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Review

Frizzled Receptors in Tumors, Focusing on Signaling, Roles, Modulation Mechanisms, and Targeted Therapies

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Wnt molecules play crucial roles in development and adult homeostasis through their receptors Frizzled proteins (Fzds). Fzds mediate canonical β -catenin pathway and various noncanonical β -catenin-independent pathways. Aberrant Fzd signaling is involved in many diseases including cancer. Wnt/ β -catenin is a well-established oncogenic pathway involved in almost every aspect of tumor development. However, Fzd-mediated noncanonical Wnt pathways function as both tumor promoters and tumor suppressors depending on cellular context. Fzd-targeted therapies have proven to be effective on cultured tumor cells, tumor cell xenografts, mouse tumor models, and patient-derived xenografts (PDX). Moreover, Fzd-targeted therapies synergize with chemotherapy in preclinical models. However, the occurrence of fragility fractures in patients treated with Fzd-targeted agents such as OMP-54F28 and OMP-18R5 limits the development of this combination. Along with new insights on signaling, roles, and modulation mechanisms of Fzds in human tumors, more Fzd-related therapeutic targets will be developed.

Key words: Wnt; Frizzled; β -Catenin; Tumor

INTRODUCTION

Frizzled proteins (Fzds) belong to the superfamily of G protein-coupled receptor (GPCR), with extracellular N-terminus, seven-transmembrane domain, and intracellular C-terminus. The extracellular N-terminus contains a cysteine-rich domain (CRD), which is highly conserved and can bind Wnt ligands. A flexible linker region connects the CRD to the transmembrane domain. The C-terminus contains several conserved motifs, which can bind the PDZ domain of Dishevelled (Dvl). The C-terminus also interacts with G proteins. Ten Fzds have been identified in humans. Phylogenetic analysis indicates that these Fzds can be divided into four or five subfamilies: Fzd1/2/7, Fzd3/6, Fzd5/8, Fzd4/9/10 or Fzd4 and Fzd9/10^{1–3}.

Fzd SIGNALOSOME

Canonical Signalosome

Canonical Wnt/ β -catenin pathway is mediated by a signalosome consisting of Fzd receptors, Lrp5/6 coreceptors, and Dvl and Axin adapters. Upon binding Wnt

ligands, Fzds and Lrp5/6 oligomerize to create a scaffold in which Dvl is phosphorylated and copolymerizes with Axin, leading to the dissociation of β -catenin destruction complex⁴. Notably, a recent study has shown that oligomerization of Fzds and Lrp5/6 can activate β -catenin pathway in the absence of Wnt ligands⁵.

Noncanonical Signalosome

Several Wnt ligands such as Wnt5a generally fail to induce Lrp6 phosphorylation and β -catenin pathway activation due to the lack of functional interaction with Lrp5/6^{6–8}. Binding of Wnt5a to Fzds results in Fzd oligomerization and Dvl phosphorylation independent of Lrp5/6^{9,10}. In some circumstances, Wnt5a signaling requires Ror1/2, which can form a receptor complex with Fzds^{8,11–13}.

DOWNSTREAM SIGNALING OF Fzds IN TUMORS

β -Catenin Pathway

Activation of canonical Fzd signalosome causes β -catenin destruction complex dissociation. Therefore,

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β -catenin in the cytoplasm escapes from phosphorylation by GSK3 β and degradation by proteasome. Accumulated β -catenin translocates into the nucleus, promoting the transcription of TCF/LEF target genes (Fig. 1A). MMTV/WNT1 transgenic mice that can develop mammary hyperplasia and adenocarcinoma clearly demonstrate the capacity of β -catenin pathway to induce malignant transformation^{14,15}. In colorectal cancer, adenomatous polyposis coli (APC), a component in β -catenin destruction complex, is frequently mutated, leading to activation of β -catenin pathway¹⁶. Actually, activation of β -catenin pathway is involved in almost all aspects of human tumor initiation and progression, including stemness, growth, survival, drug resistance, angiogenesis, immune evasion, and metastasis^{17–19}.

G Protein–Ca²⁺–PKC Pathway

Binding of Wnt5a to several Fzds such as Fzd1, Fzd2, Fzd5, Fzd6, and Fzd9 has been shown to activate G protein signaling^{20–24}. Consistently, Fzd-mediated β -catenin pathway seems not to require the involvement of heterotrimeric G proteins²⁵. Although the structure of Fzds is distinct from that of other GPCRs²⁶, Fzds still can mediate intracellular Ca²⁺ release and PKC activation through phospholipases C (PLC) signaling (Fig. 1B). Wnt5a–Fzd promotes melanoma metastasis via PKC^{27–29}, and colitis-associated cancer via CaMKII³⁰.

