Targeting the Wnt/β-catenin signaling pathway in cancer

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Abstract

The aberrant Wnt/β-catenin signaling pathway facilitates cancer stem cell renewal, cell proliferation and differentiation, thus exerting crucial roles in tumorigenesis and therapy response. Accumulated investigations highlight the therapeutic potential of agents targeting Wnt/β-catenin signaling in cancer. Wnt ligand/receptor interface, β-catenin destruction complex and TCF/β-catenin transcription complex are key components of the cascade and have been targeted with interventions in preclinical and clinical evaluations. This scoping review aims at outlining the latest progress on the current approaches and perspectives of Wnt/β-catenin signaling pathway targeted therapy in various cancer types. Better understanding of the updates on the inhibitors, antagonists and activators of Wnt/β-catenin pathway rationalizes innovative strategies for personalized cancer treatment. Further investigations are warranted to confirm precise and secure targeted agents and achieve optimal use with clinical benefits in malignant diseases.

Keywords: Wnt/β-catenin signaling pathway, Cancer, Targeted therapy, Cancer stem cell

Introduction

The Wnt/β-catenin signaling pathway, also called the canonical Wnt signaling pathway, is a conserved signaling axis participating in diverse physiological processes such as proliferation, differentiation, apoptosis, migration, invasion and tissue homeostasis [1–3]. Increasing evidence indicates that dysregulation of the Wnt/β-catenin cascade contributed to the development and progression of some solid tumors and hematological malignancies [4–8].

In the Wnt/β-catenin pathway, abnormal regulation of the transcription factor β-catenin, which is the pivotal component of the Wnt signaling pathway, leads to early events in carcinogenesis [9–12]. Within the degradation complex, glycogen synthase kinase 3β (GSK3β) and casein kinase 1α (CK1α) mediate the phosphorylation of β-catenin, promoting its ubiquitination and subsequent proteasomal degradation [13, 14]. The β-catenin-dependent signaling pathway is triggered by the binding of secreted cysteine-rich glycoprotein ligands Wnts to the LRP-5/6 receptors and FZD receptors. In the presence of Wnt ligand, the binding of Wnt ligand and receptors on the cell surface induces disheveled (DVL), causing the aggregation of the complex (AXIN, GSK3β, CK1, APC) to the receptor [15]. Subsequently, the phosphorylation and inhibition of GSK3β ensure an elevation of cytosolic β-catenin concentration. Un-phosphorylated β-catenin in the cytosol migrates to the nucleus and accumulates, interacting with T cell-specific factor (TCF)/lymphoid enhancer-binding factor (LEF) and co-activators, such as Pygopus and Bcl-9, to trigger the Wnt target genes like c-Myc, cyclin D1 and CDKN1A, resulting in the upregulation of TCF/LEF target gene.

In addition, multiple regulatory mechanisms have been identified on the phosphorylation and ubiquitination of β-catenin by the degradation complex. Notum, which removes palmitoleate from Wnt proteins, blocks their extracellular secretion. Dickkopf (DKK) negatively regulates the initiation of Wnt protein-mediated signaling by competitively binding to LRP5/6 receptors. Besides, secreted FZD-related proteins (sFRPs), which bind to
FZD receptors also blocking the initiation of Wnt protein-mediated signaling. Moreover, Wnt inhibitory factor (WIF) inhibits signaling by binding directly to Wnt proteins [16]. The transmembrane molecules ZNRF3 and RNF43 act on FZD molecules with E3 ubiquitin ligase activity [14, 17]. The 7-transmembrane receptor LGR4, LGR5 and LGR6 bind to R-spondins (RSPO) with high affinity to enhance the Wnt signal at a low dose of Wnt ligand [14, 18]. To elucidate the mechanism of Wnt/β-catenin signaling pathway activation and inhibition, a schematic diagram was depicted in Fig. 1.

