt Stem Cells as the Cells-Of-Origin of Intestinal Cancer. Nature 457 (7229), 608–611. doi:10.1038/nature07602

Buchert, M., Athineos, D., Abad, H. E., Burke, Z. D., Faux, M. C., Samuel, M. S., et al. (2010). Genetic Dissection of Differential Signaling Threshold Requirements for the Wnt/β-Catenin Pathway In Vivo. Plos Genet. 6 (1), e1000816. doi:10.1371/journal.pgen.1000816

Chan, Y.-T., Cheung, F., Zhang, C., Fu, B., Tan, H.-Y., Norimoto, H., et al. (2020). Ancient Chinese Medicine Herbal Formula Huanglian Jiedu Decoction as a Neoadjuvant Treatment of Chemotherapy by Improving Diaherrea and Tumor Response. Front. Pharmacol. 11, doi:10.3389/fphar.2020.00252

Chao, D.-C., Lin, L.-J., Kao, S.-T., Huang, H.-C., Chang, C.-S., Liang, J.-A., et al. (2011). Inhibitory Effects of Zuo-Jin-Wan and its Alkaloidal Ingredients on Activator Protein 1, Nuclear Factor-κ, and Cellular Transformation in HepG2 Cells. Fitoterapia 82 (4), 696–703. doi:10.1016/j.fitote.2011.02.009

Cho, Y.-H., Cha, P.-H., Kaduwala, S., Park, J.-C., Lee, S.-K., Yoon, J.-S., et al. (2016). KY1022, A Small Molecule Destabilizing Ras via Targeting the Wnt/β-Catenin Pathway, Inhibits Development of Metastatic Colorectal Cancer. Oncotarget 7 (49), 81727–81740. doi:10.18632/oncotarget.13172

Christie, M., Jorissen, R. N., Mouradov, D., Sakhitanandenswaren, A., Li, S., Day, F., et al. (2013). Different APC Genotypes in Proximal and Distal Sporadic Colorectal Cancers Suggest Distinct WNT/β-catenin Signaling Thresholds for Tumourigenesis. Oncogene 32 (39), 4675–4682. doi:10.1038/onc.2012.486

Clara, J. A., Monge, C., Yang, Y., and Takebe, N. (2020). Targeting Signalling Pathways and the Immune Microenvironment of Cancer Stem Cells - a Clinical Update. Nat. Rev. Clin. Oncol. 17 (4), 204–232. doi:10.1038/s41571-019-0293-2

Deguchi, A. (2015). Curcumin Targets in Inflammation and Cancer. Emidt 15 (2), 88–96. doi:10.2174/18715310156661053160120458

Diwanji, N., and Bergmann, A. (2018). An Unexpected Friend – ROS in Apoptosis-Induced Compensatory Proliferation: Implications for Regeneration and Cancer. Semin. Cell Dev. Biol. 80, 74–82. doi:10.1016/j.semcdb.2017.07.004

Drost, J., van Jaarsveld, R. H., Ponsioen, B., Zimberlin, C., van Boxtel, R., Buijs, A., Ewan, K., Pajak, B., Stubbs, M., Todd, H., Barbeau, O., Quevedo, C., et al. (2010). A Small Molecule Approach to Identify Novel Small-Molecule Inhibitors of Wnt/β-Catenin Signaling Pathway. Oncotarget 1 (11), 1–17003.

He, B., Gao, J., Luo, X., Luo, J., Shen, J., Wang, L., et al. (2011). Ginsenoside Rg3 Inhibits Colorectal Tumor Growth through the Down-Regulation of Wnt/β-Catenin Signaling. Int. J. Oncol. 38 (2), 437. doi:10.3892/ijo.2010.858

He, B.-C., Gao, J.-L., Zhang, B.-Q., Luo, Q., Shi, Q., Kim, S. H., et al. (2011). Tetrandrine Inhibits Wnt/β-Catenin Signaling and Suppresses Tumor Growth of Human Colon Cancer. Mol. Pharmacol. 79 (2), 211–219. doi:10.1124/mol.110.068668