Planar Cell Polarity (PCP) Pathway

The Wnt/PCP pathway controls tissue polarity and cell movement through downstream signaling such as Rho GTPases and JNK^{31–33} (Fig. 1C). During development, Wnt5a/b and Wnt11 activate the PCP pathway through Fzd3/6 and Ror1/2, involving Vangl1/2 and Dvl³³. In human tumors, the Wnt5a–Fzd/PCP pathway promotes the progression of chronic lymphocytic leukemia (CLL) and ovarian cancer^{34–37}.

Stat3 Pathway

The oncogenic pathway interleukin-6 (IL-6)/Jak/Stat3 is involved in many types of tumors. Binding of Wnt5a/b to Fzd2 recruits and phosphorylates Stat3 in cancer cells, which seems not to be related to G proteins and Dvl³⁸. In liver cancer cells, Fyn kinase but not IL-6/Jak is responsible for Stat3 phosphorylation. However, Wnt5a activates IL-6/Stat3 signaling in CLL and breast cancer^{39,40}. Furthermore, Wnt5a and IL-6 form a positive feedback loop to activate Stat3 in melanoma^{41–43}, and Stat3 transcriptional upregulates Wnt5a expression in CLL⁴⁴. These studies indicate that Wnt5a–Fzd cross-talks with IL-6/Stat3 in some human tumors (Fig. 2A).

Yap/Taz Pathway

Activation of LATS1/2 in the Hippo pathway induces phosphorylation of Yap and Taz, two transcriptional

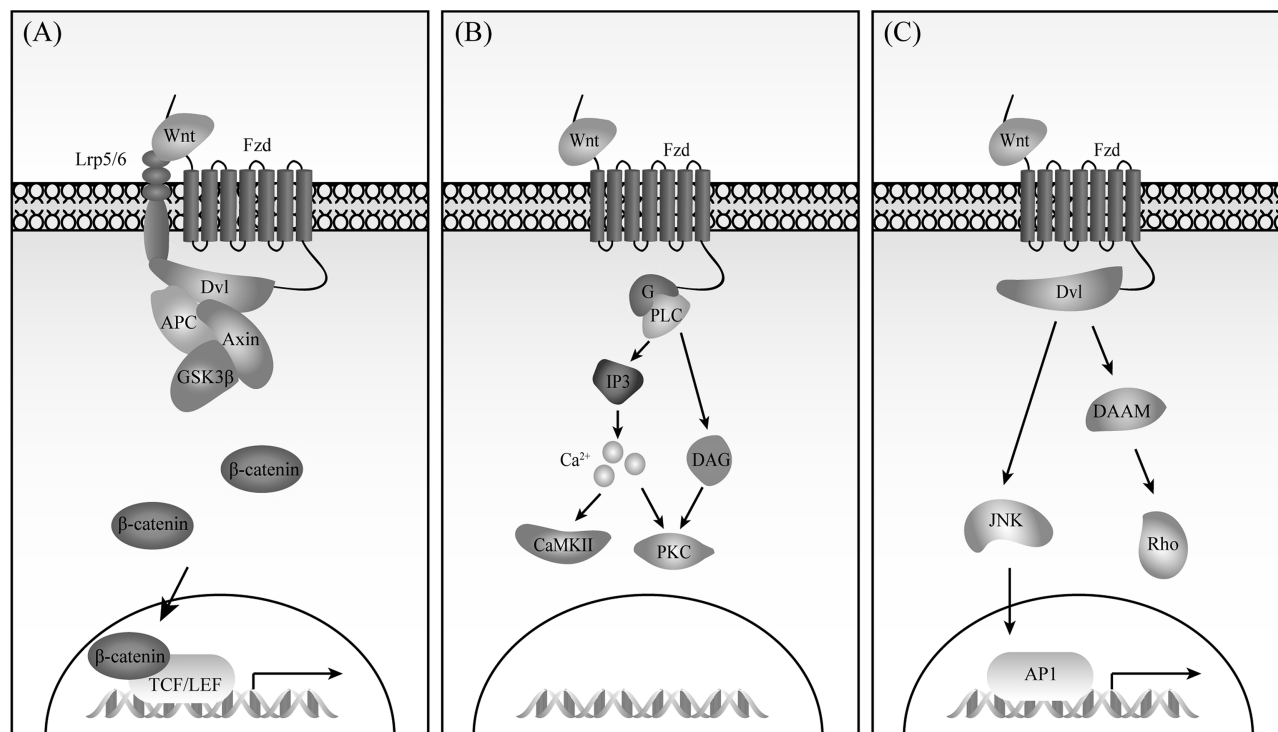


Figure 1. Downstream signaling of Frizzled proteins (Fzds). (A) β -Catenin pathway. (B) G protein–Ca²⁺–PKC pathway. (C) PCP pathway.

