Abstract. Cancer stem cells (CSCs), which have the potential for self-renewal, differentiation and de-differentiation, undergo epigenetic, epithelial-mesenchymal, immunological and metabolic reprogramming to adapt to the tumor microenvironment and survive host defense or therapeutic insults. Intra-tumor heterogeneity and cancer-cell plasticity give rise to therapeutic resistance and recurrence through clonal replacement and reactivation of dormant CSCs, respectively. WNT signaling cascades cross-talk with the FGF, Notch, Hedgehog and TGFβ/BMP signaling cascades and regulate expression of functional CSC markers, such as CD44, CD133 (PROM1), EPCAM and LGR5 (GPR49). Aberrant canonical and non-canonical WNT signaling in human malignancies, including breast, colorectal, gastric, lung, ovary, pancreatic, prostate and uterine cancers, leukemia and melanoma, are involved in CSC survival, bulk-tumor expansion and invasion/metastasis. WNT signaling-targeted therapeutics, such as anti-FZD1/2/5/7/8 monoclonal antibody (mAb) (vantictumab), anti-LGR5 antibody-drug conjugate (ADC) (mAb-mc-vc-PAB-MMAE), anti-PTK7 ADC (PF-06647020), anti-ROR1 mAb (cirmtuzumab), anti-RSPO3 mAb (rosmantuzumab), small-molecule porecupine inhibitors (ETC-159, WNT-C59 and WNT974), tankyrase inhibitors (AZ1366, G007-LK, NVP-TNKS656 and XAV939) and β-catenin inhibitors (BC2059, CWP232228, ICG-001 and PRI-724), are in clinical trials or preclinical studies for the treatment of patients with WNT-driven cancers. WNT signaling-targeted therapeutics are applicable for combination therapy with BCR-ABL, EGFR, FLT3, KIT or RET inhibitors to treat a subset of tyrosine kinase-driven cancers because WNT and tyrosine kinase signaling cascades converge to β-catenin for the maintenance and expansion of CSCs. WNT signaling-targeted therapeutics might also be applicable for combination therapy with immune checkpoint blockers, such as atezolizumab, avelumab, durvalumab, ipilimumab, nivolumab and pembrolizumab, to treat cancers with immune evasion, although the context-dependent effects of WNT signaling on immunity should be carefully assessed. Omics monitoring, such as genome sequencing and transcriptome tests, immunohistochemical analyses on PD-L1 (CD274), PD-1 (PDCD1), ROR1 and nuclear β-catenin and organoid-based drug screening, is necessary to determine the appropriate WNT signaling-targeted therapeutics for cancer patients.

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1. Introduction

Cancer stem cells (CSCs), which show the potential for self-renewal and differentiation, have been identified in a variety of human cancers based on their tumor initiating potential in vivo (1-3). Clonal expansion of a minor CSC population with a drug-resistant mutation causes early recurrence, whereas reactivation of dormant CSCs into cycling CSCs owing to tumor plasticity leads to late relapse (4-6). CSCs or bulk tumor cells undergo epigenetic reprogramming (7), epithelial-mesenchymal reprogramming [epithelial-to-mesenchymal transition (EMT) and mesenchymal-to-epithelial transition (MET)] (8,9), immunological reprogramming (immuno-editing) (10,11) and metabolic reprogramming (12) to adapt to the tumor microenvironment, which is collectively defined here as ‘omics reprogramming’ (Fig. 1). Since cycling CSCs that depend on aerobic glycolysis converge into quiescent mesenchymal CSCs through omics reprogramming to survive therapeutic insult for later recurrence, CSC targeting is necessary to avoid relapse...
after cancer therapy and improve the cost-effectiveness ratio of cancer precision medicine.

CD44, CD133 (PROM1), EPCAM and LGR5 (GPR49) are representative cell-surface markers of CSCs (2,13-16). LGR5, encoding an R-spondin (RSPO) receptor, is a target gene of the canonical WNT/β-catenin signaling cascade in quiescent as well as cycling stem cells, whereas CD44 and CD133 are further upregulated by WNT and RSPO signals in LGR5+ cycling stem/progenitor cells (17-19). EPCAM can potentiate the canonical WNT/β-catenin signaling cascade through intra-membrane proteolysis and subsequent nuclear translocation of its intracellular C-terminal domain (20). WNT signaling cascades cross-talk with the FGF, Notch, Hedgehog and TGFβ/BMP signaling cascades to constitute the stem cell signaling network, which regulates expression of functional CSC markers (21-24).