Furthermore, Wnt/β-catenin signaling orchestrates multiple cell signaling cascades, such as epidermal growth factor receptor (EGFR), Hippo/YAP, nuclear factor kappa-B (NF-κB), Notch, Sonic Hedgehog and PI3K/Akt pathway, which contribute to pivotal molecular mechanism in cancer development [19–24]. EGFR could form a complex with β-catenin and promotes the invasion and metastasis of cancer cells [25, 26]. Moreover, the Hippo pathway has been shown to inhibit Dvl phosphorylation, nuclear accumulation of β-catenin and transcription of β-catenin/TCF-target genes in the Wnt/β-catenin signaling [21, 27]. Besides, the activation of Wnt/β-catenin pathway interacted with PI3K/AKT/GSK-3 cascade in glioblastoma cells and further provided mechanistic basis for the chemoresistance to
In addition, Fig. 3 is plotted to present a panoramic overview of the endoplasmic reticulum, which subsequently present PORCN preventing the palmitoylation of Wnt proteins in targeted agents in malignancies are illustrated in Fig. 2. In phase 1 clinical trial (NCT02675946) in solid tumors. The single-dose escalation of CGX1321 is invested in a canonical and non-canonical Wnt signaling pathways. CGX1321, another PORCN inhibitor, inhibits both with pancreatic cancer, triple-negative breast cancer trials investigating WNT974 monotherapy for patients presents enhanced anti-tumor effects with the combination 32]. In EOC preclinical mouse models, WNT974 prevents their secretion [13, 24]. Blocking the acylation of Porcupine (PORCN), a family member of membrane-bound O-acyltransferases (MBOAT), is key for the secretion of Wnt ligands [30, 31]. Several inhibitors that target PORCN prevent the palmitoylation of Wnt proteins in the endoplasmic reticulum, which subsequently prevents their secretion [13, 24]. Blocking the acylation of WNT with a PORCN inhibitor to abolish WNT secretion becomes an effective treatment strategy. WNT974 (LGK974) is an orally available small molecule inhibitor that decreases epithelial ovarian cancer (EOC) cell viability in vitro and inhibits tumor growth in vivo [24, 32]. In EOC preclinical mouse models, WNT974 presents enhanced anti-tumor effects with the combination of paclitaxel [33]. There is currently a phase I clinical trial investigating WNT974 monotherapy for patients with pancreatic cancer, triple-negative breast cancer and cervical squamous cell carcinoma (NCT01351103). CGX1321, another PORCN inhibitor, inhibits both canonical and non-canonical Wnt signaling pathways. The single-dose escalation of CGX1321 is invested in a phase I clinical trial (NCT02675946) in solid tumors. In an EOC mouse model, treatment with CGX1321 led to prolonged overall survival, decreased tumor burden and increased immune cell infiltration. Furthermore, effects of some other PORCN inhibitors were evaluated in preclinical studies [34, 35]. It was reported that the combination of the PORCN inhibitor ETC-159 and the PI3K inhibitor GDC-0941 decreased RNF43-mutant pancreatic cancer cell proliferation and xenograft growth in vivo [36]. Besides, IWP-O1 was observed with significantly improved metabolic stability and inhibit the phosphorylation of DVL in Hela cells [37]. Moreover, GNF-6231 demonstrated potent inhibition activities and induced robust anti-tumor efficacy in a breast cancer mouse model [38].

Wnt/β-catenin signaling pathway interventions for cancer
The deregulation of Wnt/β-catenin signaling pathway is closely related to the initiation and progression of various types of cancers [4, 5, 29]. Thus, inhibitors, antagonists and agonists were designed to target this cascade in solid tumors (Table 1) and hematological malignancies (Table 2). Formulas and structures of agents targeted Wnt/β-catenin signaling pathway are listed in Additional file 1. Hallmarks of diverse categories of Wnt/β-catenin targeted agents in malignancies are illustrated in Fig. 2. In addition, Fig. 3 is plotted to present a panoramic overview of Wnt/β-catenin signaling pathway targeted interventions in cancer therapy, which was deciphered in the following aspects.

Inhibitors targeting Wnt ligand/receptor interface
Porcupine inhibitors
Porcupine (PORCN), a family member of membrane-bound O-acyltransferases (MBOAT), is key for the secretion of Wnt ligands [30, 31]. Several inhibitors that target PORCN prevent the palmitoylation of Wnt proteins in the endoplasmic reticulum, which subsequently prevents their secretion [13, 24]. Blocking the acylation of WNT with a PORCN inhibitor to abolish WNT secretion becomes an effective treatment strategy. WNT974 (LGK974) is an orally available small molecule inhibitor that decreases epithelial ovarian cancer (EOC) cell viability in vitro and inhibits tumor growth in vivo [24, 32]. In EOC preclinical mouse models, WNT974 presents enhanced anti-tumor effects with the combination of paclitaxel [33]. There is currently a phase I clinical trial investigating WNT974 monotherapy for patients with pancreatic cancer, triple-negative breast cancer and cervical squamous cell carcinoma (NCT01351103). CGX1321, another PORCN inhibitor, inhibits both canonical and non-canonical Wnt signaling pathways. The single-dose escalation of CGX1321 is invested in a phase I clinical trial (NCT02675946) in solid tumors. In an EOC mouse model, treatment with CGX1321 led to prolonged overall survival, decreased tumor burden and increased immune cell infiltration. Furthermore, effects of some other PORCN inhibitors were evaluated in preclinical studies [34, 35]. It was reported that the combination of the PORCN inhibitor ETC-159 and the PI3K inhibitor GDC-0941 decreased RNF43-mutant pancreatic cancer cell proliferation and xenograft growth in vivo [36]. Besides, IWP-O1 was observed with significantly improved metabolic stability and inhibit the phosphorylation of DVL in Hela cells [37]. Moreover, GNF-6231 demonstrated potent inhibition activities and induced robust anti-tumor efficacy in a breast cancer mouse model [38].

Wnt/FZD antagonists
With the antagonism of Wnt ligands and FZD receptors, canonical Wnt signaling pathway was suppressed and indicated potential strategy in cancer therapy. Ipafricept (OMP54F28; IPA) is a recombinant fusion protein, including the cysteine-rich domain of FZD8 fused to a human IgG1 Fc fragment [39]. This structure could bind directly to Wnt ligands, competing for the binding of Wnt ligands with FZD8 receptor, thereby inhibiting Wnt regulated processes [40]. In patient-derived ovarian cancer xenograft mice models, ipafricept displayed activity to decrease the population of stem cells, suppress tumor development and promote differentiation. In addition, in preclinical studies, ipafricept exhibits synergistic anti-tumor effects combined with taxanes when given prior to chemotherapy two to three days, with 82% of the patients achieved a partial or complete response [41]. Ipafricept was also investigated in a phase 1b dose-escalation study in combination with paclitaxel and carboplatin in patients with recurrent platinum-sensitive ovarian cancer. The combination of these three agents produced similar response rates and survival outcomes compared with historical treatment regimens. Nevertheless, bone toxicities at efficacy doses prevented further testing of this treatment regimen. A phase 1b clinical trials suggested that ipafricept could also be administered with nab-paclitaxel and gemcitabine with reasonable tolerance in patients with previously untreated stage IV pancreatic cancer [42].

