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REVIEW

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Emerging roles and biomarker potential of WNT6 in human cancers

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Abstract

The WNT6 ligand is a well-known activator of the WNT signaling pathway, considered a vital player in several important physiologic processes during embryonic development and maintaining homeostasis throughout life, regulating the proliferation and differentiation of multiple stem/progenitor cell types. More recently, as it is the case for many key molecular regulators of embryonic development, dysregulation of WNT6 has been implicated in cancer development and progression in multiple studies. In this review, we overview the most significant recent findings regarding WNT6 in the context of human malignancies, exploring its influence on multiple dimensions of tumor pathophysiology and highlighting the putative underlying WNT6-associated molecular mechanisms. We also discuss the potential clinical implications of WNT6 as a prognostic and therapeutic biomarker. This critical review highlights the emerging relevance of WNT6 in multiple human cancers, and its potential as a clinically-useful biomarker, addressing key unanswered questions that could lead to new opportunities in patient diagnosis, stratification, and the development of rationally-designed precision therapies.

Keywords WNT6, WNT pathway, Cancer, Aggressiveness, Precision oncology, Tumor, Oncogene, Cancer prognosis and diagnosis

Background

WNT ligands comprise a family of secreted molecules that are able to activate the WNT signaling pathway, which have raised a multidisciplinary interest due to their wide range of physiological functions [1]. The human genome contains 19 WNT genes, conserved among mammals and also found in several simpler multicellular organisms. WNT ligands are conserved cysteine-rich proteins of approximately 40 kDa, which present similar structural motifs and general physiological functions [1].

In the endoplasmic reticulum, WNTs are translated and undergo post-transcriptional glycosylation and PORCN-mediated palmitoylation [2–4]. These lipid-modified WNT ligands are then transported to the cell membrane by the Wntless protein and secreted into the extracellular space, ultimately interacting with various receptor complexes [5].

Several receptors and co-receptors interact with WNT ligands to initiate signal transduction [6–13]. These interactions can activate different signaling pathways, depending on the specific ligand-receptor combination, in a cell- and stage-dependent manner [5]. It has been suggested that rather than linear and distinct signaling pathways, multiple WNT signaling variations may be activated simultaneously, in a complex integration of various inputs [14–16].

WNT signaling has been historically divided into canonical (β-catenin-dependent) and non-canonical (β-catenin-independent) WNT pathways [17, 18]. The β-catenin-dependent WNT pathway, the most widely studied [17], involves the stabilization and nuclear translocation of β-catenin, which then modulates gene

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expression via TCF/LEF family of transcription factors [19–22]. Non-canonical pathways are a diverse and less-characterized group of pathways that act independently of β-catenin [18, 23], of which the most well-defined are planar cell polarity (PCP) and the WNT-Ca2 + pathways [5].

The WNT signaling pathways play a multitude of roles in various stages of development and throughout life, including embryogenesis, organogenesis and tissue homeostasis [24–31]. Remarkably, recent research continues to uncover novel roles to these pathways in diverse physiological processes, such as neural development, osteoblast metabolic regulation, and cardiac physiology [32–35].

Conversely, dysregulation of WNT signaling often leads to disease, including cancer [36–38]. Aberrant β-catenin expression, along with the dysregulation of other WNT pathway components, has been implicated in the oncogenic process of several human tumors [39–50]. Notably, the first mammalian WNT gene, *WNT1*, was identified in the context of a virally-induced mouse breast tumor [51–54], and later recognized as an oncogene [55]. Since then, numerous WNT genes have been implicated in various cancers [56–59].

WNT6 and cancer

Until recently, WNT6 was among the less-explored members of the WNT ligand family. However, it has emerged as a significant molecule in both physiological and pathological contexts (as reviewed in Wei et al. [60]). WNT6 activates WNT signaling pathways and plays pivotal roles in various human physiological processes, including embryonic development (e.g., neural crest induction [61]), organogenesis of heart muscle and kidneys [62, 63], maintenance and differentiation of adult progenitor cells [64, 65], and in immune contexts (e.g., macrophage M2-like polarization and proliferation [66]). Understandably, as a crucial orchestrator of several homeostatic processes, its dysregulation has been recently linked to various oncogenic processes.

In recent years, studies have increasingly described WNT6 as a key player in the development, progression, and aggressiveness of several tumor types. The first studies exploring WNT6 in cancer reported its upregulation in colorectal and gastric cancer in vitro models [67, 68]. Since then, WNT6 has been studied across a wide range of cancers, where it has been shown to activate multiple WNT signaling variations (Fig. 1). Significant findings have been reported at the molecular level, including gene expression and epigenetic alterations, as well as on dysregulation of downstream pathways. These studies also drew connections between WNT6 and key clinical features of cancer patients (Fig. 2 and Table 1). Below are summarized the major findings related to the relevance of WNT6 in various human cancers, and discussed the future challenges for the translation of this body of knowledge to the clinical settings.

Colorectal cancer

The majority of colorectal cancer cases present alterations in the WNT signaling pathway, mostly through *APC* inactivating mutations [37], compromising the negative regulation of this pathway. This association has led to extensive research on this pathway's influence in colorectal cancer, with numerous studies describing the

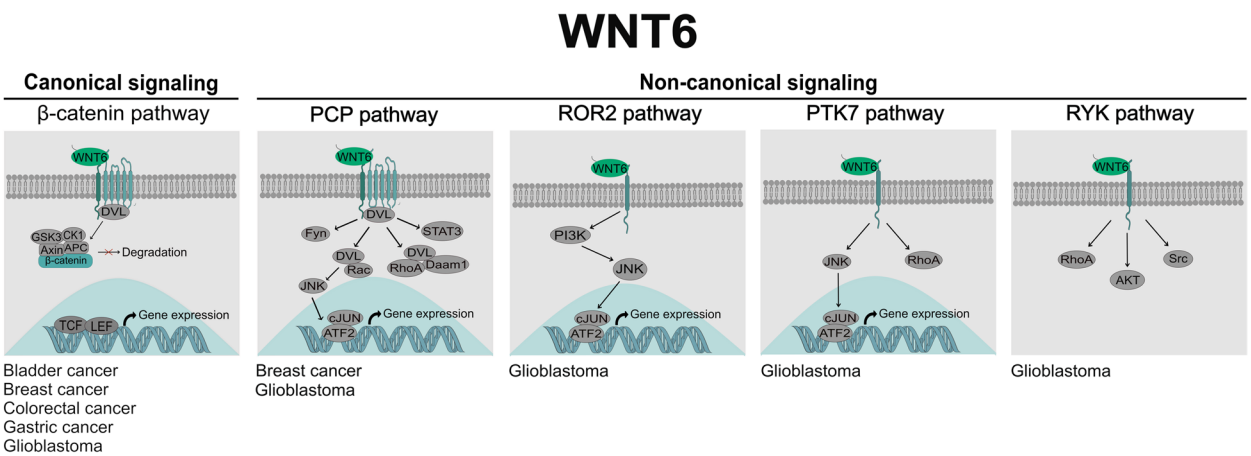

Fig. 1 WNT signaling pathways affected by WNT6 in cancer. Both β-catenin-dependent (canonical [69–76]) and -independent (non-canonical [74, 75]) WNT signaling pathways have been linked to WNT6 in cancer, including ROR2, RYK, PTK7, and planar cell polarity (PCP). Upon engagement with its receptor molecules, WNT6 may trigger multiple signaling cascades, which ultimately result in the activation and upregulation of specific target genes

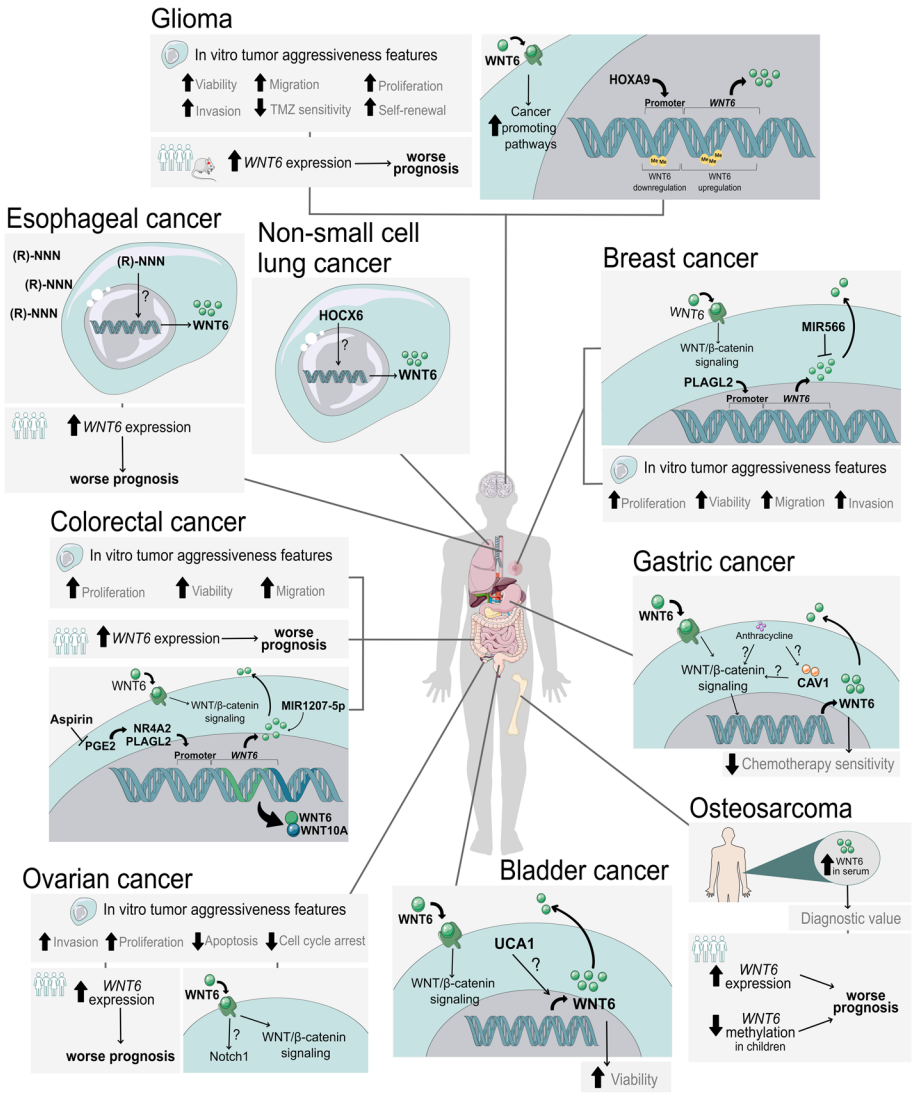

Fig. 2 Summary of key findings regarding novel roles and clinical relevance of WNT6 in cancer, highlighting functional effects affecting tumor aggressiveness, molecular regulatory and effector mechanisms of WNT6 in each tumor type, and its relevance in the clinical setting. Multiple studies described WNT6 as an oncogenic factor in various tumor types, being associated with increased tumor aggressiveness features and worse patient prognosis. The regulation of WNT6 expression by different molecules and its ability to activate WNT/β-catenin signaling, as well as other relevant mechanisms, underscore its multiple molecular partners and identify potential therapeutic opportunities

relevance of abnormal WNT ligand expression in this context [98–101].

Initial reports over 20 years ago identified *WNT6* and *WNT10A* to be clustered in close proximity in the human genome, and highly co-expressed in the SW480 colorectal tumor cell line [67]. Subsequently, SNPs within the *WNT6* gene were linked to colorectal adenoma risk, namely the rs6747776 variant, where individuals with the CC genotype had an increased risk compared to those with the GG genotype [102]. Moreover, the risk associated with this polymorphism was found to be further influenced by dietary factors, particularly the proportion of calories derived from fat, with the greatest risk observed for those with one or more minor alleles that consumed less than 30% of daily calories from fat [102].