The WNT family proteins transduce signals through the Frizzled (FZD) and LRPS/6 receptors to the WNT/β-catenin and WNT/STOP (stabilization of proteins) signaling cascades (also known as the canonical WNT signaling cascades) and through the FZD and/or ROR/R/ROR2/RYK receptors to the WNT/PCP (planar cell polarity), WNT/RTK (receptor tyrosine kinase) and WNT/Ca2+ signaling cascades (also known as the non-canonical WNT signaling cascades) (21,25-29). The canonical WNT/β-catenin signaling cascade is involved in self-renewal of stem cells and proliferation or differentiation of progenitor cells (30-33), whereas non-canonical WNT signaling cascades are involved in maintenance of stem cells, directional cell movement or inhibition of the canonical WNT signaling cascade (34-37). Both canonical and non-canonical WNT signaling cascades play key roles in the development and evolution of CSCs.

By contrast, tumors consist of heterogeneous populations of cancer cells and non-cancerous stromal/immune cells (38,39). Intra-tumor heterogeneity of cancer cells is caused by the evolution of CSCs based on epigenetic and genetic alterations (40-42), as well as the differentiation of CSCs into bulk tumor cells (1-3), niche-like cancer supporting cells (43), endothelial-like cancer cells (44) and fibroblast-like cancer cells (45). On the other hand, intra-tumor heterogeneity of non-cancerous stromal/immune cells is orchestrated by and reciprocally orchestrates CSCs and their descendants (39,45-47). Interaction and co-evolution of CSCs and niche cells are driving forces of cancer progression. Herein, canonical and non-canonical WNT signaling in CSCs will be described, with a focus on the heterogeneity of cancer and stromal/immune cells in the tumor microenvironment; then, anti-CSC mono- and combination therapies using WNT signaling-targeted therapeutics will be reviewed with emphases on omics reprogramming and tumor plasticity.

2. Canonical WNT signaling in CSCs and their niches

Canonical WNT signaling through the FZD-LRPS/6 receptor complex leads to de-repression of β-catenin as well as STOP-target proteins, such as ATOH1, CCND1 (Cyclin D1), FOXM1, MYC (c-MYC), NRF2 (NFE2L2), PLK1, SMAD3/3, SNAI1 (Snail) and YAP/TAZ, from proteasomal degradation induced by GSK-3β-dependent phosphorylation and subsequent ubiquitylation (27-29,48) (Fig. 2). β-catenin stabilization and subsequent nuclear translocation leads to transcriptional activation of β-catenin-TCF/LEF target genes, such as ATOH1, CCND1, CD44, FGF20, JAG1, LGR5, MYC and SNAI1, although transcriptional outputs of the WNT/β-catenin signaling cascade are determined in a cellular context-dependent manner (e.g., epigenetic status of target genes and activities of other transcriptional regulators). ATOH1, CCND1, MYC and SNAI1 are upregulated transcriptionally and post-translationally by the β-catenin and STOP signaling cascades, respectively. Canonical WNT signals control cell fate and function through transcriptional and post-translational regulation of the omics network.

Canonical WNT signaling in CSCs is activated by WNT2B, WNT3 and other canonical WNT ligands derived from cancerous supporting cells or non-cancerous stromal cells (49-52), as well as genetic alterations in the canonical WNT/β-catenin signaling components, such as ERF3E-RSPO2 fusions, PTPRK-RSPO3 fusions, gain-of-function mutations in the CTNNB1 (β-catenin) gene and loss-of-function mutations in the APC, AXIN1, AXIN2, NRF3 and ZNF3 genes (29,53-55). Canonical WNT signals increase the LGR5 receptor level on CSCs for the maintenance of the canonical WNT responsive state but also upregulate AXIN2, DKK1, NOTUM, NRF3 and ZNF3 for negative feedback regulation (18-21,29). Loss-of-function mutations in the APC, AXIN2, NRF3 and ZNF3 genes release CSCs from the constraints of the negative feedback regulation.