OMP-18R5 (vantictumab) is a monoclonal antibody targeting FZD1, FZD2, FZD5, FZD7 and FZD8 [43–45]. OMP-18R5 blocks tumor growth in xenograft mouse models of breast, pancreatic, colon, lung, and head and neck cancers and is being evaluated in a number of phase I trials for these tumor types [43, 46]. In a clinical trial, OTSA-101 was demonstrated that radioimmunotherapy targeting FZD10 is feasible in synovial sarcoma patients [47]. Besides, Pavlovic et al. utilized
| Agents | Mechanism | Phase | Cancer type | Side effects | Identifier |
|--------|-----------|-------|-------------|--------------|------------|
| WNT974 | PORCN inhibitor | Phase 2 | Head and neck squamous cell cancer | NR | NCT02649530 |
| WNT974 | PORCN inhibitor | Phase 1 | Pancreatic cancer; colorectal cancer; melanoma; breast cancer; head and neck squamous cell cancer; cervical squamous cell cancer; esophageal squamous cell cancer; lung squamous cell cancer | NR | NCT01351103 |
| *WNT974 (with LGX818 and Cetuximab) | PORCN inhibitor | Phase 1 | Colorectal cancer | NR | NCT02278133 |
| ETC-159 | PORCN inhibitor | Phase 1 | Solid tumor | Reversible hematological disorders | NCT02521844 |
| CGX1321 | PORCN inhibitor | Phase 1 | Colorectal adenocarcinoma; gastric adenocarcinoma; pancreatic adenocarcinoma; bile duct carcinoma; hepatocellular carcinoma, esophageal carcinoma, Gastrointestinal cancer | NR | NCT03507998 |
| *CGX1321 (with pembrolizumab) | PORCN inhibitor | Phase 1 | Solid tumors; Gastrointestinal cancer | NR | NCT02675946 |
| GNF-6231 | PORCN inhibitor | Preclinical | Breast cancer | NR | – |
| 9Gy-OTSA-101 | FZD10 antagonist | Phase 1 | Synovial sarcoma | NR | NCT01469975 |
| OMP-18R5 | Monoclonal antibody against FZD receptors | Phase 1 | Breast cancer | Nausea, alopecia, fatigue, peripheral neuropathy | NCT01973309 |
| OMP-18R5 | Monoclonal antibody against FZD receptors | Phase 1 | Solid tumors | NR | NCT01345201 |
| *OMP-18R5 (with docetaxel) | Monoclonal antibody against FZD receptors | Phase 1 | Solid tumors | NR | NCT01957007 |
| *OMP-18R5 (with nab-paclitaxel and gemcitabine) | Monoclonal antibody against FZD receptors | Phase 1 | Pancreatic cancer | NR | NCT02005315 |
| OMP-54F28 | FZD8 decoy receptor | Phase 1 | Solid tumors | Dysgeusia, muscle spasms, hypophosphatemia | NCT01608867 |
| *OMP-54F28 (with sorafenib) | FZD8 decoy receptor | Phase 1 | Hepatocellular cancer | Diarrhea, neutropenia and decreased appetite | NCT02069145 |
| *OMP-54F28 (with paclitaxel and carboplatin) | FZD8 decoy receptor | Phase 1 | Ovarian cancer | NR | NCT02092363 |
| *OMP-54F28 (with nab-paclitaxel and gemcitabine) | FZD8 decoy receptor | Phase 1 | Pancreatic cancer | NR | NCT02050178 |
| Fz7-21 | FZD7 antagonist | Preclinical | Gastroenteric tumor | – | – |
| Salinomycin | LRPS/6 inhibitor | Preclinical | Hepatocellular carcinoma; gastric cancer; colorectal cancer; bladder cancer; breast cancer | – | – |
| FJ9 | DVL inhibitor | Preclinical | Lung cancer; melanoma | – | – |
| 3289-8625 | DVL inhibitor | Preclinical | Ovarian cancer; lung cancer | – | – |
| XAV939 | Tankyrase inhibitor | Preclinical | Ovarian cancer; breast cancer | – | – |
| JW74/ JW55 | Tankyrase inhibitor | Preclinical | Osteosarcoma, colon carcinoma | – | – |
| NVP-TNK5656 | Tankyrase inhibitor | Preclinical | Hepatocellular carcinoma; colorectal cancer | – | – |
| LZZ-02 | Tankyrase inhibitor | Preclinical | Colonic carcinoma | – | – |
| SSTC3 | CK1α activator | Preclinical | Colorectal cancer | – | – |
| LF3 | β-catenin/TCF | Preclinical | Colon cancer | – | – |
### Table 1 (continued)