He, X., Zhang, W., Yan, C., Nie, F., Li, C., Liu, X., et al. (2017). Chemical Biology Reveals CARF as a Positive Regulator of Canonical Wnt Signaling by Promoting TCF/β-catenin Transcriptional Activity. Cell Discov 3, 17003. doi:10.1038/celldisc.2017.7

Huang, S. M., Mishina, Y., M., Liu, S., Cheung, A., Stegmeier, F., Michaud, G. A., et al. (2009). Tankyrase Inhibition Stabilizes Axin and Antagonizes Wnt Signalling. Nature 461 (7264), 614–620. doi:10.1038/nature08356

Isobe, T., Hisamori, S., Hogan, D. J., Zabala, M., Hendrickson, D. G., Dalpera, A., et al. (2014). miR-142 Regulates the Tumorigenicinity of Human Breast Cancer Stem Cells through the Canonical Wnt Signaling Pathway. Elite 3, e01977. doi:10.15544/elite1977

Jiang, X., Li, S., Qiu, X., Cong, J., Zhou, J., and Miu, W. (2019). Curcumin Inhibits Cell Viability and Increases Apoptosis of SW620 Human Colon Adenocarcinoma Cells via the Caudal Type Homeobox-2 (CDX2)/Wnt/β-Catenin Pathway. Med. Sci. Monit. 25, 7451–7458. doi:10.12659/MSM.918364

Jiang, Z., Cao, Q., Dai, G., Wang, J., Liu, C., Lv, L., et al. (2019). Celastrol Inhibits Colorectal Cancer through TGFB-β/Smad Signaling. Onco Targets Ther. 12, 509–518. doi:10.2147/OTT.S187817

Katoh, M. (2018). Multi-layered P-revention and T-reatment of C-hronic I-nflammation, O-ragan F-ibrosis and Cancer A-associated with C-anonical Wnt/β-catenin S-signaling A-ctivation (Review). Int. J. Mol. Med. 42 (2), 713–725. doi:10.3892/ijmm.2018.3689

Kaur, M., Velmurugan, B., Tyagi, A., Agarwal, C., Singh, R. P., and Agarwal, R. (2010). Silibinin Suppresses Growth of Human Colon Carcinoma SW480 Cells in Culture and Xenograft through Down-Regulation of β-Catenin-Dependent Signaling. Neoplasia 12 (5), 415–424. doi:10.1593/neo.10188

Kinzler, K., Nilbert, M., Su, L., Vogelstein, B., Bryant, T., Levy, D., et al. (1991). Identification of FAP Locus Genes from Chromosome 5q22. Science 253 (5020), 661–665. doi:10.1126/science.1651562

Krishnamachary, B., Subramanian, D., Dandawate, P., Ponnurunngam, S., Srihivason, P., Ramamoorthy, P., et al. (2019). Targeting Transcription Factor TCF4 by Gamma-Mangostin, a Natural Xanthone. Oncotarget 10 (54), 5576–5591. doi:10.18632/oncotarget.27159

Lau, T., Chan, E., Callow, M., Waaler, J., Boggs, J., Blake, R. A., et al. (2013). A Novel Tankyrase Small-Molecule Inhibitor Suppresses APC Mutation-Driven Colorectal Tumor Growth. Cancer Res. 73 (10), 3132–3144. doi:10.1158/0008-5472.CAN-12-4562

Lee, S.-K., Cho, Y.-H., Cha, P.-H., Yoon, J.-S., Ro, E. J., Jeong, W.-J., et al. (2018a). A Small Molecule Approach to Depress RAS with EGFR Repression Is a Potential Therapy for KRAS Mutation-Driven Colorectal Cancer Resistance to Cetuximab. Exp. Mol. Med. 50 (11), 1–12. doi:10.1038/s12276-018-0182-2