In clinical samples from patients with sporadic colorectal cancer, *WNT6* was found to be hypermethylated along with several other genes related to β-catenin-dependent WNT signaling [103]. Posteriorly, WNT6 expression was linked to increased features of tumor aggressiveness, demonstrated by increased in vitro cell proliferation, viability, migration, and decreased cell cycle arrest [69–71, 77]. In vivo, it was implicated in mechanisms that promote tumor subcutaneous growth [69] and orthotopical

Table 1 Overview of studies describing WNT6 association with several types of cancer

Tumor type	Key WNT6-related findings	References
Colorectal	**Clinical data**	
	• Higher WNT6 expression associated with patient shorter disease-free survival (HR(High/Low) = 2.1)	[70]
	Experimental data	
	• *WNT6* and *WNT10A* are clustered and highly co-expressed in SW480 cells	[67]
	• PLAGL2 and NR4A2 bind the promoter region of WNT6, induce its expression and enhance WNT signaling in HCT116 and DLD1 cells, respectively	[69, 70]
	• In vitro, WNT6 associated with increased aggressiveness features	[69–71, 77]
	• In vivo, WNT6 associated with increased aggressiveness features	[69, 70]
	• *hsa-miR-1207-5p* binds *WNT6* and enhances WNT signaling in Caco-2 cells	[71]
Colorectal liver metastasis	**Clinical data**	
	• High expression of WNT6 in 49.1% of tumor samples • High WNT6 associated with higher mortality rate after liver resection in 5-year monitoring (WNT6-high: 65.5% vs WNT6-low = 46.3%) • WNT6 expression decreased in patients receiving preoperative chemotherapy • WNT6 high expression is an independent negative predictor of 5-year overall survival (31.0% vs 62.2% in WNT6-low)	[79]
Gastric	**Clinical data**	
	• *WNT6* expressed at similar levels in 6 patient tumor and non-tumor tissue samples	[68]
	• WNT6 protein is upregulated in patient samples (3/4), and its mRNA expression differs between patients, 27% presenting an upregulation (7/26) and 73% a downregulation (19/26)	[72]
	• WNT6 associated with patients' tumor stage and nodal status • Large number of non-responders to chemotherapy (10/11) presented WNT6 expression in the tumor area	[72]
	Experimental data	
	• WNT6 is expressed in different gastric cancer cell lines	[68, 72]
	• In vitro and in vivo, anthracycline treatment induces WNT6 expression and WNT signaling • In vitro, WNT6-induced chemoresistance is dependent on Caveolin-1 expression	[72]
Breast	**Clinical data**	
	• WNT6 is strongly expressed in infiltrating ductal carcinoma samples, and in corresponding non-tumor breast epithelium	[80]
	• *WNT6* levels correlated negatively with hsa-miR-566 levels in tumor samples	[81]
	• *WNT6* included in a model that predicts 1-, 3-, and 5-year survival status in patients with breast cancer and bone metastasis • WNT6 positively associated with infiltrating T-regulatory cells in breast cancer patients with bone metastasis	[82]
	Experimental data	
	• WNT6 strongly expressed in ER positive and negative cell lines	[80, 83]
	• *WNT6* is upregulated upon NME1 overexpression in WM1158 and WRO82 cells	[84]
	• In vitro, WNT6 expression associated with increased tumor aggressiveness features	[81]
	• *WNT6* increased in doxorubicin-resistant cells, along with other components of WNT signaling • PLAGL2 binds the promoter region of *WNT6* and induces its expression in MCF7 and MDA-MB-231 cells	[75]
Bladder	**Clinical data**	
	• *WNT6* expression positively correlates with *UCA1* expression in tumor samples	[73]
	Experimental data	
	• Ectopic expression of lncRNA *UCA1* leads to the upregulation of *WNT6* in RT4 and T24 cells • WNT6 expression associated with increased viability and decreased chemotherapy effectiveness in T24 cells	[73, 85]
Neuroblastoma	**Clinical data**	
	• *WNT6* was among the most highly expressed WNT ligands in high-risk neuroblastoma without *MYCN* amplification	[86]
Glioma	**Clinical data**	
	• High WNT6 expression associated with glioma malignancy grade	[74, 87]
	• *WNT6* correlates positively with *HOXA9* in tumor samples	[87]
	• *WNT6* expression was included in a five-gene hypoxia risk score, associated to worse lower grade glioma IDH1-mutant patient survival	[88]

Table 1 (continued)

Tumor type	Key WNT6-related findings	References
Glioblastoma	**Clinical data**	
	• High *WNT6* expression considered an independent predictor of shorter patient overall survival (HR(High/Low) = 1.288)	[74, 87]
	• In patients, methylation levels of specific CpGs within *WNT6* promoter and gene body regions are negatively and positively correlated, respectively, with its expression	[87]
	Experimental data	
	• In vitro and in vivo, WNT6 expression associated with increased tumor aggressiveness features • In vitro, WNT6 expression associated with increased activation of several cancer-promoting pathways	[74, 89]
	• HOXA9 binds the promoter region of *WNT6,* induces its expression and the activation of the β-catenin-dependent pathway	[87]
Esophageal	**Clinical data**	
	• *WNT6* expression was associated to age, gender, tumor stage, and histopathological type • 50.7% of patients presented high WNT6 expression • *WNT6* expression considered an independent predictor of shorter patient overall survival and disease-free survival in univariate (HR (Low/High) = 0.412 and 0.447, respectively) and multivariate analyses (HR (Low/High = 0.464 and 0.576, respectively)	[90]
	Experimental data	
	• Upon (S)-NNN exposure, *WNT6* increased in immortalized oral keratinocyte cells and in the esophagus of an in vivo model	[91]
Bone	**Clinical data**	
	• Pediatric patients with unmethylated *WNT6* present a shorter 5-year survival rate (unmethylated = 52% vs methylated = 78%) • *WNT6* is largely unmethylated in pediatric osteosarcoma tissues (82%)	[92]
	•*WNT6* expression was associated with adult patients' age, tumor grade, and distant metastasis • High *WNT6* expression in osteosarcoma patients, was considered an independent predictor of shorter overall-survival (HR(High/Low) = 2.227) • *WNT6* serum levels may present diagnostic value	[93]
	Experimental data	
	• WNT6 upregulated and largely unmethylated in pediatric osteosarcoma MG63 cells	[92]
Post-transplant smooth muscle	**Clinical data**	
	• Increased *WNT6* expression, compared to visceral leiomyomas, leiomyosarcomas, angioleiomyomas, and endothelial haemangiomas • *WNT6* and *WNT10A* were co-expressed at similar levels	[94]
Small cell lung	**Clinical data**	[95]
	• *WNT6* levels did not differ significantly between tumor and non-tumor adjacent tissues	
	Experimental data	
	• *WNT6* negatively correlated to lncRNA NCRNA00173 in in vitro HD69 chemotherapy resistant model	
Non-small cell lung	**Experimental data**	
	• *WNT6* was upregulated upon HOXC6 overexpression in A549 cell line	[96]
Testicular germ cell	**Experimental data**	
	• *WNT6* was found to be downregulated in the majority of cisplatin-resistant cell lines	[97]
Ovarian	**Clinical data**	[76]
	• WNT6 increased in tumor samples • High *WNT6* expression associated to worse patient overall-survival (HR(High/Low) = 1.18)	
	Experimental data	
	• WNT6 increased in CAOV3 and OVCAR3 cell lines • In vitro, WNT6 expression associated with increased tumor aggressiveness features	

tumorigenesis [70]. Concordantly, a study exploiting samples from patients in clinical settings have also established a prognostic value for *WNT6*, being associated with reduced disease-free survival [70]. Curiously, another study reported a prevalent deleterious *WNT6* mutation in locally recurrent rectal cancer, and suggested

that WNT6 protein and mRNA levels were decreased in tumor samples compared to adjacent non-tumor tissues in rectal adenocarcinoma and colorectal cancer patients [104].

WNT6 has been associated to oncogenic molecules in colorectal cancer [69, 70]. Specifically, the transcription factor PLAGL2, a zinc-finger protein, was found to interact with *WNT6* promoter region, promoting its expression. This interaction ultimately, activates the β-catenin-dependent WNT signaling (Fig. 2), through which it may be inciting tumor aggressiveness [69]. Additionally, nuclear NR4A2, induced by prostaglandin E2 (PGE2) – an inflammatory molecule recurrently implicated in colorectal cancer and often linked to WNT signaling [105, 106] – has also been shown to interact with *WNT6* promoter region, inducing its expression and, consequently, activating β-catenin-dependent WNT signaling (Fig. 2) [70]. This mechanism can be suppressed by aspirin treatment, which inhibits cyclooxygenase signaling, decreasing PGE2, NR4A2 and WNT6, thus reducing colorectal cancer tumorigenesis [70]. In colorectal cancer, PLAGL2 was also reported to promote tumor aggressiveness in vitro and in vivo, via a β-catenin-dependent mechanism, in an interplay with AKT/GSK-3β signaling [107]. Similarly, NR4A2, induced by PGE2, has been shown to promote chemotherapy resistance in vitro and is considered an independent predictor of unfavorable prognosis in colorectal cancer [108]. More recently, the long non-coding RNA (lncRNA) *PVT1* was shown to interact with the *hsa-miR-1207-5p* microRNA, which in turn can bind *WNT6*, activating the β-catenin-dependent WNT pathway (Fig. 2) [71]. These ncRNAs were reported to be increased in patient samples and associated with increased tumor aggressiveness in vitro [71]. Nevertheless, complementary molecular and functional assays are still necessary to more precisely define the molecular mechanisms through which WNT6 influences colorectal cancer, and the phenotypic impact of its manipulation.

Moreover, in vitro, WNT6 expression has been associated to a decreased expression of the pro-apoptotic molecule Bax and an increase in the expression of caspase-3 precursor, indicating a possible inhibition of cell apoptosis [77]. WNT6 expression has been also linked to an increased MMP2 expression, a molecule often involved in epithelial-mesenchymal transition, a crucial event in metastasis, frequently reported to be promoted by β-catenin-dependent WNT signaling [77].

Colorectal cancer metastasis prevails as a significant colorectal cancer-driven cause of death, with the liver being the most usual long distance metastatic site [109]. Interestingly, WNT6 was found to be highly expressed in colorectal liver metastases, primarily localized in the cytoplasm, and considered relevant in patients' prognosis [79]. High WNT6 expression was associated to a higher mortality rate upon liver resection, and it was defined as an independent negative predictor of patients' 5-year overall survival, more so in patients with low-risk of recurrence [79]. In addition, WNT6 expression was significantly lower in colorectal cancer patients with liver metastasis who had received preoperative chemotherapy than in those who had not received it [79].

Globally, this body of data positions WNT6 as a novel key molecule with biomarker potential in colorectal cancer, warranting further studies to better define its underlying molecular mechanisms, which may be amenable to rational targeted therapeutic interventions.

Gastric cancer

The aberrant expression of WNT ligands, including WNT6, and the subsequent overactivation of WNT signaling, have been frequently reported in gastric cancer [72, 110–113].

WNT6 was found to be expressed in both gastric cancer cell lines derived from primary tumors and from distant metastasis [68, 72]. Moreover, in a subset of patient tumor samples, *WNT6* mRNA was upregulated in comparison to non-tumoral gastric tissue and, interestingly, WNT6 protein was upregulated as well in the tumor tissue lysates of 3 out of 4 patients [72]. These findings would benefit from validation in larger cohorts to confirm this putative differential expression. WNT6 was identified in multiple subcellular locations within these cell lines, namely in the membrane, cytoplasm, and nucleus, and was also present in conditioned media from gastric cancer cell lines, confirming its secretion [72]. In normal gastric tissue, WNT6 was observed in the apical region of the foveolar epithelium and in areas of intestinal metaplasia, as well as in macrophages and plasma cells present in the lamina propria [72]. Interestingly, in a retrospective analysis, it was reported that the majority of gastric cancer patients non-responsive to preoperative standard chemotherapy regimen (epirubicin, cisplatin and 5-fluorouracil (5-FU)) exhibited high levels of WNT6 within the tumor area, whereas an elevated number of responsive patients were essentially WNT6-negative [72]. Complementarily, WNT6 was also indirectly associated with a worse patient prognosis, associating positively with tumor stage and nodal metastatic status, both important prognostic factors for poor gastric cancer patient survival [72].

WNT6 expression was also associated to caveolin-1 (CAV1), their levels varying accordingly, both in parental cell lines and upon CAV1 manipulation [72]. Interestingly, WNT6 expression and β-catenin-dependent WNT signaling activation were induced upon anthracycline

treatment, which potentiated β-catenin binding to the distal promoter region of *WNT6*, ultimately leading to decreased chemotherapy efficacy, exclusively in cells expressing CAV1 [72]. Importantly, these effects were recapitulated in a spontaneous gastric cancer mouse model, in which epirubicin exposure upregulated both WNT6 and CAV1 expression [72]. It is suggested that chemotherapy-induced DNA damage may trigger a specific cell response involving p53 [72], a well-established regulator of CAV1 [114] and of various WNT-related genes [115, 116], leading to the upregulation of CAV1 and WNT6. Ultimately, activating β-catenin-dependent WNT signaling (Fig. 2) and inducing pro-survival genes [72].

These findings highlight an eminent role of WNT6 as an oncogenic molecule in gastric cancer, and underline its potential therapeutic interest, together with CAV1, given their upregulation upon anthracycline treatment and association with decreased sensitivity to this therapy.