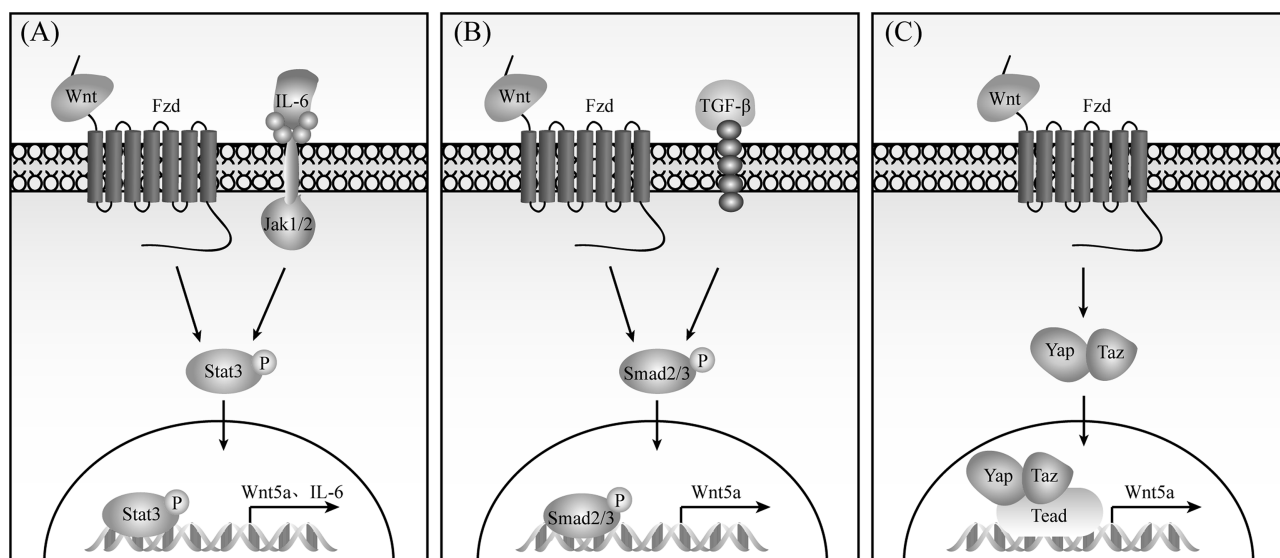


Figure 2. Fzd signaling cross-talks with Stat3 (A), Yap/Taz (B), and TGF- β (C) pathways.

coactivators, which are subsequently degraded by proteasome. When Yap and Taz are not phosphorylated, they enter into the nucleus to bind transcription factor Tead. Both Wnt5a/b and Wnt3a activate Yap/Taz–Tead signaling independent of Lrp5/6 and β -catenin; Fzd1, Fzd2, and Fzd5 all can mediate this signaling, but Ror1 is required to coordinate with Fzd2 and Fzd5²². Yap/Taz–Tead signaling antagonizes β -catenin pathway, while it promotes Wnt5a transcription, thereby forming a positive feedback loop (Fig. 2B). Wnt5a/b–Fzd2/5 modulates Yap expression in a series of human malignant tumors including breast cancer, hepatocellular carcinoma (HCC), melanoma, and squamous subtype pancreatic adenocarcinoma^{40,45–47}.

Transforming Growth Factor- β (TGF- β) Pathway

Both TGF- β and Wnt5a play dual roles in human tumors. As strong inducers of epithelial–mesenchymal transition (EMT), they increase cell motility and promote tumor metastasis. On the other hand, they have the potential to suppress cell growth, functioning as tumor suppressors. Cross-talk between Wnt5a and TGF- β exits in breast cancer; TGF- β maintains Wnt5a expression, and inversely, Wnt5a stimulates Smad2 phosphorylation in a TGFBR1-dependent manner^{40,48,49}. Moreover, Fzd8 forms a receptor complex with TGFBR1 to mediate both Wnt11 and TGF- β signaling in prostate cancer⁵⁰. Wnt5a and TGF- β also coordinate in the pathogenesis of organ fibrosis^{51–53}. TGF- β can induce Fzd expression in fibroblasts^{54,55}. Together, these studies indicate that Fzds interact with TGF- β receptors to enhance TGF- β signaling, which further upregulates Wnt5a/Fzds (Fig. 2C).

TUMOR-PROMOTING ROLES OF Fzds

All Fzds except Fzd9 have been found to play tumor-promoting roles. Through both canonical and noncanonical Wnt pathways, Fzds contribute to tumor initiation, growth, chemoresistance, and metastasis (Table 1).

Fzd1/2/7 Subfamily

Comparative studies between neuroblastoma (NB) cell lines and their chemoresistant counterparts revealed that Fzd1 contributes to NB chemoresistance⁵⁶. Fzd1 expression in chemoresistant NB cells is associated with Wnt/ β -catenin activity and multidrug resistance gene MDR1 expression. Fzd1 knockdown enhances the sensitivity of chemoresistant NB cells to chemical drugs. Moreover, Fzd1 expression is significantly upregulated in NB tumors from relapsed patients after chemotherapy. Fzd1 is also overexpressed in chemoresistant breast cancer and leukemic cell lines, and Fzd1 knockdown downregulates MDR1 expression, suppresses Wnt/ β -catenin activity, and restores the sensitivity to chemotherapy^{57,58}. These studies have clearly demonstrated that Fzd1 overexpression induces chemoresistance through activation of the Wnt/ β -catenin–MDR1 pathway, potentially facilitating tumor recurrence.