Canonical WNT signals can directly promote CSC proliferation through upregulation of CCND1, FOXM1, MYC and YAP/TAZ as described above. By contrast, canonical WNT signaling in CSCs induces expression and secretion of growth factors, such as FGFs, KIT ligand (KITLG or SCF) and VEGF (VEGFA), to fine-tune the tumor microenvironment (18,21,29). For example, MET (HGF receptor) is upregulated in human basal-like breast cancers with TP53 mutations as well as mouse basal-like breast tumors with compound gain-of-function Cmnb1 mutation and homozygous Tp53 deletion (56), and combined activation of the canonical WNT/β-catenin and HGF/MET signaling cascades induces SHH upregulation in mouse mammary CSCs and subsequent activation of cancer-associate fibroblasts for the synergistic proliferation of CSCs and cancer-associate fibroblasts (57).

Together, these findings indicate that canonical WNT signaling is involved in the maintenance and expansion of CSCs through direct effects on CSCs themselves and indirect effects via CSC-stromal/immune interactions.

3. Non-canonical WNT signaling in CSCs and their niches

Non-canonical WNT signaling through FZD receptors and/or ROR/R/ROR2/RYK co-receptors activates the PCP, RTK or Ca2+ signaling cascades (Fig. 2).

Non-canonical WNT/PCP signaling through FZD receptors and Dishevelled (DVL) adaptor proteins regulates the coordinated cellular orientation within an epithelial plane, collective cell movements during gastrulation and neurulation stages of embryogenesis and directional cell movement during invasion and metastasis of cancer cells (58-62). WNT/PCP signals are converted to actin cytoskeletal dynamics via the small G-proteins RAC and RHO (Fig. 2), and then, RAC and RHO
activate JNK-dependent transcription and YAP/TAZ-dependent transcription, respectively (63-66). WNT/PCP signaling regulates actin cytoskeletal dynamics, directional cell movement and JNK- or YAP/TAZ-dependent transcription.

Non-canonical WNT signaling through RTKs, such as ROR1, ROR2 and RYK, activates the PI3K-AKT signaling cascade (29,67-69). ROR1 and ROR2, with the extracellular WNT-binding FZD-like domain, are homologous to MUSK, NTRK1, NTRK2, NTRK3, DDR1 and DDR2 in their cytoplasmic tyrosine kinase domain, whereas RYK with an extracellular WNT-binding WIF domain is homologous to AXL, EGFR, ERBB2, ERBB3, ERBB4, MET, MERTK, MST1R and TYRO3 in its cytoplasmic tyrosine kinase domain (39,70-73). ROR1 and ROR2 are atypical RTKs that...
are defective in intrinsic tyrosine kinase activity for auto-
phosphorylation; however, ROR1 and ROR2 can be tyrosine
phosphorylated by other tyrosine kinases, such as EGFR,
ERBB3, MET and SRC, to activate the PI3K-AKT and
YAP signaling cascades, whereas non-canonical WNT signaling through FZD and/or ROR1/ROR2/RYK receptors is
transduced by the WNT/PCP (planar cell polarity), WNT/RTK (receptor tyrosine kinase) and WNT/Ca2+ signaling cascades. Antibody-based drugs, such as
anti-LGR5 antibody-drug conjugate (ADC), anti-RSPO3 monoclonal antibody (mAb), anti-ROR1 mAb and anti-PTK7 ADC, ROR1 chimeric antigen receptor-
modified T (CAR-T) cells, porcupine (PORCN) inhibitors and β-catenin inhibitors are representative WNT signaling-targeted therapeutics in clinical trials or
preclinical studies for the treatment of cancer patients.