| Agents                        | Mechanism                     | Phase     | Cancer type                          | Side effects            | Identifier      |
|-------------------------------|-------------------------------|-----------|--------------------------------------|-------------------------|-----------------|
| KYA1797K/ KY1220              | β-catenin                     | Preclinical | Colorectal cancer, breast cancer     | –                       |                 |
| iCRT3/5                       | β-catenin/TCF                 | Preclinical | Breast cancer, gastric cancer        | –                       |                 |
| ZINC02092166                  | β-catenin/TCF                 | Preclinical | Colorectal cancer                    | –                       |                 |
| NLS-StAx-h                   | β-catenin/TCF                 | Preclinical | Colorectal cancer                    | –                       |                 |
| *PRI-724 (with leucovorin calcium, oxaliplatin, or fluorouracil) | CBP/β-catenin antagonist | Phase 2   | Colorectal cancer                    | Nausea, fatigue         | NCT02413853    |
| PRI-724                       | CBP/β-catenin antagonist      | Phase 1   | Pancreatic cancer                    | NR                      | NCT01764477    |
| PRI-724                       | CBP/β-catenin antagonist      | Phase 1   | Advanced solid tumors                | Nausea, vomiting, diarrhea, alopecia, fatigue, neutropenia, thrombocytopenia, neutropenic fever | NCT01302405 |
| ICG001                        | CBP antagonist                | Preclinical | Pancreatic cancer, lung cancer, breast cancer, ovarian cancer | –                       |                 |
| Isoquercitrin                 | CBP antagonist                | Preclinical | Colorectal cancer                    | –                       |                 |

### Table 2 Clinical trials and preclinical evaluations on Wnt/β-catenin targeted agents in hematological malignancies

| Agents                        | Mechanism                     | Phase     | Cancer type                          | Side effects                                      | Identifier            |
|-------------------------------|-------------------------------|-----------|--------------------------------------|---------------------------------------------------|-----------------------|
| CWP291                        | SAM68 inhibitor               | Phase 1   | Relapsed or refractory AML and MDS   | Nausea, vomiting, diarrhea, and infusion-related reactions | NCT01398462           |
| PRI-724                       | CBP/β-catenin antagonist      | Phase 2   | AML; CML                             | NR                                                | NCT01606579           |
| GNE-781                       | CBP antagonist                | Preclinical | AML                                 | –                                                 | –                     |
| ICG001                        | CBP antagonist                | Preclinical | AML; ALL; CML; MM                    | –                                                 | –                     |
| WNT974                        | PORCN inhibitor               | Preclinical | BL                                  | –                                                 | –                     |
| Wnt-C59                       | PORCN inhibitor               | Preclinical | chL                                  | –                                                 | –                     |
| IWP-2/IWP-4                   | PORCN inhibitor               | Preclinical | AML; chL                             | –                                                 | –                     |
| XAV939                        | Tankyrase inhibitor           | Preclinical | AML; T-ALL; CML                      | –                                                 | –                     |
| IWR-1                         | Tankyrase inhibitor           | Preclinical | APL                                  | –                                                 | –                     |
| Salinomycin                   | LRP5/6 inhibitor              | Preclinical | CLL; MCL                             | –                                                 | –                     |
| iCRT14                        | β-catenin/TCF                 | Preclinical | ALL; MCL                             | –                                                 | –                     |

![Fig. 2](image-url) Hallmarks of diverse categories of Wnt/β-catenin targeted agents in cancer
combinatorial antibody engineering by phage display to generate a variant antibody F2.A with specificity of FZD4 [44]. F2.A suppresses pancreatic cancer tumor growth in xenograft mouse models. Interestingly, carbamazepine, an antiepileptic drug, was recently reported to bind the cysteine-rich domain of FZD8, which suggests being explored as a promising therapy option in cancers [48]. Additionally, Fz7-21, a selective FZD7-binding peptide, disrupts intestinal stem cells and organoids, implicating the potential of therapeutic application in malignant diseases [49].

**LRP5/6 inhibitors**

As the co-receptor of Wnt, the phosphorylation of LRP5/6 promotes the activation of Wnt/β-catenin signaling pathway. The molecular complex Wnt-FZD-LRP5/6-DVL forms a structural region for AXIN interaction that disrupts degradation of β-catenin. BMD4503-2, a quinoxaline moiety, was identified as a new small-molecule inhibitor of the LRP5/6-sclerostin interaction through pharmacophore-based virtual screening and in vitro assays. The compound BMD4503-2 could revert the down-regulated activity of the Wnt/β-catenin signaling pathway through
suggesting the tankyrase inhibitor could overcome resist-
duced apoptosis. A combination with AKT and PI3K inhibitors. A
TNKS656, 3289–8625 are some agents that block the DVL-PDZ
interaction, resulting in subsequently inhibition of the
signal transduction pathway [54, 55]. The non-electro-
philic indole-2-carbinol-based chemical scaffold of FJ9
disrupted the interaction between FZD and the PDZ
domain of DVL. NSC668036 and 3289–8625 were con-
firmed to down-regulate Wnt/β-catenin signaling and
inhibit tumor cell growth in lung, colorectal and cer-
vical cancer cell lines in vitro, as well as in a lung cancer
 xenografts [54].

