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PATHOBIOLOGY IN FOCUS

WNT signaling in glioblastoma and therapeutic opportunities

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WNTs and their downstream effectors regulate proliferation, death, and migration and cell fate decision. Deregulation of WNT signaling is associated with various cancers including GBM, which is the most malignant primary brain cancer. In this review, we will summarize the experimental evidence supporting oncogenic roles of WNT signaling in GBM and discuss current progress in the targeting of WNT signaling as an anti-cancer approach. In particular, we will focus on (1) genetic and epigenetic alterations that lead to aberrant WNT pathway activation in GBM, (2) WNT-mediated control of GBM stem cell maintenance and invasion, and (3) cross-talk between WNT and other signaling pathways in GBM. We will then review the discovery of agents that can inhibit WNT signaling in preclinical models and the current status of human clinical trials.
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WNT signaling has crucial roles in controlling self-renewal and differentiation during central nervous system (CNS) development. Neural stem cells (NSCs) are a central component of the CNS, and are located in the fetal ventricular zone, the postnatal subventricular zone, and the hippocampus. WNT signaling is required for the development of NSCs.[1,2] Aberrant activation of WNT signaling in NSCs leads to malignant transformation and development of brain tumors.[1,3,4] For example, WNT3A was demonstrated to upregulate WNT signaling activity and increase the clonogenic potential of NSCs.[1] In addition, constitutive activation of β-catenin increased the proliferation of mouse neural progenitor cells *in vivo*, whereas deletion of β-catenin decreased their proliferation.[5,6] Collectively, these studies indicate roles for WNT signaling in NSC self-renewal and proliferation.

Glioblastoma (GBM) has been designated by the World Health Organization as a grade IV cancer, and is the most common and lethal CNS tumor in adults.[7,8] Currently, the standard-of-care treatment for GBM patients consists of maximal surgical resection followed by concurrent irradiation and chemotherapy.[9] Temozolomide (Temodal), a DNA alkylating agent, is the most commonly used chemotherapeutic agent. Despite these therapies, most patients eventually relapse. There is therefore an urgent clinical need for the development of effective anti-GBM therapeutics.

The prognosis for GBM patients is uniformly poor. GBM tumors harbor a profound degree of heterogeneity; inter- and intra-tumoral heterogeneity of GBM can be attributed to genomic and molecular diversity of tumors, as well as cellular hierarchy. Recent large-scale genomic studies have provided comprehensive genetic and molecular profiles of GBM.[8,10,11] Prominent genomic alterations frequently found in GBM include loss-of-function of tumor suppressors in the p53, phosphatase and tensin homolog and neurofibromatosis 1, and hyperactivation of receptor tyrosine kinase (RTK) signaling, including epidermal growth factor receptor (EGFR), platelet-derived growth factor receptor, and the receptor for hepatocyte growth factor (MET).[8,10–12] In addition, molecular subtypes of GBM have been identified, largely based on the expression profiling analyses of GBM specimens. The most robust GBM subtypes that have consistently been identified in multiple studies are the proneural and mesenchymal subtypes.[10,13–15] On the other hand, GBM also appears to have a cellular hierarchy, whereby there exists a subpopulation of GBM cells that are enriched with the capacity for tumor initiation and propagation, and these cells drive tumor growth and treatment resistance.[16–19]

A large number of studies have suggested that WNT signaling is aberrantly activated in GBM and that it promotes GBM growth and invasion via the maintenance of stem cell properties.[20–23] Here, we will review recent studies from the

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literature that have described the functions of WNT/β-catenin signaling in development and cancer, with particular emphasis on[24–29] genetic and epigenetic alterations that lead to aberrant WNT pathway activation in GBM. We will then discuss the development of therapeutic approaches based on the inhibition of WNT activation and the current status of clinical trials based on agents targeting the WNT pathway.

OVERVIEW OF WNT SIGNALING

WNT proteins are a family of highly conserved secreted signaling molecules. Since the discovery of WNT signaling as an oncogene in mouse breast cancer models in 1982,[30] WNT signaling has emerged as a critical regulator of cell–cell interactions, cell fate decision, and migration. Mutations in WNT pathway components lead to specific developmental defects, whereas aberrant WNT signaling often leads to cancer. WNT proteins bind to receptors of the frizzled (FZD) and low-density lipoprotein receptor-related protein/alpha 2-macroglobulin receptor (LRP) families on the cell surface. Through several cytoplasmic components, the signal is transduced to β-catenin, which enters the nucleus and forms a complex with T-cell factor (TCF) to activate transcription of WNT target genes (canonical pathways). Non-canonical WNT pathways are β-catenin-independent, and are most often linked with the establishment of polarity and cytoskeleton-mediated processes. A simple diagrammatic overview of WNT signaling is shown in Figure 1.