Fzd2 is overexpressed in poorly differentiated, mesenchymal-type, and late-stage tumor samples of liver, lung, colon, and mammary gland³⁸. In vitro studies further showed that Fzd2 overexpression induces EMT and cell migration via Stat3 signaling, and consistently, Fzd2 knockdown or treatment with an anti-Fzd2 antibody suppresses tumor metastasis in mouse xenograft models³⁸. Both EMT and Stat3 signaling are related to cancer cell

Table 1. Tumor-Promoting Roles of Frizzled Proteins (Fzds)

Fzds	Wnt Pathways	Malignant Tumors	Functions	References
Fzd1	Canonical	NB, breast cancer, AML	Chemoresistance	55–58
Fzd2	Noncanonical	Liver, breast, colon, lung cancers	EMT, metastasis, stemness	38, 40, 45
Fzd7	Canonical	Liver, breast cancers	Growth, stemness	59–62
Fzd3	Noncanonical	Melanoma	Stemness, metastasis	69
Fzd6	Noncanonical	NB, TNBC	Stemness, metastasis, chemoresistance	70, 71
Fzd5	Canonical	Endometrial, ovarian, pancreatic cancers	Growth	72–74
	Noncanonical	Melanoma, cHL	Migration, invasion	27, 75
Fzd8	Canonical	HNSCC, TNBC	Stemness, metastasis, chemoresistance	76, 77
	Noncanonical	Prostate cancer	EMT, metastasis	50
Fzd4	Canonical	GBM, prostate cancer	EMT, stemness	78, 79
Fzd10	Canonical	Breast, ovarian cancers	EMT, chemoresistance	80–82

mesenchymal-like stemness. Accordingly, Fzd2 signaling stimulates stem-like properties in breast and liver cancer cells, and higher Fzd2 expression is correlated with shorter survival of these cancer patients^{40,45}.

Fzd7 is overexpressed in HCC samples⁵⁹. Transgenic HCC murine models showed that activation of Fzd7/ β -catenin signaling occurs early during the progression to HCC, suggesting an oncogenic role of Fzd7⁶⁰. Wnt3 was identified as a ligand for Fzd7 in HCC, and interaction between Wnt3 and Fzd7 is responsible for β -catenin activation and cell proliferation⁶¹. Fzd7 is also overexpressed in triple-negative breast cancer (TNBC) samples and cell lines; Fzd7 knockdown suppresses the proliferation of in vitro cultured cells and the growth of in vivo xenograft tumors⁶². Of note, Fzd7 signaling is required for the expansion of embryonic stem cells, Lgr5⁺ intestinal stem cells, mammary stem cells, and breast cancer stem cells^{63–65}.

Although Fzd1, Fzd2, and Fzd7 belong to the same subfamily, they seem to have distinct roles in human tumors. Fzd1 contributes to chemoresistance, Fzd2 promotes tumor metastasis, and Fzd7 induces carcinogenesis and tumor growth. Moreover, Fzd1 and Fzd7 act through the β -catenin pathway, whereas Fzd2 functions independent of β -catenin. Interestingly, both Fzd2 and Fzd7 are involved in cancer cell stemness even though via different mechanisms.

Fzd3/6 Subfamily

Both Fzd3 and Fzd6 have a key role in PCP during development^{66,67}. Fzd3 as well as other PCP pathway components such as Vangl2 and Celsr1 are upregulated in B lymphocytes of patients with CLL; high Fzd3 level is correlated with unfavorable prognosis³⁵. Fzd3 is also overexpressed in bone marrow cells of patients with acute lymphocytic leukemia (ALL) and myelodysplastic syndrome (MDS)⁶⁸. However, the role of Fzd3 in these

hematologic malignancies remains unknown. Recently, the regulatory function of Fzd3 in melanoma was explored⁶⁹. Global gene expression analysis using melanoma patient-derived cells identified Fzd3 as a regulator of cell cycle progression. Fzd3 knockdown inhibits the proliferation of in vitro cultured cells, and the growth, initiation, and metastasis of xenograft tumors. In melanoma patients, Fzd3 expression is associated with disease progression and reduced survival.

Fzd6 maintains stem-like phenotype and chemoresistance of NB cells; xenograft tumors derived from Fzd6-positive NB cells grow faster than those from Fzd6-negative cells. Moreover, high Fzd6 expression is correlated with poor survival of NB patients⁷⁰. Similarly, Fzd6 is associated with reduced distant relapse-free survival and has an independent prognostic significance in predicting distant TNBC relapse as shown by multivariate analysis. Fzd6 knockdown inhibits TNBC cell motility and invasion in vitro, and bone and liver metastasis in vivo⁷¹.