**Agents targeting the β-catenin-destruction complex**

**Tankyrase inhibitors**

Scaffolding protein AXIN is the rate-limiting component of
the β-catenin destruction complex, which are constantly
surveyed and regulated by tankyrases [56–58]. Tankyrases belong to the Poly (ADP-ribose)
polymerases (PARPs) family, regulating the stability of
AXIN1 and AXIN2 through directing AXIN ubiqui-
tylation by RNF146 and proteasomal degradation [59,
60]. There are two isoforms, Tankyrase 1 (PARP5a) and
Tankyrase 2 (PARP5b) involved in the Wnt/β-catenin
signaling, increasing the degradation of AXIN by the
ubiquitin–proteasome pathway [61–63]. Tankyrase
inhibitor, XAV939 and IWR-1 regulated AXIN by inhibiting
Tankyrase 1 and Tankyrase 2 [64, 65]. Treatment with
XAV939 decreased the viability of EOC cell lines and
increased radio-sensitivity in cervical cancer cells [66].
Furthermore, the tankyrase-specific inhibitor, JW74 and
JW55 affects cell cycle progression and induced apoptosis and
differentiation in osteosarcoma and colon carcinoma
cells, respectively [67, 68]. In addition, mice xenografts
and patient-derived sphere cultures of colorectal cancer
(CRC) were incubated with a Tankyrase inhibitor NVP-
TNKS656 combination with AKT and PI3K inhibitors. A
decreased nuclear β-catenin level predicted for apoptosis
suggesting the tankyrase inhibitor could overcome resist-
ance to AKT and PI3K inhibitors [61]. The same antineo-
plastic effect was observed in LZZ-02, a novel Tankyrase
1/2 inhibitor [69]. Concerns of gastrointestinal toxicity have been noted in analysis of these inhibitors, and fur-
ther studies are needed [70].

**DVL inhibitors**

DVL is important for Wnt signal transduction by
recruiting components of the β-catenin destruction complex to the cell membrane [51, 52]. DVL binds to
the cytoplasmic carboxyl terminal end of FZD proteins
through its PDZ domain [53]. NSC668036, FJ9, and
3289–8625 are some agents that block the DVL-PDZ
interaction, resulting in subsequently inhibition of the
signal transduction pathway [54, 55]. The non-electro-
philic indole-2-carbinol-based chemical scaffold of FJ9
disrupted the interaction between FZD and the PDZ
domain of DVL. NSC668036 and 3289–8625 were con-
firmed to down-regulate Wnt/β-catenin signaling and
inhibit tumor cell growth in lung, colorectal and cervi-
cal cancer cell lines in vitro, as well as in a lung cancer
 xenografts [54].

**Inhibitors targeting β-catenin/TCF transcription complex**

Several compounds targeting the downstream effec-
tors, like transcription complex and co-activators, were
identified by high through-put ELISA screening, such as
PFK115-584 and CGP049090, which can block the
β-catenin/TCF complex in a dose-dependent manner
[76]. LF3, a 4-thioureido-benzenesulfonyamide deri-

tive, robustly disrupts the critical interaction between
β-catenin and the transcription factor TCF4. Besides, LF3
reduced tumor growth and induced differentiation in a
mouse xenograft model of colon cancer [77]. KYA1797K/
KY1220 effectively suppressed the growth of colorectal
cancer and breast cancer cells via the destabilization of
both β-catenin and Ras [78–80]. Mantle cell lymphoma-
initiating cells were particularly sensitive to Wnt path-
way inhibitors. Targeting β-catenin-TCF4 interaction
with CCT036477, iCRT3, iCRT5, iCRT14 or PKF118-310
preferentially eliminated the survival of malignant cells of
acute lymphoblastic leukemia, gastric cancer, and breast
cancer [81–84]. ZINC02092166 suppresses canonical
Wnt signaling, downregulates the expression of Wnt
target genes and inhibits the growth of colorectal can-
cancer cells [85]. Based on the acylhydrazine component,
the inhibitory activities were evaluated in cellular assays.
NLS-StAx-h, a selective cell-penetrating peptide inhibi-
tor of β-catenin-transcription factor interactions sup-
pressed proliferation and migration of colorectal cancer
cells. CWP232291 (CWP291), another small molecule

**CK1 agonists**

Stabilizing the β-catenin destruction complex can block the nuclear localization of β-catenin, suggesting as an
attractive therapeutic target. Feasible strategy for the
repositioning of existing FDA approved drugs is explored
for the treatment of malignancies with deregulated Wnt
signaling. For example, pyrvinium, an existing FDA
approved drug, can bind all CK1 family members in vitro,
selectively potentiating CK1α kinase activity [71]. Colon
cancer cells with APC mutations were sensitive to pyr-
vinium treatment with a decrease in both Wnt signaling
and cell proliferation. Pyrvinium inhibits platinum-resistant
tumor growth and induces apoptosis in vitro and
in vivo, and these effects are enhanced when combined
with paclitaxel. Pyrvinium blocks Wnt signal by decreas-
ing β-catenin levels and suppressing the transcription
of β-catenin targeted genes. However, cancer cells with
increasing level of β-catenin are no longer impacted by
pyrvinium [72, 73]. In addition, a novel small-molecule
CK1α activator called SSTC3 has been proved to inhibit
the growth of CRC xenografts in mice and also attenuate
the growth of patient-derived metastatic CRC xenograft
inhibited Wnt-mediated transcriptional activity, was under evaluation on phase 1 clinical trial in patients with relapsed or refractory AML and myelodysplastic syndrome (MDS) [86]. Active form of CWP232204 binds to Src-associated substrate in mitosis of 68 kDa (SAM68), which regulates alternative splicing TCF, and promotes β-catenin degradation via apoptosis. Further investigations will explore CWP291, with a mechanism of aiming at eradication of earlier progenitors via Wnt pathway blockade, as combination therapy.