Breast cancer

WNT6 was initially identified to be strongly expressed in patient-derived infiltrating ductal carcinoma samples, and in corresponding non-tumor breast epithelium samples [80], while posterior studies reported WNT6 to be upregulated in invasive ductal carcinoma tissues compared to non-tumor tissues [81, 117]. Regarding breast cancer cell lines, *WNT6* was found to be strongly expressed in both estrogen-receptor positive and negative models [80, 81, 83].

It has been demonstrated that the microRNA *hsa-miR-566*, which has tumor suppressive functions in breast cancer, is able to downregulate WNT6 and β-catenin by targeting *WNT6* 3'-UTR, ultimately reducing cell viability, proliferation, and migration (Fig. 2) [81]. In addition, WNT6 expression, along with β-catenin and DVL1, were found to be increased in doxorubicin resistant breast cancer cell lines [75]. This abnormal WNT6 expression may be induced by PLAGL2, a transcription factor with known oncogenic functions in breast cancer. PLAGL2 is able to strongly bind the *WNT6* promoter region, inducing its expression, and resulting in increased cell proliferation, invasion and migration (Fig. 2) [75]. In fact, in PLAGL2 knockdown doxorubicin-resistant breast cancer cells, BML-284 (a pharmacological activator of β-catenin dependent WNT signaling) was able to rescue cell viability and decrease apoptosis, supporting the notion that the WNT pathway promotes aggressiveness features [75].

Interestingly, WNT6 has also recently been implicated in predicting the 1-, 3- and 5-year survival status of breast cancer patients with bone metastasis [82]. In this context, *WNT6* was also positively correlated to *KLK6, GJB3, FBN3*, and *GABBR2* [82]. Relevantly, KLK6 upregulation has been described to promote oncogenic behavior in breast cancer [118], and reported to induce nuclear accumulation of β-catenin in a mouse keratinocyte cell line, corroborating a potential link between KLK6 and WNT signaling, as well as with tumor promotion. Additionally, *WNT6* expression was also positively correlated with the extent of tumor infiltrating immune cells, namely regulatory T-cells [82]. This raises the interesting hypothesis that WNT6 can, as part of the WNT signaling cascade, influence the immune landscape and, potentially impact the response to newly-developed anticancer immunotherapies.

These relevant studies denote WNT6 roles as a promoter of tumor aggressiveness, and identify important relevant interactions that influence its expression in breast cancer.

Bladder cancer

In bladder cancer, WNT6 expression was firstly found to be upregulated following the overexpression of the lncRNA *UCA1* in vitro [85]. Later, in patient tissues, *WNT6* mRNA levels were positively correlated to *UCA1* [73]. *UCA1* has been described to be upregulated in bladder cancer tissues and to sustain oncogenic functions in vitro and in vivo [73, 85]. Interestingly, *UCA1* expression was found to increase β-catenin-dependent WNT signaling activation as well (Fig. 2) [73]. This role of WNT6 and β-catenin-dependent WNT signaling in bladder tumor aggressiveness was also demonstrated by manipulating β-catenin-dependent WNT signaling with the pharmacological inhibitor IWR-1, which led to a decrease in cell viability of *UCA1*-overexpressing cells. Conversely, genetically overexpressing WNT6 in *UCA1*-knockdown cells led to an increase in cell viability [73].

While follow-up studies are necessary to explore the direct roles of WNT6 in bladder cancer aggressiveness, these data from two independent studies strongly suggest WNT6 may be a key molecular partner for *UCA1* in sustaining its oncogenic effects in bladder cancer.

Brain cancer

In the context of malignant brain tumors, particularly gliomas, tumor aggressiveness has been often linked to the aberrant activation of the WNT signaling pathway [119–123], particularly through the abnormal expression of WNT ligands [78, 124–126]. Namely, WNT3A [124, 125] and WNT5A [126–128], which have been both associated to tumor aggressiveness in glioblastoma, the most aggressive and lethal form of glioma. However, the description of WNT6 in sustaining oncogenic properties in gliomas, particularly glioblastoma, has only recently been recognized [74].

WNT6 expression has been shown to increase alongside glioma malignancy grade, regardless of *IDH* mutations and 1p/19q co-deletion status, with glioblastoma presenting the highest WNT6 levels among gliomas [74, 87]. In glioma patient samples, WNT6 expression was predominantly cytoplasmic in tumor cells, either presenting a diffuse or more scattered pattern, while tumor infiltrating lymphocytes were considered negative for this molecule, as well as endothelial cells, presenting low to undetectable WNT6 [74]. Although its expression does not seem to be modulated by copy number alterations in lower grade gliomas or glioblastoma, DNA methylation may play an important part in the regulation of *WNT6* expression in various glioma cell lines and patients (Fig. 2) [87]. In fact, in glioblastoma, the methylation levels of two specific regions, one downstream of the *WNT6* promoter and another within the gene body, have been negatively and positively correlated with *WNT6* expression, respectively [87]. Concordantly, in another study, *WNT6* was found to be methylated in several glioblastoma cell lines, as well as in a glioblastoma and two astrocytoma patient samples [129]. However, the specific regions of CpG methylation were not disclosed, and no associations with *WNT6* expression levels were assessed.

Consistent with findings in other tumor contexts, increased WNT6 levels in gliomas were associated with higher tumor aggressiveness in vitro, reflected in increased cell viability, proliferation, migration, invasion, chemotherapy resistance, and maintenance of glioma stem cell features [74]. Interestingly, the association between WNT6 and increased chemoresistance was later corroborated in independent studies, with evidence suggesting that the p53-*hsa-miR-34a* axis sensitizes glioblastoma cells to the chemotherapeutic temozolomide by reducing WNT6 expression and β-catenin-dependent WNT signaling [89].

Additionally, in in vivo orthotopic glioblastoma models, WNT6 expression promoted accelerated tumor-related death, and the WNT6-positive tumors showed increased expression of proliferation, stem cell and anti-apoptosis markers (Fig. 2) [74].

Molecularly, in vitro phospho-proteomic assays identified that high WNT6 levels in glioblastoma are associated with the activation of several cancer-related pathways, such as, SFK, STAT, AKT, and RTK (Fig. 2) – which, ultimately contribute to the increased aggressiveness profile of this tumor subtype [74]. These results were further corroborated by patient data, which showed *WNT6* positively correlated genes were enriched for gene sets upregulated by WNT, SRC, MAPK, AKT, MYC, and JNK signaling [74]. WNT6 expression was also associated to an increased activation of the β-catenin-dependent signaling (Fig. 2), suggesting this ligand could be exerting its effects on glioblastoma through the direct activation of this pathway [74].

Moreover, HOXA9, a key mediator of glioblastoma aggressiveness [130–132], was described as an inducer of *WNT6*'s expression, interacting directly with its promoter region and activating the β-catenin-dependent WNT signaling pathway (Fig. 2) [87]. In glioblastoma patients, *WNT6* and *HOXA9* expression levels were positively correlated [87]. Interestingly, high *WNT6* expression was associated with shorter overall survival of glioblastoma patients (Fig. 2), independently of *HOXA9* expression, as well as other major prognostic variables, such as age, gender, *IDH1* mutation status, treatment regimen, and the Karnosfky Performance Score [74, 87]. Importantly, glioblastoma patients with *WNT6*- and *HOXA9*-high tumors showed a particularly dismal prognosis when compared to all other glioblastoma patients. Moreover, among *HOXA9*-low patients, those with high *WNT6* expression had a significantly shorter overall survival compared to those with low *WNT6* and *HOXA9* expression [87].

Curiously, in *IDH1*-mutant lower-grade glioma patients, *WNT6* expression, along with four other genes, was part of a hypoxia-related risk score, which was associated with shorter survival, independently of 1p/19q codeletion, age, and WHO malignancy grade (II and III) [88]. Moreover, WNT6 has also been studied in other non-glioma brain tumors, particularly in high-risk neuroblastoma without *MYCN* amplification, where *WNT6* was among the most highly expressed WNT ligands, possibly contributing to the aberrant activation of β-catenin-dependent WNT signaling in these tumors [86].

These data strongly indicate that WNT6 may have a pertinent role in brain cancers, with particularly compelling evidence in glioblastoma. Additional research is needed to explore the link between WNT6 and HOXA9, as correlations between these two key genes were also identified in several other tumors, including leukemia, melanoma, testicular germ cell tumor, and cholangiocarcinoma [87]. Finally, exploring the therapeutic inhibition of WNT6, through pharmacological or viral-mediated genetic inhibitors (e.g. adeno-associated virus (AAV)-based therapies), either as monotherapy or in combination approaches (e.g., with the standard-of-care temozolomide-based therapy, or with novel experimental therapies), could be invaluable in the context of glioblastoma, a particularly dramatic cancer in urgent need of better therapies.

Esophageal cancer

In esophageal squamous cell carcinoma patients, WNT6 was detected in the plasma of tumor cells, and nearly half the patients presented high WNT6 expression [90]. In

addition, its expression associated with various clinicopathologic characteristics, including patient age, gender, tumor stage and histopathological type. Critically, WNT6 was considered an independent prognostic factor, as high levels of WNT6 associated with shorter overall survival and disease-free survival of esophageal carcinoma patients (Fig. 2) [90].

Interestingly, *Wnt6* was found to be upregulated in the esophagi of rats treated with (S)-N'-nitrosonornicotine ((S)-NNN), as well as in immortalized oral keratinocytes after treatment with (S)-NNN and (R)-N'-nitrosonornicotine ((R)-NNN) (Fig. 2) [91]. These two carcinogenic compounds are found in tobacco, a major environmental risk factor for esophageal cancer [91], further supporting the hypothesis that WNT6 may be relevant to the pathophysiology of this tumor. Nevertheless, the differences in *WNT6* expression reported in the rat model were not recapitulated in human esophageal carcinoma patient data. However, it should be noted that this patient data analysis compared tumor and non-tumor samples, not discriminating between smokers and non-smokers, thus not fully accounting for the effects of (S)-NNN on gene expression, complicating the analysis of their exposure to (S)-NNN [91].

These studies highlight that WNT6 may be altered in a subgroup of esophageal cancer patients, and that its upregulation may identify a subset of patients with worse prognosis. Naturally, additional approaches are needed to unveil the molecular intricacies underlying this possible WNT6-driven tumor aggressiveness.

Bone cancer

In osteosarcoma, WNT6 has incited interest both in the context of infant and adult patients [92, 93]. In a pediatric osteosarcoma in vitro model, WNT6 was shown to be upregulated in comparison to a human osteoblast cell line, presenting also significantly lower overall methylation levels [92]. This was corroborated in pediatric patient tumors, in which *WNT6* was found to be expressed in a large percentage of the samples and was reported to be largely unmethylated in the majority of cases [92]. Interestingly, patients with unmethylated *WNT6* presented a significantly shorter 5-year survival rate compared to those with methylated *WNT6* [92], suggesting that *WNT6* may have potential as a prognostic biomarker in these cancers (Fig. 2). Unfortunately, in this study, the specific CpG sites whose methylation was assessed in the *WNT6* gene were not disclosed, which is crucial information as DNA methylation in different gene regions can have diverse effects on gene expression.

Concordantly, recent findings showed that adult patient tissue samples present higher WNT6 mRNA and protein levels than non-tumoral samples [93]. Additionally, *WNT6* levels were significantly higher in the serum of osteosarcoma patients compared to those with Ewing's sarcoma, osteomyelitis, and cancer-free controls [93]. The detection of *WNT6* in liquid biopsies of bone cancer patients was proposed to be a reliable diagnostic tool [93]. Moreover, *WNT6* expression was associated with patient's age, tumor grade, and presence of distant metastasis [93]. High *WNT6* expression was found to be associated with shorter overall survival, and it was considered an independent factor for worse patient prognosis [93].

While these studies underline the potential of WNT6 as a valuable biomarker with diagnostic potential in osteosarcoma, further studies in independent and larger cohorts of patients are necessary. Additionally, gaining deeper insights into the molecular mechanisms underlying these associations will be key to identify putative novel therapeutic targets.

Smooth muscle cancer

A recent study identified *WNT6* was highly expressed in post-transplant smooth muscle tumors positive for Epstein-barr virus, while visceral leiomyomas, leiomyosarcomas, angioleiomyomas, and endothelial haemangiomas presented low levels or no significant differences in *WNT6* expression [94]. Interestingly, in individual cases of post-transplant smooth muscle tumors, *WNT6* and *WNT10A* were co-expressed at similar levels [94]. However, several elements of the β-catenin-dependent and -independent WNT signaling were not altered in this tumor type, with only *CCND2* upregulated compared to angioleiomyomas and endothelial haemangiomas, and *MYC* compared to angioleiomyomas and visceral leiomyomas [94]. Thus, while the concomitant upregulation of *WNT6* and *WNT10A* in post-transplant smooth muscle tumors is interesting, whether these genes sustain effects on tumor behavior and patient clinical outcome remains to be explored.