Lee, S.-K., Hwang, J.-H., and Choi, K.-Y. (2018b). Interaction of the Wnt/β-Catenin and RAS-ERK Pathways Involving Co-stabilization of Both β-catenin and RAS Plays Important Roles in the Colorectal Tumorigenesis. Adv. Biol. Regul. 68, 46–54. doi:10.1016/j.bior.2018.01.001
Song, J., Seo, H., Kim, M.-R., Lee, S.-J., Ahn, S., and Song, M. (2020). Active Compound of Pharbitis Semen (Pharbitis Nil Seeds) Suppressed KRAS-Driven Colorectal Cancer and Restored Muscle Cell Function during Cancer Progression. *Molecules* 25 (12), 2864. doi:10.3390/molecules25122864

Spaderna, S., Schmalhofer, O., Hlbek, F., Berz, G., Eger, A., Merkel, S., et al. (2006). A Transient, EMT-Linked Loss of Basement Membranes Indicates Metastasis and Poor Survival in Colorectal Cancer. *Gastroenterology* 131 (3), 830–840. doi:10.1053/j.gastro.2006.06.016

Sun, M.-Y., Wang, D.-D., Sun, J., Zhao, X.-H., Cai, S., Wu, Q.-X., et al. (2019). The Zhu Jin Wan Formula Increases Chemosensitivity of Human Primary Gastric Cancer Cells by Akt Mediated Mitochondrial Translocation of Cofilin-1. *Chin. J. Nat. Medicines* 17 (3), 198–208. doi:10.16187/s675-5364(19)30022-6

Sun, X., Xu, X., and Song, L. (2020). TKP, a Serine Protease Extracted from Trichosanthes Kirilowii, Inhibits the Migration and Invasion of Colorectal Adenocarcinoma Cells by Targeting Wnt/β-catenin and Hedgehog/Gli1 Signaling. *Phytotherapy Research* 34 (4), 867–878. doi:10.1002/ptr.6569

Tan, H., Li, X., Yang, W. H., and Kang, Y. (2019). A Flavone, Wogonin From Scutellaria Baicalensis Inhibits the Proliferation of Human Colorectal Cancer Cells by Inducing of Autophagy, Apoptosis and G2/M Cell Cycle Arrest via Modulating the PI3K/AKT and STAT3 Signalling Pathways. *J. BUON* 24 (3), 1143–1149.

Tanaka, N., Mashima, T., Mizutani, A., Sato, A., Aoyama, A., Gong, B., et al. (2017). APC Mutations as a Potential Biomarker for Sensitivity to Tankyrase Inhibitors in Colorectal Cancer. *Mol. Cancer Ther.* 16 (4), 752–762. doi:10.1158/1535-7163.MCT-16-0578

Tao, L., Gu, Y., Zheng, J., Yang, J., and Zhu, Y. (2019). Weichang’an Suppressed Migration and Invasion of HCT116 Cells by Inhibiting Wnt/β-catenin Pathway while Upregulating ARHGAP25. *Biotechnol. Appl. Biochem.* 66 (5), 787–793. doi:10.1002/bab.1784

Tian, W., Han, X., Yan, M., Xu, Y., Dugginieni, S., Lin, N., et al. (2012). Structure-Based Discovery of a Novel Inhibitor Targeting the β-Catenin/Tcf4 Interaction. *Biochemistry* 51 (2), 724–731. doi:10.1021/bi201428h

Todaro, M., Gaggianesi, M., Catalano, V., Benfante, A., Iovino, F., Biffoni, M., et al. (2014). CD44v6 Is a Marker of Constitutive and Reprogrammed Cancer Stem Cells Driving colon Cancer Metastasis. *Cell Stem Cell* 14 (3), 342–356. doi:10.1016/j.stem.2014.01.009

Vincan, E., and Barker, N. (2008). The Upstream Components of the Wnt Signalling Pathway in the Dynamic EMT and MET Associated with Colorectal Cancer Progression. *Clin. Exp. Metastasis* 25 (6), 657–663. doi:10.1007/s10585-008-9156-4