There are several co-activators of β-catenin-dependent transcription, including CREB binding protein (CBP). The CBPs are key transcriptional co-activators essential for a multitude of cellular processes and involved in human pathological conditions and cancer [87, 88]. Several CBP inhibitors have been developed in recent years and have shown promising antineoplastic effects in preclinical models with minimal off-target effects, such as PRI-724, ICG-001, GNE-781, 1-(1H-indol-1-yl)ethene, JW67, JW74, NLS-StAx-h, et al. [89–91]. PRI-724 is a first-in-class small molecule antagonist that inhibits the interaction between β-catenin and CBP [92]. It was phosphorylated-C-82 and was rapidly hydrolyzed to its active form C-82 in vivo [93]. In chemotherapy resistant EOC with hyperactivated CBP/β-catenin signaling, PRI-724 increased sensitization to platinum chemotherapy and preclinical studies had shown considerable toxicity profile [93, 94]. Monotherapy with ICG-001 led to the reduction of tumor-related characteristics [95, 96]. GNE-781 displayed anti-tumor activity in an acute myeloid leukemia (AML) model and was also shown to decrease Foxp3 transcript levels in a dose-dependent manner [90]. 1-(1H-indol-1-yl) ethene markedly inhibited cell growth in several prostate cancer cell lines [89]. JW67 and JW74 were identified specifically inhibiting canonical Wnt pathway at the level of the destruction complex and inhibited the growth of colorectal cancer mouse xenograft model and multiple intestinal neoplasia mice [97]. Moreover, isoquercitrin showed anti-tumor effects on colon cancer cells (SW480, DLD-1 and HCT116), whereas exerting no significant effect on non-tumor colon cell (IEC-18), suggesting a specific effect in tumor cells in vitro [98].

Natural agents and new activity of old drugs
It is notable that some of the natural agents exert anti-tumor activities via regulating canonical Wnt signaling pathway [99, 100]. Curcumin, isolated from the rhizome of Curcuma longa, modulates Wnt signaling pathway and exerts anti-tumor activities in melanoma, lung cancer, breast cancer, colon cancer, endothelial carcinoma, gastric carcinoma and hepatocellular carcinoma [101]. 3,3′-diindolylmethane (DIM), a natural compound derived from cruciferous vegetables inhibited proliferation of colon and colorectal cancer cells via Wnt/β-catenin pathway, highlighting as a promising chemo-preventive agent or chemo-radio-sensitizer for the prevention of tumor recurrence in cancer therapy [102]. Formononetin, isolated from the red clover, displayed anti-tumor activities in breast cancer and glioma cells with high-level IC50 values. To achieve high potency, formononetin was modified with a coumarin unit to design a derivate 10 via the molecular hybridization strategy. The analog 10 presented anti-proliferative effects through Wnt/β-catenin pathway in gastric cancer [103]. Besides, Wogonin, a major flavonoid compound isolated from Scutellaria radix, decreased intracellular levels of Wnt proteins and activated degradation β-catenin for proteasomal degradation [104]. Gigantol, a benzyl compound from orchid species, was also reported to inhibit Wnt/β-catenin signaling through down-regulation of phosphorylated LRP6 and cytosolic β-catenin in breast cancer cells [105]. Additionally, treatment of echinacoside, a phenylethanoid glycoside from Tibetan herbs, significantly reduced tumor growth and regulation of Wnt/β-catenin signaling [106]. Besides, nimbolide, a limonoid present in leaves of the neem tree, concurrently abrogated canonical Wnt signaling and induced intrinsic apoptotic in hepatocarcinoma cells [107]. Moreover, isoquercitrin, a natural flavonol compound, exerted an inhibitory effect on Wnt/β-catenin, where the flavonoid regulated downstream of β-catenin translocation to the nucleus [108]. It was also noted that triptolide, a diterpenoid epoxide presented in Tripterygium wilfordii, could effectively inhibit canonical Wnt/β-catenin signaling by targeting the downstream C-terminal transcription domain of β-catenin or a nuclear component associated with β-catenin and induced apoptosis of Wnt-dependent cancer cells [109]. Moreover, the fungus Exobasidium vexans and its subcomponent atranorin were reported to inhibit lung cancer cell motility and tumorigenesis by affecting nuclear import of β-catenin and downregulating β-catenin/Lef downstream target genes [110].

In addition, researchers had found some old drugs performed new tricks, which play important roles in tumor growth, invasion and metastasis via regulating Wnt/β-catenin signaling pathway. Carbamazepine, an antiepileptic drug, was recently reported to bind the cysteine-rich domain of FZD8, which suggested to been explored as a promising therapy option in cancers [48]. It was also reported that psychiatric agent hexachlorophene attenuated Wnt/β-catenin signaling through suppressing β-catenin degradation in colon cancer cells [111]. Salinomycin, a type of antibiotics, was reported to trigger ionic changes to inhibit proximal Wnt signaling by interfering with LPR6 phosphorylation, and thus impairing.
the survival of cells that depend on Wnt signaling at the plasma membrane [112–116]. Besides, hematein was found to inhibit cancer cell growth and increased apoptosis through Wnt/TCF pathway [117]. Trifluoperazine (TFP), used as an antipsychotic and antiemetics, had been found to inhibit lung CSC spheroid formation ability and suppress expression of lung CSC markers (e.g., CD44/CD133) by inhibiting Wnt/β-catenin signal transduction [118]. The similar activities were also investigated in thioridazine, pimozide and diphenylbutylpiperidine class, other antiangiogenic agents [119–121]. It is notable that cyclooxygenases (COX1 and 2) inhibitors (e.g., aspirin, celecoxib, sulindac and ursolic acid) could inhibit Wnt/β-catenin pathway in cancer cells [122–124]. Aspirin increased expression of the Wnt antagonist Dickkopf-1, which suppressed activities of cancer stem cells in CRC cells [125].