Lung cancer

In non-small cell lung cancer, a study found *WNT6* was one of the genes upregulated upon HOXC6 overexpression in vitro [96]. Considering HOXC6 is a transcription factor with known oncogenic roles in non-small cell lung cancer, promoting cell proliferation and migration, these preliminary findings raise the hypothesis that HOXC6 may enhance the expression of other oncogenes, such as *WNT6*, ultimately increasing tumor aggressiveness (Fig. 2) [96]. Nonetheless, it is critical to firstly validate this putative HOXC6-WNT6 molecular link in additional preclinical models of non-small cell lung cancer, both in vitro and in vivo, and in human tumor specimens.

In small cell lung cancer, in a microarray analysis, *WNT6* was reported to be downregulated in

cisplatin-resistant in vitro models and its expression was inversely correlated to the lncRNA *NCRNA00173* [95]. This lncRNA has been considered a promoter of aggressiveness in several tumors [133–136], being also associated with chemoresistance in small cell lung cancer [137]. However, *WNT6* expression did not differ significantly between tumor and non-tumor adjacent tissues in a small cohort of these patients [95]. The results herein reported are particularly interesting, since WNT signaling had been previously described to induce resistance to platinum-based chemotherapy in this tumor type [138]. Hence, it would be important to validate this association in a larger cohort, and to compare cisplatin-resistant and -sensitive tumor data, to understand the exact role of the WNT pathway and WNT6 in small cell lung cancer chemoresistance.

While these results indicate that WNT6 may be of interest in both non-small cell and small cell lung cancer, to ascertain its pertinence, it is necessary to further study the phenotypical and molecular implications of its aberrant expression.

Reproductive cancers

The WNT signaling pathway has also been recurrently linked to the onset and progression of ovarian cancer, with certain WNT ligands implicated in such processes [139–141]. Recently, WNT6 was found to be increased in ovarian cancer patient samples in comparison to non-tumor tissues, and this upregulation was also observed in ovarian cancer in vitro models relative to non-tumor ovarian cells [76]. Furthermore, WNT6 expression was associated to increased cell proliferation, invasion, as well as decreased cell cycle arrest, and inhibition of apoptosis in vitro [76]. Interestingly, high *WNT6* expression was also associated with decreased progression-free survival in ovarian cancer patients [76]. Molecularly, WNT6 associated with β-catenin and NOTCH1 expression in ovarian cancer cells, suggesting WNT6 may exert its oncogenic effects through the β-catenin-dependent WNT and Notch pathways (Fig. 2) [76], whose interconnection has been often reported as important for the aggressiveness of different tumor types [142–145]. This important study revealed a biomarker potential of WNT6 in ovarian cancer patients, identifying as well possible mechanisms through which it may be inciting its oncogenic effects. Future efforts should focus on better defining the molecules and pathways that act upstream and downstream of WNT6, possibly opening new therapeutic opportunities.

Additionally, in testicular germ cell tumors, *WNT6* was identified to be downregulated in a small number of cisplatin-resistant cell lines when compared to cisplatin-sensitive cells [97]. While this study suggested that WNT6 could be of interest in testicular tumors, it would be noteworthy to complement this data with functional and molecular analyses to provide mechanistic and causality insights.

Emerging pro-tumoral roles of WNT6 in multiple cancers

WNT6 exerts its physiological functions through the activation of WNT signaling pathways, encompassing both β-catenin-dependent and independent cascades, in a manner specific to cell type and developmental stage [61, 62, 64–66]. Emerging evidence has highlighted WNT6 aberrant expression as a prevalent alteration in various tumor types [67, 70, 72–74, 76, 77, 79–81, 83, 84, 86–88, 90–94, 96]. More significantly, extensive evidence emphasizes its pertinent role in tumor aggressiveness across several malignancies (Fig. 2 and Table 1) [69–77, 79, 81, 82, 85, 87–90, 92, 93, 102]. Nonetheless, curiously, contrasting findings in a subset of tumor types suggest a possible dual role for WNT6 in those contexts [95, 97]. At the light of this increasing body of data, it is critical to understand the putative relevance of WNT6 in multiple aspects of cancer, namely cancer cell functions, sensitivity to anticancer therapies, and patient prognosis. Equally relevant is to dissect whether WNT6 may be a novel therapeutic target in specific cancer types.

In vitro, WNT6 has been classified has a cancer-promoting molecule (Fig. 2), sustaining multiple tumorigenic capabilities across a range of cancer types [69–77, 81, 85]. Although a limited number of studies have reported in vivo approaches, they corroborate an association between higher WNT6 expression and increased tumor aggressiveness [69, 70, 72, 74]. Of note, clinical data from several tumors reveal that high WNT6 may be a predictor of unfavorable patient outcome [74, 76, 79, 82, 88, 90, 93]. Complementarily, data from multiple tumors suggests WNT6 may be involved in increased chemoresistance to anthracyclines [72, 75] and to alkylating agents [73, 74, 89]. Interestingly, patients with colorectal cancer liver metastasis who received preoperative chemotherapy presented significantly lower WNT6 expression, in comparison to those who did not receive it [79]. This raises the hypothesis that preoperative treatment may reduce WNT6 expression in colorectal cancer liver metastasis, and/or that WNT6-positive cells could be more susceptible to chemotherapy-induced cell death.

The apparent dual effects of WNT6 expression in anticancer therapy response warrant further investigation, namely clarifying whether this is dependent on the mechanism of action of the particular therapeutic agent used in each study, or if it is tumor-type/subtype related. To further unravel this, the use of ever-increasing bioinformatic tools (e.g. Connectivity map and multi-omics data combined with clinical data), and drug screening

assays could be interesting approaches. Additionally, it remains to be studied whether WNT6 may affect sensitivity to radiotherapy, which would be particularly critical given its wide clinical use in multiple cancer types.

Molecular regulators and interactors of WNT6 in cancer

Mechanistically, WNT6 may be promoting tumor aggressiveness through the activation of both β-catenin-dependent and -independent WNT pathways (Fig. 1). Although WNT6 is more frequently associated with β-catenin-dependent signaling activation [69–76], this may be due to the limited analysis of other possible signaling cascades in which WNT6 could be involved. Indeed, when this was analyzed, as, for example, in glioblastoma [74] and post-transplant smooth muscle tumors [94], WNT6 was also associated with the activation of β-catenin-independent WNT signaling pathways.

Curiously, *WNT6* and *WNT10A* co-expression was reported in colorectal [67] and post-transplant smooth muscle tumors [94], but whether this *WNT6-WNT10A* association also occurs in other malignancies remains to be explored.

Depending on the tumor type, various molecules have been identified as regulators of WNT6 (Table 2), many of which are known oncogenes [107, 108, 132], such as PLAGL2 [69, 75], HOXA9 [87], and NR4A2 [70]. These molecules function as transcription factors directly interacting with the *WNT6* promoter, inducing its expression, and consequently promoting tumor aggressiveness [69, 70, 75, 87]. Notably, PLAGL2 has been reported in both colorectal and breast cancers [69, 75], indicating that it could be valuable to study this association beyond the in vitro models, particularly exploring whether these effects can be replicated in vivo, and ultimately validated in primary tumor patient samples.

Additionally, *WNT6* has been described to interact with and be regulated by non-coding RNAs, such as lncRNA *UCA1*, *hsa-miR-566* and *hsa-miR-1207-5p* [71, 73, 81], as well as other molecular players whose interactions with WNT6 remains incompletely understood [72, 82, 84, 96], such as CAV1 [72]. Importantly, further molecular assays are required to unveil causative associations, assess functional implications, and understand the nature of these interactions. It would be interesting to assess whether these molecules may be associated with WNT6 in other tumor types, as it has been done for glioblastoma, where it was found that molecules such as *CAV1*, *PLAGL2*, and *UCA1* have no significant correlation to *WNT6* in this context [87].

Of note, some putative associations were also revealed through transcriptomic analyses (e.g., *KLK6* in breast [82] and *HOXC6* in non-small cell lung cancer [96]), but further investigations must be conducted for comprehensive validation of such molecular relations.

WNT6 as a putative novel therapeutic target in cancer

Considering WNT6 was shown to promote tumor aggressiveness and progression in most cancer types in which it was studied [77, 78, 87, 93, 104, 105, 116, 146], it would be interesting to explore its relevance as a novel therapeutic target. Unfortunately, structural similarities and functional redundancy of WNT ligands pose additional challenges for the development of specific inhibitors of WNT6. Nonetheless, considering some of the already-known signaling pathways activated by WNT6 in particular cancers, a legitimate therapeutic strategy would be to test targeted therapies already developed to inhibit those WNT6-related (upstream or downstream) pathways (Fig. 3). For example, PORCN inhibitors [147–151] prevent WNT ligand extrusion (e.g., IWP2 [147], IWP-L6 [148], ETC-159 [149], C59 [150], or LGK974 [151]), of which LGK974 has shown great promise in clinical studies for safety and tolerability in various tumor types, both in monotherapy and in combination with other therapeutics [146, 152, 153]. Currently, a phase I clinical trial is testing LGK974 in patients whose tumors present alterations in the WNT signaling pathway

Table 2 Summary of the main molecules found to be associated with WNT6 in different tumor types

Tumor type	Molecule	Function	Association to WNT6	References
Gastric	**Caveolin-1**	Oncogene	*WNT6*-CAV1 axis promotes chemoresistance to anthracycline drugs	[72]
Bladder	**lncRNA *UCA1***	Oncogene	Its expression positively correlates with *WNT6* expression	[73]
Colorectal	**PLAGL2**	Oncogene	Binds *WNT6* promoter region and activates its expression	[69]
	NR4A2	Oncogene	Binds *WNT6* promoter region and activates its expression	[70]
	hsa-miR-1207-5p	Oncogene	Binds *WNT6* mRNA and promotes its activity	[71]
Glioblastoma	**HOXA9**	Oncogene	Binds *WNT6* promoter region and activates its expression	[87]
Breast	***hsa-miR-566***	Tumor suppressor	Its expression negatively correlates with *WNT6* expression. Binds *WNT6* mRNA and decreases its activity	[81]
	PLAGL2	Oncogene	Binds *WNT6* promoter region and activates its expression	[75]

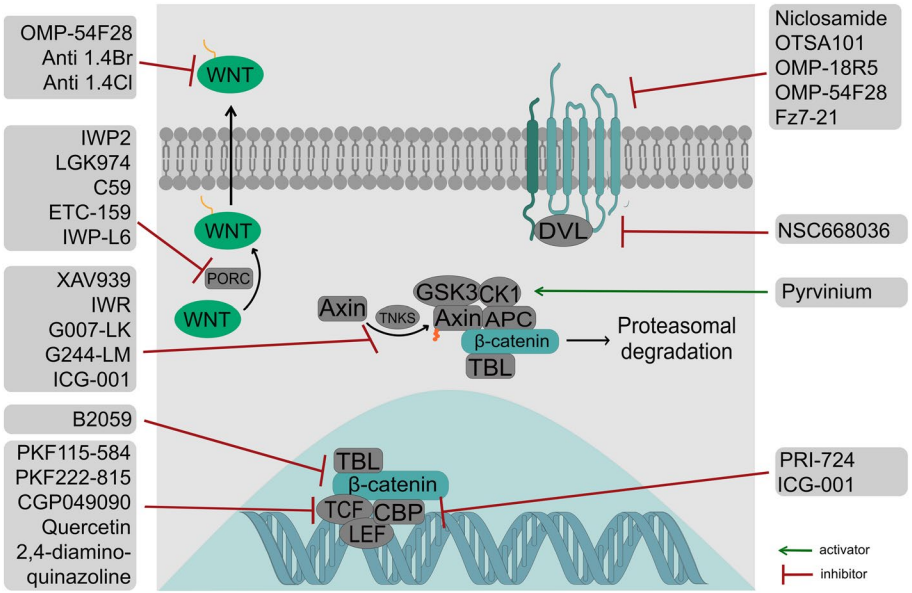

Fig. 3 Compounds that target components of WNT pathway. Schematic representation of the β-catenin-dependent WNT signaling pathway alongside pharmaceutical modulators. These are strategically designed to inhibit WNT pathway activity, acting at various stages, including inhibition of WNT ligand maturation and secretion, of WNT ligand-receptor complex interactions, and of key molecular players within the β-catenin-dependent intracellular WNT signaling cascade. These include small molecule inhibitors, peptide antagonists, and monoclonal antibodies

(NCT01351103). The safety and tolerability of ECT159 has also been clinically tested in patients with advanced/unresponsive solid tumors (NCT02521844).