Figure 2. Overview of WNT signaling cascades and WNT signaling-targeted therapeutics. WNT signals are transduced by multiple downstream signaling
cascades in a cell context-dependent manner. Canonical WNT signaling through Frizzled (FZD) and LRP5/6 receptors is transduced by the WNT/β-catenin
and WNT/STOP (stabilization of proteins) signaling cascades, whereas non-canonical WNT signaling through FZD and/or ROR1/ROR2/RYK receptors is
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the endoplasmic reticulum via small G-protein- or SEC14L2-
mediated activation of phospholipase C (PLC) and subsequent
generation of inositol-1,4,5-triphosphate (IP3) (21,79-81).
WNT signaling through Polycystin 1 (PKD1) is proposed
to induce Ca2+ influx through a TRPP2 Ca2+ channel (82).
Ca2+/Calmodulin-dependent protein kinase II (CAMK2) and
Calcineurin are representative downstream effectors of the
WNT/Ca2+ signaling cascade (Fig. 2). For example, WNT/Ca2+
signaling-dependent CAMK2 activation leads to phosphoryla-
tion and activation of Nemo-like kinase (NLK), which can
inhibit canonical WNT/β-catenin signaling in some cells (83).
PI3K-AKT signaling activation and YAP/TAZ-mediated transcription promotes survival and therapeutic resistance of CSCs through cascades. For example, canonical WNT/β-catenin signaling cascades and non-canonical WNT signaling βγ-clearly indicate that canonical WNT/β-catenin promotes invasion, survival and metastasis of CSCs or circumventing E-cadherin (CDH1) repression through transcriptional activation of NFAT-target genes, de-repression of MEF2-target genes and repression WNT/β-catenin-target genes in a cellular context-dependent manner.

Non-canonical WNT signaling in CSCs is activated by WNT5A, WNT11 and other non-canonical WNT ligands that are secreted from cancer cells (86,87) or stromal/immune cells (88,89), as well as genetic alterations that trans-activate non-canonical WNT signaling cascades, such as E2A-PBX1 fusion and MET amplification (74-76). Non-canonical WNT signaling through FZD7 activates the PI3K-AKT signaling cascade as a result of Daple (CCDC88C)-mediated dissociation of Gβγ from Gai (90), whereas non-canonical WNT signaling through ROR1 activates PI3K-AKT signaling cascade owing to ROR1 trans-phosphorylation by other tyrosine kinases, such as MET and SRC (67,75). ROR1 is involved in HER3-Y1307 trans-phosphorylation and subsequent NSUN6-dependent MST1-K59 methylation, which induces YAP/TAZ-dependent transcriptional activation through LATS1/LATS2 inhibition (78). WNT/PCP signaling can also induce Rho-mediated LATS1/LATS2 inhibition for transcriptional activation of YAP/TAZ-target genes (91,92), whereas non-canonical WNT signaling through FZD10 induces YAP/TAZ activation through Gai3 (93). Non-canonical WNT signaling promotes survival and therapeutic resistance of CSCs through PI3K-AKT signaling activation and YAP/TAZ-mediated transcriptional activation.

By contrast, invasion and metastasis are driven by canonical WNT signaling cascades and non-canonical WNT signaling cascades. For example, canonical WNT/β-catenin and WNT/STOP signaling cascades synergistically upregulate SNAI1 to repress epithelial genes, such as CDH1 (E-cadherin), for the initiation of EMT of CSCs, and non-canonical WNT signals promote invasion, survival and metastasis of CSCs or circulating tumor cells (28,29,35,62,87). Together, these findings clearly indicate that canonical WNT/β-catenin signaling as well as WNT signaling cascades are critically involved in the malignant features of CSCs.

4. Anti-CSC mono-therapy targeting WNT signaling cascades

WNT signaling cascades are hot and cutting-edge topics in the field of translational oncology and medicinal chemistry (29,94-96). Therapeutics directly targeting WNT signaling cascades are classified into i) ligand/receptor-targeted drugs binding to ligands or transmembrane proteins involved in WNT signaling, ii) porcupine (PORCN) inhibitors abrogating WNT secretion and FZD-dependent signaling, iii) tankyrase (TNKS) inhibitors repressing WNT/β-catenin and WNT-independent signaling cascades and iv) β-catenin inhibitors blocking TCF/LEF-dependent transcription (Table I).