Cancer stem cells -Wnt/β-catenin signaling pathway inhibitors

CSCs display many characteristics of embryonic or tissue stem cells and often show continuous activation of highly conserved signaling pathways related to development and tissue homeostasis [126, 127]. The Wnt/β-catenin signaling pathway is associated with regulating the pluripotency, self-renewal of stem cells and differentiation ability [1, 128].

Abnormal activation of the Wnt/β-catenin pathway promotes CSC progression and thus leads to the deterioration and metastasis of cancer [129]. For instance, abnormal activation of Wnt signaling disrupted the normal growth and differentiation of colonic crypt stem cells, resulting in a colorectal CSC phenotype by upregulating expression of target genes such as c-MYC and cyclin D [130]. Moreover, one study showed that experimental knockdown of CD146 could dedifferentiate colorectal cancer cells to acquire a stem cell phenotype through inhibiting GSK-3β which in turn promoted nuclear translocation of β-catenin for Wnt signaling activation [131]. Recent studies identified SAM68 as a novel transcriptional modulator selectively targeting CSCs over healthy stem cells via Wnt/β-catenin signaling [132]. Wnt/β-catenin signaling also exerts a crucial role in early hematopoiesis, notably in hematopoietic stem cells (HSCs). Loss- and gain-of-function studies demonstrated that Wnt signaling and β-catenin activity were necessary for proper function and cellularity control of hematopoietic cells including HSCs and MKs12-15 [133]. Overactive Wnt/β-catenin signaling led to exhaustion of HSCs, causing multilineage differentiation block and compromised hematopoietic stem cell maintenance [134].

Table 3: Small-molecule compounds targeting Wnt/β-catenin cascade to inhibit cancer stem cells

| Agents          | Target                   | Phase     | Type of cancer              | Side effects              | References                      |
|-----------------|--------------------------|-----------|-----------------------------|---------------------------|---------------------------------|
| WNT974          | PORCN inhibitor          | Phase I   | Breast cancer               | Not reported              | Solzak JP et al. [136]          |
| Niclosamide     | Wnt/β-catenin            | Phase II  | Colorectal cancer           | Vomiting, diarrhea, and colitis | Burock S et al. [140] |
|                 | Wnt/β-catenin            | Preclinical| Ovarian cancer             | Not reported              | Lin CK et al. [137]          |
|                 | LRP6, β-catenin          | Preclinical| Basal-like breast cancer    | Not reported              | Ye T et al. [139]          |
| ONC201          | Wnt/β-catenin            | Phase I/ II | Glioblastoma cancer        | Not reported              | Arrilaga-Romany I et al. [144] |
|                 |                          | Preclinical| Prostate cancer             | Not reported              | Lev A et al. [143]          |
|                 | Tankyrase inhibitor      | Preclinical| Colon cancer                | Not reported              | Wu X et al. [147]          |
|                 |                          | Preclinical| Head and neck squamous cell carcinoma | Not reported | Roy S et al. [146]          |
| IWR-1           | Tankyrase inhibitor      | Preclinical| Osteosarcoma                | Not reported              | Martins-Neves SR et al. [148] |
| TFP             | Wnt/β-catenin            | Preclinical| Lung cancer                 | Not reported              | Yeh CT et al. [118]          |
| AD and Ts       | Wnt/β-catenin            | Preclinical| Lung cancer                 | Not reported              | Lamture G et al. [165]         |
| Chelerythrine   | β-catenin                | Preclinical| Non-small cell lung carcinoma | Not reported             | Medvetz D et al. [150]        |
| Wnt-CS9         | PORCN inhibitor          | Preclinical| Nasopharyngeal carcinoma    | Not reported              | Cheng Y et al. [152]          |
| IC-2            | Wnt                      | Preclinical| Hepatocellular carcinoma    | Not reported              | Seto K et al                 |
|                 |                          | Preclinical| Colorectal cancer           | Not reported              | Utushibara S et al           |
| JIB-04          | β-catenin                | Preclinical| Colorectal cancer           | Not reported              | Kim M et al. [153]           |
| FH535           | Wnt/β-catenin            | Preclinical| Pancreatic cancer           | Not reported              | Razak S et al. [155]         |
| Docetaxel and sulforaphane | β-catenin            | Preclinical| Breast cancer               | Not reported              | de Bessa Garcia SA et al. [157] |
| Pyrvinium pamoate | β-catenin              | Preclinical| Breast cancer               | Not reported              | Xu L et al. [158]           |
| SKL2001         | Axin/β-catenin           | Preclinical| Mesenchymal stem cell       | Not reported              | Jiwon Choi et al. [159]       |


Several compounds have been identified to target CSCs via Wnt/β-catenin signaling pathway (Table 3, Fig. 4). It has been reported that PORCN inhibitor WNT974 (LGK-974) inhibited the proliferation of breast CSCs [135, 136]. Niclosamide, an FDA approved anti-helminthic agent, was identified as an inhibitor of the Wnt/β-catenin pathway and showed anti-tumor properties to selectively target ovarian CSCs [137]. In addition, niclosamide decreased the level of CSCs by reducing the expression of LRP6 and β-catenin in basal-like breast cancer [138, 139]. Notably, in a phase 2 trial, the safety and effectiveness of niclosamide was proved in the treatment of colorectal cancer [140]. Furthermore, niclosamide can reduce the expression of many components in the Wnt/β-catenin signaling pathway, the self-renewal ability and population of CSCs in CRC [141]. Additionally, ONC201, which is in a phase I/II study for patients with advanced cancer (NCT02038699), induced significant CSC-suppression and repress the expression of CSC-related genes in prostate and glioblastoma tumors through suppressing the Wnt signaling pathway [142–144].