Other therapeutic strategies include the inhibition of WNT interactions with receptor complexes using peptide antagonists (e.g., Fz7-21 [154]), monoclonal antibodies (e.g., OMP-18R5 [155], OTSA-10 [156]) or decoy receptors (e.g., OMP-54F28 [157]). OMP-18R5 [158] and OMP-54F28 [157, 159, 160] were already tested in multiple early-phase clinical trials, and while the efficacy results of many are still unpublished, general good tolerability was shown, despite some reports of bone toxicity when combined with certain chemotherapies [157–161]. Of note, as the full spectrum of WNT6 receptors remains to be identified, a single drug affecting only a particular ligand/receptor interaction may not be able to inhibit all the WNT6-mediated signaling cascades.

Other putative approaches consist of targeting the dishevelled protein, a critical cytoplasmic partner that interacts with the majority of WNT co-receptors (NSC668036 [162]), or inhibiting specifically β-catenin-dependent WNT signaling [69–76]. Several compounds have been developed to target various intermediates of this pathway, aiming to stabilize the β-catenin destruction complex [147, 163, 164] or to inhibit the β-catenin-TCF/LEF complex [165–169]. Particularly, BC2059 (tegavivint [169], an inhibitor of β-catenin and TBL interaction; NCT03459469, NCT04851119, NCT04874480, NCT04780568) and PRI-724 (an inhibitor of β-catenin-CBP interaction [170]; NCT01302405, NCT01606579, NCT01764477) have been under clinical investigation for their efficacy in various malignancies. The full completion of these clinical trials may provide invaluable findings, potentially impacting human cancers with WNT6-driven activation of WNT signaling.

Nonetheless, while specific inhibitors of WNT6 are not yet available, the landscape of cancer treatment offers multiple potential strategies that could be adapted to target WNT6. As above-described for many other molecules that take part in WNT signaling, the development of small-molecule inhibitors and antibody-based therapies offer promising avenues. A particularly intriguing approach is the use of bispecific antibodies, which may simultaneously target WNT6 together with other key tumor oncogenic biomarkers or immune-system activating molecules. This strategy may not only interfere with the ability of WNT6 to bind its receptor complex, but may also enhance the immune system's response against tumor cells. Interestingly, a recent study explored the effects of targeting LRP6 along with cell-type specific antigens, to inhibit WNT signaling in a cell-type specific manner [171]. Additionally, recent advances in gene-based therapies may hold significant potential by precisely targeting WNT6 expression in tumor tissues,

such as those based on CRISPR-Cas9 editing and optimized viral delivery systems. For example, gene therapies are already FDA-approved for rare, non-oncogenic conditions, such as dystrophic epidermolysis bullosa [172], and haemophilia B [173], and similar approaches may be exploited in malignant contexts.

Thus, in the challenging quest to successfully inhibit the pro-tumoral effects of WNT6 in cancer, several possible courses of action can and should be explored, possibly rationally adapted to the specificities of each particular tumor type. Given some of these human cancers are particularly prevalent, these novel therapeutic tools hold great clinical potential. In a time where precision medicine holds immense promise for improved cancer treatment outcomes, it would also be important that both the scientific community and pharmaceutical industry join efforts and prioritize the development of novel molecules capable of directly targeting WNT6, paving the way for more effective and precise interventions in cancer therapy.

Future directions

Despite the growing body of evidence supporting the relevance of WNT6 in the promotion of oncogenic processes, and of the putative molecular interactions in which it may be involved, there are still many unanswered questions, mostly in what regards the causative or associative nature of particular molecular links with WNT6, the identification of upstream regulatory mechanisms, as well as the dissection of its receptors, downstream effector pathways and molecular partners. These insights would be highly relevant, as they could contribute to the development and selection of more precise targeted-therapies. This could be achieved by performing, for example, systematic and complementary bioinformatic analyses, which are becoming widely available and of increased power, such as through gene set enrichment analysis (GSEA; https://www.gsea-msigdb.org) [174], and it would be particularly useful to validate some of the reported findings in well-characterized cohorts (e.g., querying data from The Cancer Genome Atlas), establishing important correlations between the expression of WNT6 and its potential molecular partners, as well as further assessing their impact in the survival outcome of patients with different tumor types. Additionally, complementing this knowledge with more integrative approaches, exploiting comprehensive transcriptomic analysis, such as RNA-sequencing, ChIP-sequencing, as well as epigenomics, and proteomics analyses, would be particularly informative, allowing the concomitant characterization of multiple layers of molecular data, potentially contributing to a more integrated understanding of how they interact and relate to WNT6 expression and function. Indeed, for some tumors, such as glioblastoma, upregulation of WNT6 expression was already shown to depend, at least partly, on specific gene methylation patterns, and the presence of a particular transcription factor [87]. Importantly, more advanced methodologies should also be explored, such as single-cell sequencing and spatial transcriptomics, since these consider tumor heterogeneity and offer a more in-depth comprehensive analysis of the different cell populations present in the tumor microenvironment, possibly revealing specific populations exhibiting altered WNT6 levels. Moreover, these would be extremely valuable to explore the effects of WNT6 on cancer stem cell (CSC) populations and stem-cell like behavior of tumor cells, considering the significance of WNT signaling and WNT6 in the regulation of progenitor and stem cell fate under physiological conditions, and the recent reported influence of WNT6 expression in a stem-cell like phenotype in glioblastoma cells [74]. Additionally, given the relevance of CSCs in therapy resistance, it is also important to assess how modulation of WNT6 in these cells could affect their malignant phenotype and investigate how it may be combined with particular molecularly-targeted therapies.

Despite a few exceptions with studies reporting in vivo findings [69, 70, 72, 74, 91], several articles present only in vitro data, highlighting a need for validation in more refined and relevant preclinical in vivo models that would undoubtedly strengthen the validity and translational impact of the findings. For example, using more sophisticated animal models, particularly orthotopic models, or xenograft models, would be immensely informative in what regards the impact of WNT6 in tumor progression, molecular interactions, and specificities in particular niches of the tumor microenvironment. Of these, syngeneic and/or humanized mouse models would be of particular relevance, given their immunocompetent contexts, considering the described role of WNT6 in immune response, specifically in macrophage differentiation and proliferation [66], as well as its reported correlation to regulatory T-cell infiltration in breast cancer patients with bone metastasis [82], it is reasonable to hypothesize that WNT6 may also affect the immune system.

Furthermore, posterior to these validations, it may be pertinent to explore the implications of WNT6 modulation in the response of tumor cells to particular anticancer immunotherapies, such as, for example, immune checkpoint inhibitors. In this context, it would be relevant to perform analyses in large patient cohorts, associating WNT6 expression with patient responsiveness to immunotherapies.

Of note, given that most of these studies collectively suggest WNT6 as a promoter of cancer aggressiveness,

it would be invaluable to deploy innovative strategies to investigate its potential role in tumor development. One approach could involve manipulating non-tumor cells in vitro to overexpress WNT6, and assess how it may influence transformation and tumorigenesis. Complementarily, a more sophisticated and informative methodology could use syngeneic genetically-modified mice presenting modulated expression levels of human *WNT6*, taking advantage of inducible temporal- and tissue-specific conditional models. Exploring WNT6-driven tumorigenesis in a more complex living organism would offer key insights at multiple levels. For example, it could reveal how WNT6 may affect intricate intercellular interactions within the various niches of the tumor microenvironment, and help uncover whether WNT6 effects are tissue-specific or, as a secreted ligand, if it may also be broader at systemic levels. Additionally, tracking tumor-initiating cells could provide crucial information on WNT6's role in tumorigenesis. For example, studying clonal evolution and lineage tracing may also reveal whether WNT6 can drive the expansion of specific tumor subpopulations, particularly those associated with aggressive cancer behavior. These methods would help determine whether WNT6 is among the key drivers of tumorigenesis in multiple tumor types.

In conclusion, WNT6 has recently emerged as a key player in multiple oncogenic processes of a significant variety of tumors. Its elevated expression has been associated with tumor aggressiveness, sensitivity to chemotherapeutics, and patient prognosis. While frequently being reported to activate the β-catenin-dependent WNT cascade, various other molecular inter-players have been found to interact with and modulate the expression of WNT6. Yet, there is still much to unveil for a comprehensive understanding of its role in cancer, as well as its therapeutic potential. Undoubtedly a clearer understanding will contribute with invaluable insights, paving the way for new breakthroughs in the field.

Abbreviations

5-FU	5-Fluorouacil
(S)-NNN	(S)-N'-nitrosonornicotine
APC	Adenomatous polyposis coli
CAV1	Caveolin-1
CK1	Casein kinase 1
CSC	Cancer stem cell
FZD	Frizzled
GSK3	Glycogen synthase kinase 3
lncRNA	Long non-coding RNA
MuSK	Muscle skeletal receptor Tyr kinase
PGE2	Protaglandin E2
PORCN	Porcupine
PTK7	Protein Tyr kinase 7
ROR	Tyr kinase-like orphan receptor
RYK	Tyr kinase receptor

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Data availability
No datasets were generated or analysed during the current study.

Declarations

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Not applicable.

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References

1. Nusse R, Clevers H. Wnt/β-catenin signaling, disease, and emerging therapeutic modalities. Cell. 2017;169(6):985–99.
2. Tanaka K, Kitagawa Y, Kadowaki T. Drosophila segment polarity gene product porcupine stimulates the posttranslational N-glycosylation of wingless in the endoplasmic reticulum. J Biol Chem. 2002;277(15):12816–23.
3. Willert K, Brown JD, Danenberg E, Duncan AW, Weissman IL, Reya T, et al. Wnt proteins are lipid-modified and can act as stem cell growth factors. Nature. 2003;423(6938):448–52.
4. Van den Heuvel M, Harryman-Samos C, Klingensmith J, Perimon N, Nusse R. Mutations in the segment polarity genes wingless and porcupine impair secretion of the wingless protein. EMBO J. 1993;12(13):5293–302.
5. Niehrs C. The complex world of WNT receptor signalling. Nat Rev Mol Cell Biol. 2012;13(12):767–79.
6. Yang-Snyder J, Miller JR, Brown JD, Lai CJ, Moon RT. A frizzled homolog functions in a vertebrate Wnt signaling pathway. Curr Biol. 1996;6(10):1302–6.
7. He X, Semenov M, Tamai K, Zeng X. LDL receptor-related proteins 5 and 6 in Wnt/β-catenin signaling: arrows point the way. Development. 2004;131(8):1663–77.
8. Wehrli M, Dougan ST, Caldwell K, O'Keefe L, Schwartz S, Valzel-Ohayon D, et al. Arrow encodes an LDL-receptor-related protein essential for Wingless signalling. Nature. 2000;407(6803):527–30.