Human/humanized monoclonal antibody (mAb) drugs, such as anti-FZD1/2/5/7/8 mAb (vantictumab/OMP-18R5) (97), anti-FZD5 mAb (lgG-2919) (52), anti-FZD10 antibody-drug conjugate (ADC) (OTS101-DTPA- 90Y) (98), anti-LGR5 ADC (mAb-mc-vc-PAB-MMAE) (99), anti-PTK7 ADC (PF-06647020) (100), anti-ROR1 mAb (cirtumuzumab/ UC-961) (101) and anti-RSPO3 mAb (rosmantuzumab/ OMP-131R10) (102) have been developed as large-molecule cancer therapeutics. ROR1 CAR-T cells (103) and WNT-trapping FZD8-Fc chimeric protein (ipafricept/OMP-54F28) (104) are also classified as WNT ligand/receptor-targeted drugs. Among this class of therapeutics, cirtumuzumab, ipafricept, PF-06647020, rosmantuzumab and vantictumab, which showed anti-CSC effects in preclinical model experiments, are in clinical trials to treat cancer patients (Table I).

PORCN inhibitors restrain PORCN-dependent palmitoylation of WNT family ligands in the endoplasmic reticulum, which obstructs WNT signaling through blockade of WNT secretion as well as palmitoylated WNT-mediated oligomerization of FZD receptors (105-108). ETC-159 (109), IWP-2 (110), WNT-C59 (111) and WNT974 (LGK974) (112) are small-molecule PORCN inhibitors. A preclinical study of IWP-2 on organoids derived from colorectal cancer patients revealed that PORNC inhibitors are applicable for the treatment of cancers with RNF43 mutations but not APC mutations (52). By contrast, preclinical studies of WNT974 indicated that PORNC inhibitors repress the survival and tumor initiating potential of CSCs (43,112). ETC-159 and WNT974 are in clinical trials for the treatment of cancer patients (Table I).

TNKS inhibitors repress TNKS-dependent poly-ADP-riboseylation and subsequent degradation of negative regulators of oncogenic signaling cascades, such as AXIN family proteins, AMOT family proteins, PTEN and TERTF1 (TRF1), which results in inhibition of WNT/β-catenin signaling, repression of YAP-dependent transcription, suppression of PI3K signaling and telomere shortening, respectively (113-116). AZI366 (117), G007-LK (118), JW55 (119), NVP-TNKS656 (120) and XAV939 (121) are representative TNKS inhibitors that abrogate WNT/β-catenin signaling and tumorigenesis in preclinical mouse model experiments. TNKS inhibitors show synergistic antitumor effects with other therapeutics, such as an AKT inhibitor (API2), EGFR inhibitors (gefitinib and erlotinib), a MEK inhibitor (AZD6244), a PI3K inhibitor (BKMI120) and irinotecan (117,118,120,122-124). TNKS inhibitors are promising candidates for CSC-targeted therapeutics; however, because of diverse on-target effects, TNKS inhibitors stalled in their preclinical stage.

β-catenin inhibitors block TCF/LEF-dependent transcription through inhibition of protein-protein interactions (PPI) between β-catenin and other transcriptional regulators (29,125), promotion of β-catenin degradation (126) or inhibition of β-catenin kinases, such as TNIK (127-129). BC0259 (130), CGP049090(131), CWP232228 (132), ICG-001 (133), LF3 (134), PKF115-584 (135), PRI-724 (136) and SAH-BCL9 (137) are small-molecule β-catenin PPI inhibitors. MSAB is a small-molecule compound that binds to β-catenin and promotes proteosomal degradation of β-catenin (126), KY-05009 (128),
Table I. WNT signaling inhibitors and anti-CSC effects.