Furthermore, many potential compounds targeting CSCs through inhibiting Wnt/β-catenin signaling pathway have been undertaken in preclinical evaluations. For example, XAV939 inhibited β-catenin signaling, thus attenuated CSC progression, thereby eliminating the CSC-mediated chemical resistance in head and neck squamous cell carcinoma (HNSCC) and colon cancer cells [145–147]. IWR-1, a tankyrase inhibitor, can hamper the expression of key stem markers in osteosarcoma, impair osteosarcoma CSC self-renewal and enhance doxorubicin sensitivity by affecting β-catenin translocation in vivo [148]. Trifluoperazine (TFP), used as an antipsychotic and antiemetics, has been found to inhibit lung CSC spheroid formation ability and suppress expression of lung CSC markers (e.g., CD44/CD133) by inhibiting Wnt/β-catenin signal transduction [118]. Additionally, actinomycin D (AD) and telmisartan (TS) can also attenuate the number and activity of CSC and reduce CSC marker expression (such as ALDH1, SOX2 and NO2) in lung cancer by blocking the Wnt/β-catenin signaling pathway. Besides, chelerythrine was identified to down-regulate the level of β-catenin and inhibited CSC invasion, spheroid formation and the expression of the stem marker SOX2 in non-small cell lung carcinoma (NSCLC) [149, 150]. Wnt-C59 (C59), an inhibitor of Wnt, decreased the sphere formation ability of CSCs dose-dependently in nasopharyngeal carcinoma (NPC) [151]. IC-2, a novel small-molecule Wnt inhibitor, reduced the population of CD44+ cells (liver CSCs) and the sphere-forming ability of hepatocellular carcinoma (HCC) cells, as well as in CRC and bladder cancer cells [152]. In addition, IC-2 increased the sensitivity of 5-FU in the DLD-1 cells, a CRC cell line. Moreover, JIB-04, a selective inhibitor of histone demethylase, significantly attenuated CSC tumor sphere formation, migration and invasion in vitro by regulating the recruitment of β-catenin [153]. A similar phenomenon was noted in FH535, which could...
suppress the expression of the liver CSC marker CD24 and CD44 [154, 155]. The combination of docetaxel (DTX) and sulforaphane (SFN) and pyrvinium pamoate (PP) can both inhibit the EMT (epithelial–mesenchymal transition), CSC self-renewal ability and drug resistance by decreasing β-catenin expression in BCSCs [156–158]. Additionally, SKL2001, an agonist of the Wnt/β-catenin pathway, stabilizes intracellular β-catenin via disruption of the AXIN/β-catenin interaction [159]. The treatment of mesenchymal stem cells with SKL2001 promoted osteoblastogenesis and suppressed adipocyte differentiation, providing a new strategy to regulate mesenchymal stem cell differentiation by modulation of the Wnt/β-catenin pathway. Besides, 5-FU was reported to promote stemness of colorectal cancer via p53-mediated WNT/β-catenin pathway activation [160]. Anti-progastrin humanized antibodies were investigated to decrease self-renewal of CSCs via Wnt signaling and represent potential novel strategies for K-RAS-mutated colorectal cancer [161].

Challenges of Wnt/β-catenin signaling targeted agents in cancer

Aberrant activation of Wnt/β-catenin signaling drives oncogenic transformation in a wide range of cancers, indicating the key pathway modulators as attractive therapeutic targets in malignancies. Despite that Wnt/β-catenin targeted therapies are varied and clinical experience nascent, with the development of the targeted agents and combination strategies under investigation, the risk for off-targeting effectivity, side effects and toxicities are not allowed to be neglected. Of note, the critical role of Wnt/β-catenin signaling in stem cell maintenance raised concerns regarding the dose-limiting toxicity of targeted agents in bone, hair and gastrointestinal tract as well as in hematopoiesis, which limited of its clinical application [162–164]. Besides, considerable cross talks between the Wnt/β-catenin signaling pathway with other pathways are critical to designing effective therapeutic approaches. The combination therapy with agents that have impacts on multiple pathways in solid and hematologic malignancies needs long-term follow-up observation. Therefore, further exploration and evaluation are warranted to identify precise and safe targeted agents and achieve optimal use with clinical benefits in cancer.

Conclusions

Novel strategies are imperative to improve the outcome of cancer patients. With great advances in the knowledge of molecular basis and the constant effort for improvement, preclinical investigations and clinical trials have been conducted on the Wnt/β-catenin signaling targeted interventions in malignancies. The Wnt/β-catenin signaling targeted regimens have been proved to represent promising candidates of individualized approaches in the treatment of cancer patients. Further investigations are expected on confirming the safety, efficacy, patient stratification and drug delivery of innovative Wnt/β-catenin targeted therapies in cancer.
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