9. Jing L, Lefebvre JL, Gordon LR, Granato M. Wnt signals organize synaptic prepattern and axon guidance through the Zebrafish unplugged/MuSK receptor. Neuron. 2009;61(5):721–33.
10. Minami Y, Oishi I, Endo M, Nishita M. Ror-family receptor tyrosine kinases in noncanonical Wnt signaling: their implications in developmental morphogenesis and human diseases. Dev Dyn. 2010;239(1):1–15.
11. Fradkin LG, Dura JM, Noordermeer JN. Ryks: new partners for Wnts in the developing and regenerating nervous system. Trends Neurosci. 2010;33(2):84–92.
12. Peradziryi H, Tolwinski NS, Borchers A. The many roles of PTK7: a versatile regulator of cell-cell communication. Arch Biochem Biophys. 2012;524(1):71–6.
13. Baeg GH, Selva EM, Goodman RM, Dasgupta R, Perrimon N. The Wingless morphogen gradient is established by the cooperative action of Frizzled and Heparan Sulfate Proteoglycan receptors. Dev Biol. 2004;276(1):89–100.
14. van Amerongen R, Nusse R. Towards an integrated view of Wnt signaling in development. Development. 2009;136(19):3205–14.
15. Halleskog C, Schulte G. Pertussis toxin-sensitive heterotrimeric Gαi/o proteins mediate WNT/β-catenin and WNT/ERK1/2 signaling in mouse primary microglia stimulated with purified WNT-3A. Cell Signal. 2013;25(4):822–8.
16. Thrasivoulou C, Millar M, Ahmed A. Activation of intracellular calcium by multiple Wnt ligands and translocation of β-catenin into the nucleus: a convergent model of Wnt/Ca^{2+} and Wnt/β-catenin pathways. J Biol Chem. 2013;288(50):35651–9.
17. MacDonald BT, Semenov MV, He X. SnapShot: Wnt/β-catenin signaling. Cell. 2007;131(6):1204.e1-1204.e2.
18. Semenov MV, Habas R, MacDonald BT, He X. SnapShot: noncanonical Wnt signaling pathways. Cell. 2007;131(7):1378.e1-1378.e2.
19. Molenaar M, Van De Wetering M, Oosterwegel M, Peterson-Maduro J, Godsave S, Korinek V, et al. XTcf-3 transcription factor mediates β-catenin-induced axis formation in xenopus embryos. Cell. 1996;86(3):391–9.
20. Behrens J, Von Kries JP, Kühl M, Bruhn L, Wedlich D, Grosschedl R, et al. Functional interaction of β-catenin with the transcription factor LEF- 1. Nature. 1996;382(6592):638–42.
21. Van de Wetering M, Cavallo R, Dooijes D, Van Beest M, Van Es J, Loureiro J, et al. Armadillo coactivates transcription driven by the product of the Drosophila segment polarity gene dTCF. Cell. 1997;88(6):789–99.
22. Nakamura Y, De Paiva AE, Veenstra GJC, Hoppler S. Tissue-and stage-specific Wnt target gene expression is controlled subsequent to β-catenin recruitment to cis-regulatory modules. Development. 2016;143(11):1914–25.
23. Van Amerongen R, Mikels A, Nusse R. Alternative Wnt signaling is initiated by distinct receptors. Sci Signal. 2008;1(35):re9.
24. Shi DL. Wnt/planar cell polarity signaling controls morphogenetic movements of gastrulation and neural tube closure. Cell Mol Life Sci. 2022;79(12):586.
25. Niehrs C. On growth and form: a cartesian coordinate system of Wnt and BMP signaling specifies bilaterian body axes. Development. 2010;137(6):845–57.
26. Axelrod JD. Planar cell polarity signaling in the development of left–right asymmetry. Curr Opin Cell Biol. 2020;62:61–9.
27. Shi DL. Planar cell polarity regulators in asymmetric organogenesis during development and disease. J Genet Genomics. 2023;50(2):63–76.
28. Wray J, Hartmann C. WNTing embryonic stem cells. Trends Cell Biol. 2012;22(3):159–68.
29. Reya T, Clevers H. Wnt signalling in stem cells and cancer. Nature. 2005;434(7035):843–50.
30. Loh KMM, Chen A, Koh PWW, Deng TZZ, Sinha R, Tsai JMM, et al. Mapping the pairwise choices leading from pluripotency to human bone, heart, and other mesoderm cell types. Cell. 2016;166(2):451–67.
31. Niehrs C, Acebron SP. Mitotic and mitogenic Wnt signalling. EMBO J. 2012;31(12):2705–13.
32. Marinou K, Christodoulides C, Antoniades C, Koutsilieris M. Wnt signaling in cardiovascular physiology. Trends Endocrinol Metab. 2012;23(12):628–36.
33. Noelanders R, Vleminckx K. How Wnt signaling builds the brain: bridging development and disease. Neuroscientist. 2017;23(3):314–29.
34. Russell JO, Monga SP. Wnt/β-catenin signaling in liver development, homeostasis, and pathobiology. Annu Rev Pathol. 2018;13(1):351–78.
35. Karner CM, Long F. Wnt signaling and cellular metabolism in osteoblasts. Cell Mol Life Sci. 2017;74(9):1649–57.
36. Karim RZ, Tse GMK, Putti TC, Scolyer RA, Lee CS. The significance of the Wnt pathway in the pathology of human cancers. Pathology. 2004;36(2):120–8.
37. Zhan T, Rindtorff N, Boutros M. Wnt signaling in cancer. Oncogene. 2017;36(11):1461–73.
38. Parsons MJ, Tammela T, Dow LE. WNT as a driver and dependency in cancer. Cancer Discov. 2021;11(10):2413–29.
39. Nishisho I, Nakamura Y, Miyoshi Y, Miki Y, Ando H, Horii A, et al. Mutations of chromosome 5q21 genes in FAP and colorectal cancer patients. Science. 1991;253(5020):665–9.
40. Morin PJ, Sparks AB, Korinek V, Barker N, Clevers H, Vogelstein B, et al. Activation of β-catenin-Tcf signaling in colon cancer by mutations in β-catenin or APC. Science. 1997;275(5307):1787–90.
41. Rubinfeld B, Robbins P, El-Gamil M, Albert I, Porfiri E, Polakis P. Stabilization of β-catenin by genetic defects in melanoma cell lines. Science. 1997;275(5307):1790–2.
42. Liu W, Dong X, Mai M, Seelan RS, Taniguchi K, Krishnadath KK, et al. Mutations in AXIN2 cause colorectal cancer with defective mismatch repair by activating β-catenin/TCF signalling. Nat Genet. 2000;26(2):146–7.
43. Lammi L, Arte S, Somer M, Järvinen H, Lahermo P, Thesleff I, et al. Mutations in AXIN2 cause familial tooth agenesis and predispose to colorectal cancer. Am J Hum Genet. 2004;74(5):1043–50.
44. Yang S, Liu Y, Li MY, Ng CSH, Yang S li, Wang S, et al. FOXP3 promotes tumor growth and metastasis by activating Wnt/β-catenin signaling pathway and EMT in non-small cell lung cancer. Mol Cancer. 2017;16(1):124.
45. Leung HW, Leung CON, Lau EY, Chung KPS, Mok EH, Lei MML, et al. EPHB2 activates β-catenin to enhance cancer stem cell properties and drive sorafenib resistance in hepatocellular carcinoma. Cancer Res. 2021;81(12):3229–40.
46. Wan X, Guan S, Hou Y, Qin Y, Zeng H, Yang L, et al. FOSL2 promotes VEGF-independent angiogenesis by transcriptionally activating Wnt5a in breast cancer-associated fibroblasts. Theranostics. 2021;11(10):4975.
47. Kim KB, Kim DW, Kim Y, Tang J, Kirk N, Gan Y, et al. WNT5A-RHOA signaling is a driver of tumorigenesis and represents a therapeutically actionable vulnerability in small cell lung cancer. Cancer Res. 2022;82(22):4219–33.
48. Shu Z, Fan M, Tu B, Tang Z, Wang H, Li H, et al. The Lin28b/Wnt5a axis drives pancreas cancer through crosstalk between cancer associated fibroblasts and tumor epithelium. Nat Commun. 2023;14(1):6885.
49. Uysal-Onganer P, Kawano Y, Caro M, Walker MM, Diez S, Darrington RS, et al. Wnt-11 promotes neuroendocrine-like differentiation, survival and migration of prostate cancer cells. Mol Cancer. 2010;9(1):55.
50. Murillo-Garzón V, Gorroño-Etxebarria I, Åkerfelt M, Puustinen MC, Sistonen L, Nees M, et al. Frizzled-8 integrates Wnt-11 and transforming growth factor-β signaling in prostate cancer. Nat Commun. 2018;9(1):1747.
51. Nusse R, Varmus HE. Many tumors induced by the mouse mammary tumor virus contain a provirus integrated in the same region of the host genome. Cell. 1982;31(1):99–109.
52. Nusse R, Van Ooyen A, Cox D, Fung YKT, Varmus H. Mode of proviral activation of a putative mammary oncogene (int-1) on mouse chromosome 15. Nature. 1984;307(5947):131–6.
53. van Ooyen A, Nusse R. Structure and nucleotide sequence of the putative mammary oncogene int-1; proviral insertions leave the protein-encoding domain intact. Cell. 1984;39(1):233–40.
54. Fung YK, Shackleford GM, Brown AM, Sanders GS, Varmus HE. Nucleotide sequence and expression in vitro of cDNA derived from mRNA of int-1, a provirally activated mouse mammary oncogene. Mol Cell Biol. 1985;5(12):3337–44.
55. Tsukamoto AS, Grosschedl R, Guzman RC, Parslow T, Varmus HE. Expression of the int-1 gene in transgenic mice is associated with mammary gland hyperplasia and adenocarcinomas in male and female mice. Cell. 1988;55(4):619–25.