| Category                        | Drug                          | Preclinical Anti-CSC TX | (Refs.) | Drug development stage | Details of clinical trials for cancer patients          |
|---------------------------------|-------------------------------|--------------------------|---------|-------------------------|---------------------------------------------------------|
| Ligand/receptor-targeted drug   | Anti-FZD1/2/5/7/8 mAb (Vantictumab, OMP-18R5) | Breast CSC | (97) | P1 (NCT01345201)       | Solid tumors, Mono                                      |
|                                 | Anti-FZD5 mAb (IgG-2919)     | Panc CSC                 |         | P1 (NCT01957007)       | Solid tumors. Combo                                      |
|                                 | Anti-FZD10 ADC (OTSA101-DTPA-90Y) | |         | P1 (NCT01973309)       | Breast, Combo                                           |
|                                 | Anti-LGR5 ADC (mAb-nc-vc-PAB-MMAE) | |         | P1 (NCT02005315)       | Panc, Combo                                              |
|                                 | Anti-PTK7 ADC (PF-06647020) | Breast CSC | (100) | P1 (NCT02222922)       | Solid tumors, Mono                                      |
|                                 | Anti-ROR1 mAb (Cirtmutuzumab, UC-961) | |         | P1 (NCT0222688)        | CLL, Mono                                                |
|                                 | Anti-LGR5 ADC (mAb-nc-vc-PAB-MMAE) | Lung CSC | (100) | P1 (NCT02776917)       | Breast, Combo                                            |
|                                 | Anti-RSPO3 mAb (Rosmantuzumab, OMP-131R10) | |         | P1/2 (NCT03088878)     | CLL/MCL/SLL, Combo                                      |
|                                 | ROR1 CAR-T cells (Ipafriccept, OMP-54F28) | Panc CSC | (104) | P1 (NCT02482441)       | Solid tumors, Combo                                      |
|                                 | PORCN inhibitor               | ETC-159                  | (109)  | P1 (NCT02521844)       | Solid tumors, Mono                                      |
|                                 | IWP-2                         | WNT-C59                  | (110)  | Preclinical             |                                                         |
|                                 | WNT974 (LGK974)               | CML CSC                  | (112)  | P1 (NCT01351103)       | Solid tumors, Mono                                      |
|                                 |                               | Lung CSC                 | (43)   | P1/2 (NCT02728133)     | mCRC, Combo                                              |
|                                 | TNKS inhibitor                | AZ1366                   | (117)  | Preclinical             |                                                         |
|                                 | G007-LK                       | JW55                     | (119)  | Preclinical             |                                                         |
|                                 | NVP-TNKS656                   | XAV939                   | (121)  | Preclinical             |                                                         |
|                                 | β-catenin inhibitor           | BC2059                   | (130)  | Preclinical             |                                                         |
|                                 |                               | CGP049090                | (131)  | Preclinical             |                                                         |
|                                 |                               | CWP232228                | (132)  | Preclinical             |                                                         |
|                                 |                               | ICG-001                  | (133)  | Preclinical             |                                                         |
|                                 |                               | KY-05009                 | (138)  | Preclinical             |                                                         |
|                                 |                               | LF3                      | (134)  | Preclinical             |                                                         |
|                                 |                               | Mebendazole              | (129)  | P1 (NCT01729260)       | Glioma, Mono                                             |
|                                 |                               |                         |         | P1 (NCT02644291)       | Glioma, Mono                                             |
|                                 |                               |                         |         | P1/2 (NCT01837862)     | Glioma, Combo                                            |
|                                 | MSAB                          |                         | (126)  | Preclinical             |                                                         |
|                                 | PF-794                        |                         | (138)  | Preclinical             |                                                         |
|                                 | PKF115-584                    | PRI-724                  | (136)  | P1 (NCT01764477)       | Panc, Combo                                              |
|                                 |                               |                         |         | P1/2 (NCT01606579)     | AML/CML/Combo                                            |
|                                 | PRI-724                       |                         | (137)  | Preclinical             |                                                         |

PORCN, porcupine; TNKS, tankyrase; PPI, protein-protein interaction; mAb, monoclonal antibody; bsAb, bispecific antibody; ADC, antibody-drug conjugate; P1, phase I; P2, phase II; AML, acute myeloid leukemia; Breast, breast cancer; CLL, chronic lymphocytic leukemia; CML, chronic myeloid leukemia; Liver, hepatocellular carcinoma; MCL, mantle cell lymphoma; mCRC, metastatic colorectal cancer; NPC, nasopharyngeal carcinoma; NSCLC, non-small cell lung cancer; Ovary, ovarian cancer; Panc, pancreatic cancer; SLL, small lymphocytic lymphoma; Mono, mono-therapy; Combo, combination therapy.
mendezole (129) and PF-794 (138) are TNIK inhibitors that repress phosphorylation of TNIK substrates, such as TCF4, FMNL2, PRICKLE1, SMAD1 and SMAD2, which leads to inhibition of β-catenin-TCF/LEF-dependent transcription and a variety of cellular processes. Among the β-catenin inhibitors mentioned above, BC2059, CWP232228 and ICG-001 repress the expansion of CSCs. The β-catenin inhibitors PRI-724 and mebendazole are in phase I/II clinical trials for cancer patients (Table I), whereas other β-catenin inhibitors are still in the preclinical stage of drug development. β-catenin inhibitors are challenging therapeutics for cancer patients.