Fzd3 and Fzd6 seem to be less frequently involved in human tumors. Moreover, Fzd3 and Fzd6 signaling in these tumors is not related to β -catenin pathway.

Fzd5/8 Subfamily

Wnt7a and Wnt7b specifically bind to Fzd5 among the 10 Fzds². Wnt7a–Fzd5 induces the proliferation of endometrial and ovarian cancer cells through the β -catenin pathway^{72,73}. Wnt7b–Fzd5 promotes RNF43-mutant pancreatic ductal adenocarcinoma (PDAC) cell in vitro proliferation and in vivo growth⁷⁴. Moreover, Fzd5 promotes the proliferation of a patient-derived PDAC cell line that harbors an *RNF43* variant. Upon binding to Wnt5a, Fzd5 mediates noncanonical Wnt pathways. Wnt5a–Fzd5 increases melanoma cell motility and invasion through PKC²⁷. This pair also stimulates classical Hodgkin lymphoma (cHL) cell migration through RhoA⁷⁵.

Fzd8 was shown to be a downstream component of c-Met signaling and responsible for β -catenin activation in head and neck squamous cell carcinomas (HNSCC); ectopic expression of Fzd8 rescues c-Met inhibition-induced impairment of tumor incidence, growth, and metastasis⁷⁶. Treatment with chemical agents upregulates Fzd8 expression in TNBC cells and tumors; Fzd8 knockdown in TNBC cells reduced β -catenin and survivin levels and increased the sensitivity to chemical agents, suggesting that canonical Fzd8 signaling mediates TNBC chemoresistance⁷⁷. Similar to Fzd5, Fzd8 mediates β -catenin-independent pathway upon binding noncanonical Wnt ligands. As a receptor of Wnt11, Fzd8 forms a complex with the TGF- β receptor, thereby cross-talking with the TGF- β pathway and promoting EMT in prostate cancer cells and invasion in prostate cancer cell organotypic 3D cultures⁵⁰.

Clearly, two members in this subfamily mediate both canonical and noncanonical Wnt pathways in human tumors depending on their binding ligands. They are involved in stemness, growth, chemoresistance, and metastasis of human tumors.

Fzd4/9/10 Subfamily

Fzd4 is upregulated in highly invasive glioblastoma (GBM) cells, maintaining stem cell properties through the β -catenin pathway, and EMT phenotype through SNAI1⁷⁸. Consistently, expression of Fzd4 and nuclear β -catenin was detected at the invasive front of primary GBM specimens. Fzd4 also induces EMT through the β -catenin pathway in ERG-positive prostate cancer cells⁷⁹. These two studies suggest that Fzd4 is a prometastatic factor through induction of EMT.

BRMS1L suppresses breast cancer cell invasion and migration in vitro and metastasis in xenograft models; these inhibitory effects are mediated by inactivation of the Fzd10- β -catenin pathway⁸⁰. Fzd10 knockdown inhibits the Wnt/ β -catenin pathway in PARPi-resistant ovarian cancer cells and increases the sensitivity of these cells to PARP inhibitors Olaparib and Rucaparib; moreover, β -catenin inhibitor XAV939 synergizes with Olaparib in suppressing PARPi-resistant cells in vitro and in vivo⁸¹. Fzd10 is highly methylated and significantly downregulated in chemoresponsive ovarian cancer samples; Fzd10

knockdown synergizes with cisplatin to inhibit growth and induce apoptosis in ovarian cancer cell lines⁸².

Together, Fzd4 and Fzd10 are involved in tumor metastasis through activation of canonical Wnt pathway. Moreover, Fzd10 contributes to the chemoresistance of ovarian cancer.

TUMOR-SUPPRESSING ROLES OF Fzds

Fzd-mediated noncanonical pathways have been demonstrated to antagonize β -catenin activity, thereby functioning as tumor suppressor depending on cellular context (Table 2).

Fzd1/2/7 Subfamily

Fzd1 functions as a putative tumor suppressor in follicular thyroid carcinoma (FTC). Fzd1 expression is downregulated in this type of tumor, and overexpression of Fzd1 reduces FTC cell proliferation, invasion, and migration⁸³. The antitumor effect of Fzd1 may be associated with its noncanonical Wnt ligand. Overexpression of Wnt5a in a thyroid tumor cell line reduces proliferation, migration, and invasion by antagonizing the β -catenin pathway⁸⁴.

Wnt5a signaling inhibits the proliferation of normal hepatocytes and HCC cells^{85,86}. The inhibitory effect of Wnt5a is mediated by Fzd2. Wnt5a-Fzd2 suppresses β -catenin-TCF activity in HCC cells, suggesting that this signaling may hinder tumor initiation and growth. However, as described above, Wnt5a-Fzd2 also functions as a prometastatic signaling, especially in the absence of β -catenin pathway activity.