56. Lo ML. A possible role for the WNT-1 pathway in oral carcinogenesis. Crit Rev Oral Biol Med. 2001;12(2):152–65.
57. Kumawat K, Gosens R. WNT-5A: Signaling and functions in health and disease. Cell Mol Life Sci. 2016;73(3):567–87.
58. Zhang Q, Pan Y, Ji J, Xu Y, Zhang Q, Qin L. Roles and action mechanisms of WNT4 in cell differentiation and human diseases: a review. Cell Death Discov. 2021;7(1):287.
59. Perkins RS, Singh R, Abell AN, Krum SA, Miranda-Carboni GA. The role of WNT10B in physiology and disease: a 10-year update. Front Cell Dev Biol. 2023;11:1120365.
60. Wei M, Zhang C, Tian Y, Du X, Wang Q, Zhao H. Expression and function of WNT6: from development to disease. Front Cell Dev Biol. 2020;8:558155.
61. Schmidt C, McGonnell IM, Allen S, Otto A, Patel K. Wnt6 controls amniote neural crest induction through the non-canonical signaling pathway. Dev Dyn. 2007;236(9):2502–11.
62. Lavery DL, Martin J, Turnbull YD, Hoppler S. Wnt6 signaling regulates heart muscle development during organogenesis. Dev Biol. 2008;323(2):177–88.
63. Itäranta P, Lin Y, Peräsaari J, Roël G, Destrée O, Vainio S. Wnt-6 is expressed in the ureter bud and induces kidney tubule development in vitro. Genesis. 2002;32(4):259–68.
64. Bonnet C, Oh D, Mei H, Robertson S, Chang D, Bourges JL, et al. Wnt6 plays a complex role in maintaining human limbal stem/progenitor cells. Sci Rep. 2021;11(1):20948.
65. Fu H, Tan X, Ye L, Wang C. The glycoprotein Wnt6 regulates human dental papilla cells differentiation by canonical Wnt signaling pathway. Arch Oral Biol. 2022;141:105469.
66. Schaale K, Brandenburg J, Kispert A, Leitges M, Ehlers S, Reiling N. Wnt6 is expressed in granulomatous lesions of mycobacterium tuberculosis –infected mice and is involved in macrophage differentiation and proliferation. J Immunol. 2013;191(10):5182–95.
67. Kirikoshi H, Sekihara H, Katoh M. WNT10A and WNT6, clustered in human chromosome 2q35 region with head-to-tail manner, are strongly coexpressed in SW480 cells. Biochem Biophys Res Commun. 2001;283(4):798–805.
68. Kirikoshi H, Sekihara H, Katoh M. Up-regulation of WNT10A by tumor necrosis factor alpha and Helicobacter pylori in gastric cancer. Int J Oncol. 2001;19(3):533–6.
69. Li N, Li D, Du Y, Su C, Yang C, Lin C, et al. Overexpressed PLAGL2 transcriptionally activates Wnt6 and promotes cancer development in colorectal cancer. Oncol Rep. 2019;41(2):875–84.
70. Feng Y, Tao L, Wang G, Li Z, Yang M, He W, et al. Aspirin inhibits prostaglandins to prevents colon tumor formation via down-regulating Wnt production. Eur J Pharmacol. 2021;906:174173.
71. Yu P, Zhang J, Zhu A, Kong W, Shen X. LncRNA PVT1 regulates miR-1207-5p to affect colon cancer proliferation and migration via the Wnt6/β-catenin2 pathway. Genet Test Mol Biomarkers. 2022;26(6):307–15.
72. Yuan G, Regel I, Lian F, Friedrich T, Hitkova I, Hofheinz RD, et al. WNT6 is a novel target gene of caveolin-1 promoting chemoresistance to epirubicin in human gastric cancer cells. Oncogene. 2013;32(3):375–87.
73. Fan Y, Shen B, Tan M, Mu X, Qin Y, Zhang F, et al. Long non-coding RNA UCA1 increases chemoresistance of bladder cancer cells by regulating Wnt signaling. FEBS J. 2014;281(7):1750–8.
74. Gonçalves CS, de Castro JV, Pojo M, Martins EP, Queirós S, Chautard E, et al. WNT6 is a novel oncogenic prognostic biomarker in human glioblastoma. Theranostics. 2018;8(17):4805–23.
75. Li Y, Liu R, Han X, Xu W, Liu Y. PLAGL2 increases adriamycin resistance and EMT in breast cancer cells by activating the Wnt pathway. Genes Genomics. 2023;45(1):49–57.
76. Bao H, Wu W, Li Y, Zong Z, Chen S. WNT6 participates in the occurrence and development of ovarian cancer by upregulating/activating the typical Wnt pathway and Notch1 signaling pathway. Gene. 2022;846:146871.
77. Zheng XL, Yu HG. Wnt6 contributes tumorigenesis and development of colon cancer via its effects on cell proliferation, apoptosis, cell-cycle and migration. Oncol Lett. 2018;16(1):1163–72.
78. Yu T, Zhou F, Tian W, Xu R, Wang B, Zeng A, et al. EZH2 interacts with HP1BP3 to epigenetically activate WNT7B that promotes temozolomide resistance in glioblastoma. Oncogene. 2023;42(6):461–70.
79. Peng J, Zhao Y, Luo Q, Chen H, Fan W, Pan Z, et al. High WNT6 expression indicates unfavorable survival outcome for patients with colorectal liver metastasis after liver resection. J Cancer. 2019;10(12):2619–27.
80. Milovanovic T, Planutis K, Nguyen A, Marsh JL, Lin F, Hope C, et al. Expression of Wnt genes and frizzled 1 and 2 receptors in normal breast epithelium and infiltrating breast carcinoma. Int J Oncol. 2004;25(5):1337–42.
81. Zhao MC, Zhang MM, Li T, Tao ZH, Du YQ, Wang LP, et al. MiR-566 protects the malignant progression of breast cancer by negatively regulating WNT6. Eur Rev Med Pharmacol Sci. 2020;24(11):6185–94.
82. Liu S, Song A, Zhou X, Huo Z, Yao S, Yang B, et al. ceRNA network development and tumour-infiltrating immune cell analysis of metastatic breast cancer to bone. J Bone Oncol. 2020;24:100304.
83. Benhaj K, Akcali KC, Ozturk M. Redundant expression of canonical Wnt ligands in human breast cancer cell lines. Oncol Rep. 2006;15(3):701–7.
84. Mccorkle JR, Leonard MK, Kraner SD, Blalock EM, Deqin M, Zimmer SG, et al. The metastasis suppressor NME1 regulates expression of genes linked to metastasis and patient outcome in melanoma and Breast carcinoma. Cancer Genomics Proteomics. 2014;11(4):175–94.
85. Wang F, Li X, Xie XJ, Zhao L, Chen W. UCA1, a non-protein-coding RNA up-regulated in bladder carcinoma and embryo, influencing cell growth and promoting invasion. FEBS Lett. 2008;582(13):1919–27.
86. Liu X, Mazanek P, Dam V, Wang Q, Zhao H, Guo R, et al. Deregulated Wnt/β-catenin program in high-risk neuroblastomas without MYCN amplification. Oncogene. 2008;27(10):1478–88.
87. Gonçalves CS, Xavier-Magalhães A, Martins EP, Pinto AA, Pires MM, Pinheiro C, et al. A novel molecular link between HOXA9 and WNT6 in glioblastoma identifies a subgroup of patients with particular poor prognosis. Mol Oncol. 2020;14(6):1224–41.
88. Trong PD, Rösch S, Mairbäurl H, Pusch S, Unterberg A, Herold-Mende C, et al. Identification of a prognostic hypoxia-associated gene set in IDH-mutant glioma. Int J Mol Sci. 2018;19(10):2903.
89. Ma Z, Cai S, Xiong Q, Liu W, Xia H, Zhu Z, et al. WNT signaling modulates chemoresistance to temozolomide in p53-mutant glioblastoma multiforme. Apoptosis. 2022;27(1–2):80–9.
90. Zhang L, Yuan G, Fang Y, Qiu M, Lin J, Sun J, et al. Increased WNT6 expression in tumor cells predicts unfavorable survival in esophageal squamous cell carcinoma patients. Int J Clin Exp Pathol. 2015;8(9):11421–7.
91. Khammanivong A, Anandharaj A, Qian X, Song JM, Upadhyaya P, Balbo S, et al. Transcriptome profiling in oral cavity and esophagus tissues from (S)-N′-nitrosonornicotine-treated rats reveals candidate genes involved in human oral cavity and esophageal carcinogenesis. Mol Carcinog. 2016;55(12):2168–82.
92. Li L, Xu C, Liu P, Huang J. Correlation study of DNA methylation of WNT6 gene with osteosarcoma in children. Oncol Lett. 2017;14(1):271–5.
93. Jiang K, Li S, Li L, Wang X, Gu Y, Jin Z. WNT6 is an effective marker for osteosarcoma diagnosis and prognosis. Medicine. 2018;97(46):e13011.
94. Teiken K, Kuehnel M, Rehkaemper J, Kreipe H, Laenger F, Hussein K, et al. Non-canonical WNT6/WNT10A signal factor expression in EBV+ post-transplant smooth muscle tumors. Clin Sarcoma Res. 2018;8(1):10.
95. Zeng FR, Zhou XY, Zeng LG, Sun JC, He F, Mo W, et al. Identification of key genes and pathway related to chemoresistance of small cell lung cancer through an integrative bioinformatics analysis. Ann Transl Med. 2022;10(18):968.
96. Yang Y, Tang X, Song X, Tang L, Cao Y, Liu X, et al. Evidence for an oncogenic role of hoxc6 in human non-small cell lung cancer. PeerJ. 2019;7:e6629.
97. Roška J, Wachsmannová L, Hurbanová L, Šestáková Z, Mueller T, Jurkovičová D, et al. Differential gene expression in cisplatin-resistant and -sensitive testicular germ cell tumor cell lines. Oncotarget. 2020;11(51):4735–53.
98. Yang D, Li Q, Shang R, Yao L, Wu L, Zhang M, et al. WNT4 secreted by tumor tissues promotes tumor progression in colorectal cancer by activation of the Wnt/β-catenin signalling pathway. J Exp Clin Cancer Res. 2020;39(1):251.
99. Ying J, Li H, Yu J, Ka MN, Fan FP, Wong SCC, et al. WNT5A exhibits tumor-suppressive activity through antagonizing the Wnt/β-catenin signaling, and is frequently methylated in colorectal cancer. Clin Cancer Res. 2008;14(1):55–61.

100. Nie X, Xia F, Liu Y, Zhou Y, Ye W, Hean P, et al. Downregulation of WNT3 suppresses colorectal cancer development through inhibiting cell proliferation and migration. Front Pharmacol. 2019;10:1110.
101. He B, Reguart N, You L, Mazieres J, Xu Z, Lee AY, et al. Blockade of Wnt-1 signaling induces apoptosis in human colorectal cancer cells containing downstream mutations. Oncogene. 2005;24(18):3054–8.
102. Galbraith RL, Poole EM, Duggan D, Muehling J, Hsu L, Makar K, et al. Polymorphisms in WNT6 and WNT10A and colorectal adenoma risk. Nutr Cancer. 2011;63(4):558–64.
103. Farkas SA, Vymetalkova V, Vodickova L, Vodicka P, Nilsson TK. DNA methylation changes in genes frequently mutated in sporadic colorectal cancer and in the DNA repair and Wnt/β-catenin signaling pathway genes. Epigenomics. 2014;6(2):179–91.
104. Yang Y, Gu X, Li Z, Zheng C, Wang Z, Zhou M, et al. Whole-exome sequencing of rectal cancer identifies locally recurrent mutations in the Wnt pathway. Aging. 2021;13(19):23262–83.
105. Goessling W, North TE, Loewer S, Lord AM, Lee S, Stoick-Cooper CL, et al. Genetic Interaction of PGE2 and Wnt signaling regulates developmental specification of stem cells and regeneration. Cell. 2009;136(6):1136–47.
106. Zhang H, Chi J, Hu J, Ji T, Luo Z, Zhou C, et al. Intracellular AGR2 transduces PGE2 stimuli to promote epithelial–mesenchymal transition and metastasis of colorectal cancer. Cancer Lett. 2021;518:180–95.
107. Wu L, Zhou Z, Han S, Chen J, Liu Z, Zhang X, et al. PLAGL2 promotes epithelial–mesenchymal transition and mediates colorectal cancer metastasis via β-catenin-dependent regulation of ZEB1. Br J Cancer. 2020;122(4):578–89.
108. Han Y, Cai H, Ma L, Ding Y, Tan X, Liu Y, et al. Nuclear orphan receptor NR4A2 confers chemoresistance and predicts unfavorable prognosis of colorectal carcinoma patients who received postoperative chemotherapy. Eur J Cancer. 2013;49(16):3420–30.
109. Zhou H, Liu Z, Wang Y, Wen X, Amador EH, Yuan L, et al. Colorectal liver metastasis: molecular mechanism and interventional therapy. Signal Transduct Target Ther. 2022;7(1):70.
110. Kurayoshi M, Oue N, Yamamoto H, Kishida M, Inoue A, Asahara T, et al. Expression of Wnt-5a is correlated with aggressiveness of gastric cancer by stimulating cell migration and invasion. Cancer Res. 2006;66(21):10439–48.
111. Gao Q, Yang L, Shen A, Li Y, Li Y, Hu S, et al. A WNT7B-m^{6}A-TCF7L2 positive feedback loop promotes gastric cancer progression and metastasis. Signal Transduct Target Ther. 2021;6(1):43.
112. Zhu Y, Zhang B, Gong A, Fu H, Zhang X, Shi H, et al. Anti-cancer drug 3,3′-diindolylmethane activates Wnt4 signaling to enhance gastric cancer cell stemness and tumorigenesis. Oncotarget. 2016;7(13):16311–24.
113. Lu J, Xu Y, Xie W, Tang Y, Zhang H, Wang B, et al. Long noncoding RNA DLGAP1-AS2 facilitates Wnt1 transcription through physically interacting with Six3 and drives the malignancy of gastric cancer. Cell Death Discov. 2021;7(1):255.
114. Bist A, Fielding CJ, Fielding PE. p53 regulates caveolin gene transcription, cell cholesterol, and growth by a novel mechanism. Biochemistry. 2000;39(8):1966–72.
115. Li Q, Lin S, Wang X, Lian G, Lu Z, Guo H, et al. Axin determines cell fate by controlling the p53 activation threshold after DNA damage. Nat Cell Biol. 2009;11(9):1128–34.
116. Lee KH, Li M, Michalowski AM, Zhang X, Liao H, Chen L, et al. A genomewide study identifies the Wnt signaling pathway as a major target of p53 in murine embryonic stem cells. Proc Natl Acad Sci. 2010;107(1):69–74.
117. Ain Q ul, Seemab U, Nawaz S, Rashid S. Integrative analyses of conserved WNT clusters and their co-operative behaviour in human breast cancer. Bioinformation. 2011;7(7):339–46.
118. Sidiropoulos KG, Ding Q, Pampalakis G, White NMA, Boulos P, Sotiropoulou G, et al. KLK6-regulated miRNA networks activate oncogenic pathways in breast cancer subtypes. Mol Oncol. 2016;10(7):993–1007.
119. Zhang N, Wei P, Gong A, Chiu WT, Te LH, Colman H, et al. FoxM1 promotes β-catenin nuclear localization and controls Wnt target-gene expression and glioma tumorigenesis. Cancer Cell. 2011;20(4):427–42.
120. Ma Q, Yang Y, Feng D, Zheng S, Meng R, Fa P, et al. MAGI3 negatively regulates Wnt/β-catenin signaling and suppresses malignant phenotypes of glioma cells. Oncotarget. 2015;6(34):35851–65.
121. Bao Z, Xu X, Liu Y, Chao H, Lin C, Li Z, et al. CBX7 negatively regulates migration and invasion in glioma via Wnt/β-catenin pathway inactivation. Oncotarget. 2017;8(24):39048–63.
122. Huang T, Alvarez AA, Pangeni RP, Horbinski CM, Lu S, Kim SH, et al. A regulatory circuit of miR-125b/miR-20b and Wnt signalling controls glioblastoma phenotypes through FZD6-modulated pathways. Nat Commun. 2016;7:12885.
123. Hao J, Han X, Huang H, Yu X, Fang J, Zhao J, et al. Sema3C signaling is an alternative activator of the canonical WNT pathway in glioblastoma. Nat Commun. 2023;14(1):2262.
124. Kaur N, Chettiar S, Rathod S, Rath P, Muzumdar D, Shaikh ML, et al. Wnt3a mediated activation of Wnt/β-catenin signaling promotes tumor progression in glioblastoma. Mol Cell Neurosci. 2013;54:44–57.
125. Riganti C, Salaroglio IC, Caldera V, Campia I, Kopecka J, Mellai M, et al. Temozolomide downregulates P-glycoprotein expression in glioblastoma stem cells by interfering with the Wnt3a/glycogen synthase-3 kinase/β-catenin pathway. Neuro Oncol. 2013;15(11):1502–17.
126. Hu B, Wang Q, Wang YA, Hua S, Sauvé CEG, Ong D, et al. Epigenetic activation of WNT5A drives glioblastoma stem cell differentiation and invasive growth. Cell. 2016;167(5):1281–95.e18.
127. Yu JM, Jun ES, Jung JS, Suh SY, Han JY, Kim JY, et al. Role of Wnt5a in the proliferation of human glioblastoma cells. Cancer Lett. 2007;257(2):172–81.
128. Binda E, Visioli A, Giani F, Trivieri N, Palumbo O, Restelli S, et al. Wnt5a drives an invasive phenotype in human glioblastoma stem-like cells. Cancer Res. 2017;77(4):996–1007.
129. Ordway JM, Bedell JA, Citek RW, Nunberg A, Garrido A, Kendall R, et al. Comprehensive DNA methylation profiling in a human cancer genome identifies novel epigenetic targets. Carcinogenesis. 2006;27(12):2409–23.
130. Costa BM, Smith JS, Chen Y, Chen J, Phillips HS, Aldape KD, et al. Reversing HOXA9 oncogene activation by PI3K inhibition : epigenetic mechanism and prognostic significance in human glioblastoma. Cancer Res. 2010;70(2):453–62.
131. Xavier-Magalhães A, Gonçalves CS, Fogli A, Lourenço T, Pojo M, Pereira B, et al. The long non-coding RNA HOTAIR is transcriptionally activated by HOXA9 and is an independent prognostic marker in patients with malignant glioma. Oncotarget. 2018;9(21):15740–56.
132. Pojo M, Gonçalves CS, Xavier-Magalhães A, Oliveira AI, Gonçalves T, Correia S, et al. A transcriptomic signature mediated by HOXA9 promotes human glioblastoma initiation, aggressiveness and resistance to temozolomide. Oncotarget. 2015;6(10):7657–74.
133. Fan H, Yuan J, Li X, Ma Y, Wang X, Xu B, et al. LncRNA LINC00173 enhances triple-negative breast cancer progression by suppressing miR-490-3p expression. Biomed Pharmacother. 2020;125:109987.
134. Yu Y, Lu X, Yang C, Yin F. Long noncoding RNA LINC00173 contributes to the growth, invasiveness and chemo-resistance of colorectal cancer through regulating MiR-765/PLP2 axis. Cancer Manag Res. 2020;12:3363–9.
135. Chen J, Liu A, Wang Z, Wang B, Chai X, Lu W, et al. LINC00173.v1 promotes angiogenesis and progression of lung squamous cell carcinoma by sponging miR-511–5p to regulate VEGFA expression. Mol Cancer. 2020;19(1):98
136. Hu CH, Yang XJ, Yu L, Wang LY, Zhao XC, Han CH. Long non-coding RNA LINC00173 serves as sponge for miR-338–3p to promote prostate cancer progression via regulating Rab25. Eur Rev Med Pharmacol Sci. 2020;24(18):9290–302.
137. Zeng F, Wang Q, Wang S, Liang S, Huang W, Guo Y, et al. Linc00173 promotes chemoresistance and progression of small cell lung cancer by sponging miR-218 to regulate Etk expression. Oncogene. 2020;39(2):293–307.
138. Wagner AH, Devarakonda S, Skidmore ZL, Krysiak K, Ramu A, Trani L, et al. Recurrent WNT pathway alterations are frequent in relapsed small cell lung cancer. Nat Commun. 2018;9(1):3787.
139. Yoshioka S, King ML, Ran S, Okuda H, MacLean JA, McAsey ME, et al. WNT7A regulates tumor growth and progression in ovarian cancer through the WNT/β-catenin pathway. Mol Cancer Res. 2012;10(3):469–82.
140. Qi H, Sun B, Zhao X, Du J, Gu Q, Liu Y, et al. Wnt5a promotes vasculogenic mimicry and epithelial-mesenchymal transition via protein kinase Cα in epithelial ovarian cancer. Oncol Rep. 2014;32(2):771–9.