WNT signaling cascades are the major driver of various types of human cancers (29), but the development of many WNT signaling-targeted therapeutics is stuck in the preclinical stage or phase I/II stages of clinical trials (Table I) because of the complexity of WNT signaling cascades and genetic alterations in non-enzymatic signaling components. MAAb-based drugs and PORCN inhibitors with the potential to target CSCs as well as bulk cancer cells are promising therapeutics for the patients with WNT signaling-driven cancers.

5. Anti-CSC combination therapy using WNT signaling-targeted drugs

Tyrosine kinase inhibitors are rational anticancer therapeutics because tyrosine kinases with intrinsic enzyme activities are aberrantly activated in cancer cells owing to genetic alterations. Tyrosine kinase inhibitors have contributed to the improved prognosis of cancer patients and are essential for genome-based precision medicine; however, unavoidable drug resistance or recurrence is a serious issue for cancer patients and health care systems (4).

Activated tyrosine kinases, such as BCR-ABL fusion kinase, EGFR-T790M mutant, FLT3 internal tandem duplication (FLT3-ITD) mutant, KIT-D814V mutant and RET, promote β-catenin phosphorylation at Y654 to release E-cadherin-bound β-catenin from the adherens junction for its stabilization and subsequent nuclear translocation (139-143). By contrast, canonical WNT signals inhibit β-catenin phosphorylation at S33, S37, T41 and S45 to release β-catenin from proteasomal degradation for its stabilization and nuclear translocation (21,25,26,29). Since canonical WNT signals and oncogenic tyrosine kinases converge to β-catenin stabilization for the maintenance and expansion of CSCs, canonical WNT signaling inhibitors can block CSC evasion of tyrosine kinase inhibitors. For example, the porcupine inhibitor WNT974 significantly reduced residual stem/progenitor cells of chronic myeloid leukemia (CML) after treatment with the BCR-ABL inhibitor nilotinib via blockade of WNT ligand secretion into the bone marrow microenvironment (112); the β-catenin inhibitors ICG-001 and PRI-724 induced synergistic effects with the BCR-ABL inhibitors imatinib and nilotinib, respectively, on CML stem/progenitor cells (136,144); and the TNKS inhibitor AZI366 and EGFR inhibitor gefitinib showed synergistic effects on lung cancer cells in vivo (124). These preclinical studies indicate that combination therapies using WNT signaling-targeted therapeutics and tyrosine kinase inhibitors might be applicable for treatment of a subset of patients with tyrosine kinase-driven cancers (Fig. 3).

Immune checkpoint blockers that abrogate interactions of ligands and inhibitory receptors on CD8+ T cells are promising antitumor drugs in the clinic or clinical trials (145-151). PD-L1 (CD274) is a representative ligand for inhibitory immune signaling, whereas PD-1 (PDCD1) and CTLA4 are representative receptors for inhibitory immune signaling. Anti-PD-L1 mAbs (atezolizumab, avelumab and durvalumab), anti-PD-1 mAbs (nivolumab and pembrolizumab) and an anti-CTLA4 mAb (ipilimumab) are approved for the treatment of patients with melanoma or other types of solid tumors. Immune checkpoint blockers result in significant therapeutic effects in a subset of patients; however, the lack of benefits in other patients owing to primary or acquired resistance to immune checkpoint blockers has resulted in a cost-effectiveness issue (152-156).

Canonical WNT signaling activation in melanoma induces immune evasion through CCL4 repression and immunological reprogramming into non-T cell-infiltrated melanoma (11). Since melanoma-derived WNT5A promotes β-catenin signaling activation and subsequent IDO upregulation in dendritic cells to induce immune evasion through accumulation of regulatory T (Treg) cells, combination immunotherapy using the porcupine inhibitor WNT-C59 and anti-CTLA4 mAb showed synergistic anti-melanoma effects in vivo. By contrast, WNT5A and ROR2 are relatively frequently upregulated in pretreatment tumors of melanoma patients that do not respond to PD-1 immune checkpoint blockade (158), which suggests involvement of non-canonical WNT signaling in resistance to immune checkpoint blockers. Since DKK1-dependent canonical WNT signaling inhibition or putative reciprocal non-canonical WNT signaling activation in tumor microenvironment induces immune evasion through accumulation of myeloid-derived suppressor cells (MDSCs) and depletion of T cells (159), combination therapy using anti-DKK1 mAb (BHQ880 or DKN-01) (160,161) and immune checkpoint blockers might show synergistic antitumor effects in vivo. WNT signaling-targeted therapeutics might be applicable for combination immunotherapy for cancer patients (Fig. 3); however, context-dependent effects of WNT signaling on immunity (4) should be kept in mind.