Fzd3/6 Subfamily

Fzd6 signaling represses proliferation and migration of gastric cancer cells⁸⁷ and stemness of prostate cancer cells by antagonizing the β -catenin pathway⁸⁸, suggesting Fzd6 as a putative tumor suppressor in these two tumors. In 293T cells, Fzd6 signaling does not affect β -catenin stabilization and β -catenin/TCF4 complex formation, but impairs the binding of TCF/LEF factor to promoters of target genes⁸⁹. In addition to antagonizing the β -catenin pathway, cross-talking with the TGF- β 1 pathway is another mechanism underlying the tumor-suppressing roles of noncanonical Fzds. In breast cancer transgenic

Table 2. Tumor-Suppressing Roles of Fzds

Fzds	Wnt Pathways	Malignant Tumors	Functions	References
Fzd1	Noncanonical	FTC	Growth, invasion, migration	83
Fzd2	Noncanonical	HCC	Growth, stemness	85
Fzd6	Noncanonical	Gastric, prostate, breast cancers	Growth, migration, stemness	87, 88
Fzd5	Noncanonical	Prostate cancer	Growth	90, 91
Fzd8	Noncanonical	Pancreatic cancer, glioma	Stemness, growth	92, 93
Fzd9	Noncanonical	NSCLC	EMT	99, 100

mouse models, Wnt5a dampens the expansion of tumor-initiating cells involving TGFR1/Smad2 and Fzd6⁴⁹. Therefore, similar to Wnt5a–Fzd2 in HCC, Wnt5a–Fzd6 may inhibit carcinogenesis but promote metastasis in breast cancer.

Fzd5/8 Subfamily

Similar to Fzd2 and Fzd6, Fzd5 exerts antiproliferative and proapoptotic effects on prostate cancer cells upon binding Wnt5a^{90,91}. Fzd8, which is repressed by oncogenic K-Ras, blocks tumorigenicity by reducing β -catenin transcriptional activity⁹². Restoration of Fzd8 reduces tumor formation capacity of K-Ras^{V12}-transformed NIH/3T3 cells and K-Ras-possessing PDAC cells in xenograft models. Fzd8 expression is epigenetically downregulated during tumorigenesis of glioma in a mouse model, and Fzd8 negatively regulates tumor cell proliferation in vitro⁹³. Both *FZD5* and *FZD8* are hypermethylated in a mouse model of AML, and the hypermethylation level increases with disease progression, suggesting their tumor-suppressing role in this tumor⁹⁴. Notably, Wnt7a functions as a putative tumor suppressor in gastric cancer and HCC^{95,96}. As the special receptor for Wnt7a, Fzd5 has the potential to transduce suppressive signaling in these two tumors.

Fzd4/9/10 Subfamily

Wnt7a maintains E-cadherin expression and inhibits EMT in non-small cell lung cancer (NSCLC) cell lines^{97,98}. The antitumor effects of Wnt7a depend on Fzd9^{98–100}. Wnt7a–Fzd9 contributes to the inhibition of NSCLC cell growth and migration. *FZD9* was frequently hypermethylated in human AML samples, and *FZD9* methylation is an independent predictor of decreased survival for AML patients, suggesting that Fzd9 is a candidate tumor suppressor in AML¹⁰¹. *FZD9* is also frequently hypermethylated in ER/PR⁺ breast cancer and GBM, but the role of *FZD9* hypermethylation in these two tumors is unexplored^{102,103}.

MODULATION OF Fzds IN TUMORS

Gene Mutation

Mutations in genes encoding Fzds are rare in tumors. Loss of heterozygosity (LOH) of *FZD1* gene at 7q21.2 results in a reduced Fzd1 expression in FTC⁸³. As Fzds play crucial roles in development, *FZD* mutations may lead to dysplasia. For instance, *FZD2* mutation causes autosomal dominant omodysplasia and Robinow syndrome-like features^{104,105}, and *FZD4* mutation causes familial exudative vitreoretinopathy¹⁰⁶.

Gene Promoter Hypermethylation

Hypermethylation of gene promoter is a common epigenetic mechanism leading to gene expression silence. In tumors, hypermethylation of *FZDs* mainly occurs in

Fzd5/8 and Fzd4/9/10 subfamilies. Both *FZD5* and *FZD8* are hypermethylated in AML compared to granulocytes and CD34⁺ cells from healthy donors⁹⁴. *FZD8* is also hypermethylated in B-cell lymphoma but not in normal B cells¹⁰⁷. *FZD9* is hypermethylated in several types of tumors including AML, ER/PR⁺ breast cancer, and GBM^{101–103}. Both *FZD4* and *FZD10* are hypermethylated in epithelial ovarian cancers^{82,108}.