141. Asem M, Young AM, Oyama C, De La Zerda AC, Liu Y, Yang J, et al. Host Wnt5a potentiates microenvironmental regulation of ovarian cancer metastasis. Cancer Res. 2020;80(5):1156–70.
142. Pannequin J, Bonnans C, Delaunay N, Ryan J, Bourgaux JF, Joubert D, et al. The Wnt target jagged-1 mediates the activation of notch signaling by progastrin in human colorectal cancer cells. Cancer Res. 2009;69(15):6065–73.
143. Camps J, Pitt JJ, Emons G, Hummon AB, Case CM, Grade M, et al. Genetic amplification of the NOTCH modulator LNX2 upregulates the WNT/β-catenin pathway in colorectal cancer. Cancer Res. 2013;73(6):2003–13.
144. Wang R, Sun Q, Wang P, Liu M, Xiong S, Luo J, et al. Notch and Wnt/β-catenin signaling pathway play important roles in activating liver cancer stem cells. Oncotarget. 2016;7(5):5754–68.
145. Fendler A, Bauer D, Busch J, Jung K, Wulf-Goldenberg A, Kunz S, et al. Inhibiting WNT and NOTCH in renal cancer stem cells and the implications for human patients. Nat Commun. 2020;11(1):929.
146. Janku F, de Vos F, de Miguel M, Forde P, Ribas A, Nagasaka M, et al. Abstract CT034: Phase I study of WNT974 + spartalizumab in patients (pts) with advanced solid tumors. Cancer Res. 2020;80(16_Supplement):CT034.
147. Chen B, Dodge ME, Tang W, Lu J, Ma Z, Fan CW, et al. Small molecule-mediated disruption of Wnt-dependent signaling in tissue regeneration and cancer. Nat Chem Biol. 2009;5(2):100–7.
148. Wang X, Moon J, Dodge ME, Pan X, Zhang L, Hanson JM, et al. The development of highly potent inhibitors for porcupine. J Med Chem. 2013;56(6):2700–4.
149. Madan B, Ke Z, Harmston N, Ho SY, Frois AO, Alam J, et al. Wnt addiction of genetically defined cancers reversed by PORCN inhibition. Oncogene. 2016;35(17):2197–207.
150. Proffitt KD, Madan B, Ke Z, Pendharkar V, Ding L, Lee MA, et al. Pharmacological inhibition of the Wnt acyltransferase PORCN prevents growth of WNT-driven mammary cancer. Cancer Res. 2013;73(2):502–7.
151. Liu J, Pan S, Hsieh MH, Ng N, Sun F, Wang T, et al. Targeting Wnt-driven cancer through the inhibition of Porcupine by LGK974. Proc Natl Acad Sci. 2013;110(50):20224–9.
152. Rodon J, Argilés G, Connolly RM, Vaishampayan U, de Jonge M, Garralda E, et al. Phase 1 study of single-agent WNT974, a first-in-class Porcupine inhibitor, in patients with advanced solid tumours. Br J Cancer. 2021;125(1):28–37.
153. Tabernero J, Van Cutsem E, Garralda E, Tai D, De Braud F, Geva R, et al. A phase Ib/II Study of WNT974 + Encorafenib + Cetuximab in patients With BRAF V600E-Mutant KRAS wild-type metastatic colorectal cancer. Oncologist. 2023;28(3):230–8.
154. Nile AH, De Sousa E Melo F, Mukund S, Piskol R, Hansen S, Zhou L, et al. A selective peptide inhibitor of Frizzled 7 receptors disrupts intestinal stem cells. Nat Chem Biol. 2018;14(6):582–90.
155. Gurney A, Axelrod F, Bond CJ, Cain J, Chartier C, Donigan L, et al. Wnt pathway inhibition via the targeting of Frizzled receptors results in decreased growth and tumorigenicity of human tumors. Proc Natl Acad Sci. 2012;109(29):11717–22.
156. Giraudet AL, Cassier PA, Iwao-Fukukawa C, Garin G, Badel JN, Kryza D, et al. A first-in-human study investigating biodistribution, safety and recommended dose of a new radiolabeled MAb targeting FZD10 in metastatic synovial sarcoma patients. BMC Cancer. 2018;18(1):646.
157. Jimeno A, Gordon M, Chugh R, Messersmith W, Mendelson D, Dupont J, et al. A first-in-human phase I study of the anticancer stem cell agent ipafricept (OMP-54F28), a decoy receptor for wnt ligands, in patients with advanced solid tumors. Clin Cancer Res. 2017;23(24):7490–7.
158. Diamond JR, Becerra C, Richards D, Mita A, Osborne C, O'Shaughnessy J, et al. Phase Ib clinical trial of the anti-frizzled antibody vantictumab (OMP-18R5) plus paclitaxel in patients with locally advanced or metastatic HER2-negative breast cancer. Breast Cancer Res Treat. 2020;184(1):53–62.
159. Moore KN, Gunderson CC, Sabbatini P, McMeekin DS, Mantia-Smaldone G, Burger RA, et al. A phase 1b dose escalation study of ipafricept (OMP—54F28) in combination with paclitaxel and carboplatin in patients with recurrent platinum-sensitive ovarian cancer. Gynecol Oncol. 2019;154(2):294–301.
160. Dotan E, Cardin DB, Lenz HJ, Messersmith W, O'Neil B, Cohen SJ, et al. Phase Ib study of wnt inhibitor ipafricept with gemcitabine and nab-paclitaxel in patients with previously untreated stage IV pancreatic cancer. Clin Cancer Res. 2020;26(20):5348–57.
161. Davis SL, Cardin DB, Shahda S, Lenz HJ, Dotan E, O'Neil BH, et al. A phase 1b dose escalation study of Wnt pathway inhibitor vantictumab in combination with nab-paclitaxel and gemcitabine in patients with previously untreated metastatic pancreatic cancer. Invest New Drugs. 2020;38(3):821–30.
162. Shan J, Shi DL, Wang J, Zheng J. Identification of a specific inhibitor of the Dishevelled PDZ domain. Biochemistry. 2005;44(47):15495–503.
163. Huang SMA, Mishina YM, Liu S, Cheung A, Stegmeier F, Michaud GA, et al. Tankyrase inhibition stabilizes axin and antagonizes Wnt signalling. Nature. 2009;461(7264):614–20.
164. Thorne CA, Hanson AJ, Schneider J, Tahinci E, Orton D, Cselenyi CS, et al. Small-molecule inhibition of Wnt signaling through activation of casein kinase 1α. Nat Chem Biol. 2010;6(11):829–36.
165. Lepourcelet M, Chen YNP, France DS, Wang H, Crews P, Petersen F, et al. Small-molecule antagonists of the oncogenic Tcf/β-catenin protein complex. Cancer Cell. 2004;5(1):91–102.
166. Chen Z, Venkatesan AM, Dehnhardt CM, Dos SO, Santos ED, Ayral-Kaloustian S, et al. 2,4-Diamino-quinazolines as inhibitors of β-catenin/Tcf-4 pathway: potential treatment for colorectal cancer. Bioorg Med Chem Lett. 2009;19(17):4980–3.
167. Emami KH, Nguyen C, Ma H, Kim DH, Jeong KW, Eguchi M, et al. A small molecule inhibitor of beta-catenin/CREB-binding protein transcription. Proc Natl Acad Sci. 2004;101(34):12682–7.
168. Gang EJ, Hsieh YT, Pham J, Zhao Y, Nguyen C, Huantes S, et al. Small-molecule inhibition of CBP/catenin interactions eliminates drug-resistant clones in acute lymphoblastic leukemia. Oncogene. 2014;33(17):2169–78.
169. Fiskus W, Smith J, Mudunuru U, Hembruff S, Reyes R, Abhyankar S, et al. Abstract C144: Treatment with β-catenin antagonist BC2059 exhibits single agent efficacy and exerts superior activity with tyrosine kinase inhibitor (TKI) or histone deacetylase (HDAC) inhibitor against human AML, CML, and myeloproliferative neoplasm (MPN) progenitor cells. Mol Cancer Ther. 2011;10(11_Supplement):C144.
170. Lenz HJ, Kahn M. Safely targeting cancer stem cells via selective catenin coactivator antagonism. Cancer Sci. 2014;105(9):1087–92.
171. Lee NK, Zhang Y, Su Y, Bidlingmaier S, Sherbenou DW, Ha KD, et al. Cell-type specific potent Wnt signaling blockade by bispecific antibody. Sci Rep. 2018;8(1):766.
172. Mullard A. FDA approves first topical gene therapy. Nat Rev Drug Discov. 2023;22:526–7.
173. Mullard A. FDA approves first haemophilia B gene therapy. Nat Rev Drug Discov. 2023;22:6–7.
174. Subramanian A, Tamayo P, Mootha VK, Mukherjee S, Ebert BL, Gillette MA, et al. Gene set enrichment analysis: a knowledge-based approach for interpreting genome-wide expression profiles. Proc Natl Acad Sci. 2005;102(43):15545–50.

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