6. Omics monitoring for WNT signaling-targeted therapy

WNT-related human cancers are classified into three major subtypes based on signaling aberrations associated with therapeutic choices (Fig. 3): APC/CTNNB1-altered cancers with WNT/β-catenin signaling activation that can be treated with β-catenin inhibitors; RNF43/ZNRF3/RSP02/RSP03-altered cancers with WNT/β-catenin and other WNT signaling activation that can be treated with PORCN inhibitors, anti-FZD mAb or anti-RSPO3 mAb; and ROR1-upregulated cancers with WNT/PCP and WNT/RTK signaling activation that can be treated with anti-ROR1 mAb, anti-ROR1 x CD3 bispecific antibody and ROR1 chimeric antigen receptor-modified T (CAR-T) cells (29). Genome sequencing, transcriptomic and/or immunohistochemical tests are necessary for the detection and subtyping of WNT signaling-driven cancers and subsequent determination of appropriate WNT signaling-targeted therapeutics (Fig. 3). WNT signaling-targeted therapeutics are also applicable for combination therapies with tyrosine kinase inhibitors or...
immune checkpoint blockers as mentioned above (Fig. 3). Since resistance to tyrosine kinase inhibitors occur owing to multiple mechanisms, such as acquired drug-resistant mutations in targeted tyrosine kinases, EMT, activation of other tyrosine kinase signaling cascades to bypass targeted tyrosine kinases (4,162) and activation of WNT/β-catenin signaling cascade (Fig. 3), genomic, transcriptomic and/or immunohistochemical monitoring during tyrosine kinase inhibitor treatment is also necessary to identify a subset of patients for combination therapy with tyrosine kinase inhibitor and WNT signaling-targeted therapeutics. By contrast, because WNT signaling in the tumor microenvironment orchestrates antitumor immunity
and immune tolerance in a context-dependent manner, immune monitoring is necessary to choose the appropriate WNT signaling-targeted therapeutics for cancer patients with immune evasion (Fig. 3).

Investigational genome medicine platforms based on nucleotide sequencing of transcribed regions are applicable for determination of targeted therapeutics only in 10-24% of cancer patients (163,164). Since alterations in non-transcribed regulatory regions also drive human carcinogenesis, whole-genome sequencing rather than whole- or partial-exome sequencing is preferable to improve the precision of genome-based medicine (4,165). In addition, organoid culture is a cutting-edge technology in the fields of oncology and stem cell biology (166-168), and organoid-based tests are also used for selecting targeted therapeutics (163,166). However, because tumor-stromal/immune interactions are not recapitulated in patient-derived organoid models, immunological monitoring in the tumor microenvironment is also necessary to improve genome-based medicine.

Together, these findings indicate that ‘omics monitoring’, including genome sequencing, transcriptomic, immunohistochemical and organoid-based tests, before and during treatment is necessary to choose and fine-tune WNT signaling-targeted therapeutics for the treatment of cancer patients (Fig. 3).

7. Conclusion

Cancer stem cells (CSCs) are part of the tumor microenvironment and survive host defense or therapeutic insult through omics reprogramming. Aberrant WNT signaling activation in human cancers promotes CSC survival, bulk-tumor expansion and invasion/metastasis. Anti-FZD mAb, anti-ROR1 mAb, anti-RSPO3 mAb, PORCN inhibitors and β-catenin inhibitors are representative WNT signaling-targeted therapeutics in clinical trials or preclinical studies. WNT signaling-targeted therapeutics are applicable for combination therapy with tyrosine kinase inhibitors or immune checkpoint blockers. Omics monitoring is necessary for therapeutic optimization of WNT signaling-targeted therapy.

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