Histone Modification

Histone modifications alter the chromatin structure, thereby affecting gene transcription. Trimethylation of histone H3 at lysine 27 (H3K27me3) is induced by Polycomb repressive complex 2 (PRC2), in which EZH1/2 catalyzes methyltransferase activity in the presence of EED and SUZ12. H3K27me3 modification results in downregulation of gene expression in all cell types. *FZD4* and *FZD8* have been shown to be downregulated by H3K27me3 modification via EZH2 recruitment in gastric cancer and glioma, respectively^{93,109}. *FZD10* is downregulated by histone H3K9 deacetylation via HDAC1 recruitment in breast cancer⁸⁰. Notably, histone deacetylation may promote subsequent H3K27me3 by PRC2^{110,111}. Moreover, EZH2 can bind DNA methyltransferases DNMT1 and DNMT3A/B to modulate DNA methylation¹¹¹.

m6A Modification

N6-methyladenosine (m6A) is the most prevalent modification of RNA, which is critical to almost every aspect of mRNA metabolism. The abundance of m6A is controlled by methyltransferases, RNA binding proteins, and demethylases. Reduced m6A demethylases FTO, and ALKBH5 increases m6A modification and stability of *FZD10* mRNA in BRCA-mutated epithelial ovarian cancer cells, contributing to PARPi resistance by upregulating β -catenin pathway⁸¹.

Posttranscriptional Modulation by MicroRNAs

MicroRNAs (miRs) are short noncoding RNAs with a length of approximate 22 nucleotides and are involved in posttranscriptional regulation of gene expression, mainly leading to mRNA degradation or translation inhibition. Aberrant expression of miRs is implicated in many diseases. Most Fzds have been reported to be negatively modulated by miRs in various tumors^{112–119} (Table 3). These miRs act as tumor suppressors or promoters. Interestingly, miR-31 is tumor suppressing in breast cancer by targeting Fzd3, whereas it is tumor promoting in lung cancer by targeting Fzd9.

Protein Ubiquitination

E3 ubiquitin-protein ligase RNF43 ubiquitinates Fzds and exerts a negative effect on the Wnt/ β -catenin pathway¹²⁰. Inactivating *RNF43* mutations are frequent in

Table 3. miRs Modulating Fzds in Tumors

Fzds	miRs	Modulation	Tumors	Function of miRs	References
Fzd2	miR-30a	Negative	HNSCC	Suppressor	112
Fzd3	miR-31	Negative	Breast cancer	Suppressor	113
Fzd4	miR-493	Negative	Bladder cancer	Suppressor	114
Fzd5	miR-23a/24	Negative	Pancreatic cancer	Suppressor	115
Fzd6	miR-194	Negative	HCC	Suppressor	116
Fzd7	miR-23b	Negative	Colon cancer	Suppressor	117
Fzd8	miR-1249	Negative	Biliary tract cancer	Promoter	118
Fzd9	miR-31	Negative	Lung cancer	Promoter	119

colorectal, endometrial, and pancreatic cancer, accompanied by increased β -catenin activity^{121,122}. Using genome-wide CRISPR-Cas9 screening, Fzd5 was identified to mediate the activation of the β -catenin pathway and the growth of several *RNF43*-mutated PDAC cells⁷⁴. Interestingly, Dvl is required for RNF43-mediated ubiquitination and degradation of Fzds. Canonical β -catenin pathway negatively regulates Fzds via RNF43 to ensure proper control of pathway activity^{122,123}. Contrary to RNF43, ubiquitin-specific protease 6 (USP6) is a deubiquitylase; ectopic expression of USP6 increases cell surface abundance of Fzds and Lrp6 and activates β -catenin pathway in tumor cells¹²⁴.

Fzd-TARGETED THERAPIES IN TUMORS

A number of small molecules, peptides, and blocking antibodies have been developed, targeting ligands, receptors, or key downstream molecules of the Wnt pathway^{125,126}. This review only focused on Fzd-targeted therapies.

Fzd7

Fzd7 acts as a tumor promoter through mediating the canonical Wnt pathway, which involves Dvl. Therefore, disrupting the binding of Fzd7 to the PDZ domain of Dvl is considered a strategy to block the β -catenin pathway. Peptide and small-molecule inhibitor that impair Fzd7–Dvl binding exhibited an antitumor effect on liver and lung cancers, respectively; they were shown to suppress the growth of cultured tumor cells, tumor cell xenografts, and mouse tumor models^{127,128}. Soluble receptors are often used to compete with membrane receptors for their ligands, leading to signaling blockade. Given the oncogenic role of Wnt3–Fzd7 in HCC, a soluble extracellular domain of Fzd7 was generated to bind HCC-derived Wnt3, accompanied by a reduction in HCC cell growth in vitro and in xenografts¹²⁹. Moreover, antibodies against Fzd7 displayed an antigrowth effect on cultured Wilms' tumor cells and TNBC cells^{130,131}.