Voorneveld, P. W., Kodach, L. L., Jacobs, R. J., van Noesel, C. J. M., Peppelenbosch, M. P., Korkmaz, K. S., et al. (2015). The BMP Pathway Either Enhances or Suppresses the Wnt Pathway Depending on the SMAD4 and P53 Status in CRC. *Br. J. Cancer* 112 (1), 122–130. doi:10.1038/bjc.2014.560

Vu, T., and Datta, P. (2017). Regulation of EMT in Colorectal Cancer: A Culprit in Intestinal Stem-Cell Self-Renewal. *Nature* 545 (7653), 238–242. doi:10.1038/nature22133

Yan, M., Li, G., and An, J. (2017). Discovery of Small Molecule Inhibitors of the Wnt/β-catenin Pathway by Targeting β-catenin/Tcf4 Interactions. *Exp. Biol. Med. (Maywood)* 242 (11), 1185–1197. doi:10.15532/0717781918

Yan, Y., Han, Z., Han, Z., Li, X., Huang, A., Shi, J., et al. (2020). Epidemiology and Risk Factors of Colorectal Cancer in China. *Chin. J. Cancer Res.* 32 (6), 729–741. doi:10.21147/jss.9006-2020.06.06

Ye, Z.-N., Yuan, F., Liu, J.-Q., Peng, X.-R., An, T., Li., X., et al. (2019). Physalis Peruviana-Derived 4′-Hydroxywithanolidine E4, a Novel Antagonist of Wnt Signaling, Inhibits Colorectal Cancer In Vitro and In Vivo. *Molecules* 24 (6), 1146. doi:10.3390/molecules24061146

Yeh, M.-H., Chiu, H.-P., Wu, M.-C., Kuo, M., Lin, N.-W., Liao, K.-K., et al. (2020). Integrated Chinese Herbal Medicine and Western Medicine on the Survival in Patients with Colorectal Cancer: A Retrospective Study of Medical Records. *Evidence-Based Complement. Altern. Med.* 2020, 1–10. doi:10.1155/2020/4561040

Zhan, T., Rindtorff, N., and Boutros, M. (2017). Wnt Signaling in Cancer. *Oncogene* 36 (11), 1461–1473. doi:10.1038/onc.2016.304

Zhang, J., Cao, H., Zhang, B., Cao, H., Xu, X., Ruan, H., et al. (2013). Berberine Potently Attenuates Intestinal Polyps Growth in ApcMin Mice and Familial Adenomatous Polyposis Patients through Inhibition of Wnt Signalling. *J. Col. Mol. Med.* 17 (11), 1484–1493. doi:10.1111/jcc.12119

Zhang, L., Ren, B., Zhang, J., Liu, L., Liu, J., Jianga, G., et al. (2017). Anti-tumor Effect of Scutellaria Barbata D. Don Extracts on Ovarian Cancer and its Phytochemicals Characterisation. *J. Ethnopharmacol.* 206, 184–192. doi:10.1016/j.jep.2017.05.032

Zhang, L., Zhang, J., Gong, Y., and Lv, L. (2020). Systematic and Experimental Investigations of the Anti-colorectal Cancer Mediated by Genistein. *Biofactors* 46 (6), 974–982. doi:10.1002/biof.1677

Zhang, T., Wang, K., Zhang, J., Wang, X., Chen, Z., Ni, C., et al. (2013). Huaiar Aqueous Extract Inhibits Colorectal Cancer Stem Cell Growth Partially via Downregulation of the Wnt/β-catenin Pathway. *Onco. Lett. 5 (4), 1171–1176. doi:10.3892/ol.2013.1145

Zhang, Y., Appleton, B. A., Wiesmann, C., Lai, T., Costa, M., Hannoush, R. N., et al. (2009). Inhibition of Wnt Signaling by Dishevelled PDZ Peptides. *Nat. Chem. Biol.* 5 (4), 217–219. doi:10.1038/nchembio.152

**Conflict of Interest:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

**Publisher's Note:** All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.