Fzd5/8

Antibodies against Fzd5 were shown to inhibit the growth of pancreatic cancer cells with *RNF43* mutation

in vitro and in xenografts, and the growth of tumor organoid cultures from colorectal cancer patients carrying *RNF43* mutations⁷⁴. In combination with various chemotherapy agents, soluble Fzd8 (OMP-54F28, ipafricept) exhibited an antitumor effect on patient-derived xenografts (PDXs) of pancreatic, ovarian, and breast cancers¹³². Consistently, patients with advanced solid tumors were not responsive to single OMP-54F28 in a first-in-human phase I study¹³³, while patients with recurrent platinum-sensitive ovarian cancer were responsive to OMP-54F28 in combination with chemotherapy in a phase 1b study¹³⁴. In the latter study, the overall response rate was 75.7%, slightly higher than historical data with chemotherapy alone on OCEANS (57%) and GOG 213 (56%); the median progression-free survival (PFS) was 10.3 months, similar to historical data on OCEANS (8.4 months) and GOG 213 (10.4 months)^{135,136}. Actually, the occurrence of fragility fractures at doses associated with efficacy may limit further development of OMP-54F28.

Fzd1/2/7/5/8

OMP-18R5 (vantictumab) is a monoclonal antibody against the Fzd1/2/7 subfamily and Fzd5/8 subfamily, which has the capacity to block the β -catenin pathway induced by multiple Wnt molecules. OMP-18R5 was shown to suppress the development of pancreatic cancer and gastric cancer in mouse models^{137,138}. Similar to OMP-54F28, OMP-18R5 was also shown to synergize with chemotherapy agents on several types of cancers in xenografts and PDXs^{132,139}. A phase 1b study evaluating OMP-18R5 in combination with chemotherapy in patients with metastatic pancreatic cancer was terminated because of fragility fractures¹⁴⁰.

Fzd1/2/7/5/8/4

By using combinatorial antibody engineering, a variant antibody of OMP-18R5 named F2.A was developed. In addition to against the Fzd1/2/7 subfamily and Fzd5/8 subfamily, F2.A also targets Fzd4. It was shown that F2.A was much more effective than OMP-18R5 in suppressing the growth of *RNF43*-mutant pancreatic cancer cells¹⁴¹.

Fzd10

Antibodies against Fzd10 displayed suppressive effects on the growth of synovial sarcoma cells in vitro and in xenografts^{142,143}.

CONCLUSIONS AND PERSPECTIVES

Fzds mediate both canonical Wnt/ β -catenin pathway and various noncanonical pathways. The Wnt/ β -catenin pathway has been fully demonstrated to be oncogenic. However, in the presence of noncanonical Wnt ligands such as Wnt5a/b and Wnt11, Fzd signaling plays a dual role in tumors. Generally, noncanonical Wnt pathways promote tumor metastasis by inducing EMT, but potentially hinder tumor initiation and growth by antagonizing the β -catenin pathway. An apparent explanation for this duality is that noncanonical Wnts compete with canonical Wnts for Fzds, further preventing β -catenin translocation into the nucleus. Study on Fzd6 may provide an alternative mechanism that noncanonical Wnt pathways interfere with the binding of transcription factor TCF/LEF to promoters of target genes. Notably, Yap/Taz and TGF- β , downstream signaling of Fzds also has a dual role in human tumors, suggesting that in the absence of β -catenin activity, noncanonical Wnt pathway still can exert tumor-suppressing effects by cross-talking with this signaling.

At present, targeting Fzds has proven to be effective on cultured tumor cells, tumor cell xenografts, mouse tumor models, and PDXs. As chemotherapy increases Wnt pathway activity, which counteracts the antitumor effect, Fzd-targeted therapies can enhance the sensitivity of tumor cells to chemical agents. Actually, Fzd-targeted therapies seem to be effective upon combining with chemotherapy in preclinical models. However, the occurrence of fragility fractures in patients treated with Fzd-targeted agents such as OMP-54F28 and OMP-18R5 limits the development of this combination. Given the crucial role of the Wnt pathway in tissue homeostasis (the intestine, hematopoietic system, bone, and so on), adverse effects with regard to Fzd-targeted therapies are anticipated. Therefore, to obtain clinical efficacy and safety, aside from specific protection and prevention such as using vitamin D3 and calcium carbonate against fragility fractures, a fine balance is required between repressing tumors and maintaining homeostasis.

Because of the heterogeneity of tumors and the lack of specific biomarkers, the Wnt/Fzd expression profile should be evaluated in individual tumor. According to the expression pattern, one can more precisely determine Fzd-targeted strategies, thereby improving efficacy. The early phase compounds OMP-54F28 and OMP-18R5 block a variety of Wnts/Fzds. The excessive blocking may aggravate the side effects, especially in patients with tumors overexpressing fewer Fzds. Therefore, therapies targeting single Fzd or Fzd subfamily should also be developed.

As noncanonical Fzd signaling has antitumor activity, targeting Fzds should be cautious and tumor and patient specific. Recently, some novel downstream pathways and modification mechanisms of Fzds have been discovered. New insights on signaling, modulation mechanisms, and roles of Fzds in human tumors undoubtedly will contribute to identifying more Fzd-related therapeutic targets.

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