Canonical WNT Signaling

The canonical WNT signaling cascade is a key regulator in embryonic and adult stem cells. This signaling is initiated by the binding of WNT ligands to cysteine-rich domains of the FZD and LRP families on the cell surface. Activation of these receptors leads to disassembly of the complex consisting of AXIN, adenomatous polyposis coli (APC), and GSK3β, thereby stabilizing β-catenin. As a result, β-catenin is translocated from the cytoplasm into the nucleus where it forms a complex with T-cell factor/lymphoid enhancer factor (TCF/LEF) and promotes transcription of multiple target genes including c-MYC and cyclin D1.[31,32] A recent report showed that FoxM1 promotes nuclear translocation and stabilization of β-catenin in GBM, via binding to cytoplasmic β-catenin, suggesting that FoxM1 can activate canonical WNT signaling in a ligand-independent manner.[33]

Non-Canonical WNT Pathway

Non-canonical WNT signaling, currently defined as the β-catenin-independent pathway, mainly affects cell polarity and WNT-Ca^{2+} pathways.[34–36] These pathways have been reported to contribute to developmental processes including planar cell polarity in *Drosophila*, convergent extension movements during gastrulation, and cell migration of neuronal and epithelial origin.[34,37,38] Binding of WNT ligands (WNT4, WNT5A, and WNT11) to the FZD receptor induces recruitment of Dishevelled (Dvl) and Dvl-associated activator of morphogenesis 1 (Daam1). This complex initiates a cascade that activates Rac and Rho GTPases to mediate asymmetric cytoskeletal organization and polarized cell migration. The other type of non-canonical WNT signaling is related to calcium signaling. Binding of WNT ligand to the FZD receptor promotes recruitment of Dvl in complex with a G-protein, resulting in G-protein-dependent release of Ca^{2+}. Intracellular calcium release activates protein kinase C and calmodulin-dependent protein kinase 2. Increased Ca^{2+} can stimulate the activation of calcineurin (Ca^{2+}-dependent serine/threonine phosphatase), leading to accumulation of nuclear factor of activated T cells in the nucleus.[39,40]

GENETIC/EPIGENETIC ALTERATIONS OF WNT SIGNALING COMPONENTS

As mentioned above, aberrant WNT pathway activation is found in various type of cancer including GBM. Mutations in WNT signaling components (APC, β-catenin, AXIN, WTX, TCF4) can be the cause of WNT pathway activation in these tumors.[41–45] In colorectal cancer, mutations in WNT signaling components have been extensively characterized. Approximately 85% of colorectal tumors have mutations in APC, whereas an activating mutation in β-catenin was observed in 50% of colorectal tumors lacking APC mutations.[46–48] APC is a negative regulator of WNT pathway activation. Accordingly, most APC mutations are loss-of-function mutations. Similar to colon cancer, mutations in WNT signaling components (β-catenin, APC, and AXIN1) have been identified in medulloblastoma (a brain tumor primarily originating in the cerebellum).[49–53] Recent large-scale genomic studies showed that β-catenin mutations in exon 3, corresponding to its phosphorylation site were found in 18–22% of medulloblastoma cases.[51,52] An additional 5% had mutations in APC or AXIN1.[52,53] β-Catenin mutations detected in hepatocellular carcinoma and medulloblastoma led to the disruption of phosphorylation and degradation of β-catenin, resulting in hyperactivation of WNT signaling.[43,44,54] Thus, the mutation status of the above WNT signaling components is an indicator of WNT activation in tumor.

In sharp contrast to colon cancer and medulloblastoma, no genomic mutations have been found in β-catenin and APC in GBM.[55,56] Recently, *Morris et al* identified a homozygous deletion of FAT Atypical Cadherin 1 (FAT1), a negative effector of WNT signaling, in GBM. Copy number loss of FAT1 was found in nearly 20% of GBMs; WNT signaling-associated genes were enriched in this subset of GBMs, suggesting that FAT1 loss is a critical molecular event for WNT activation in GBM. The frequency of FAT1-inactivating mutations in GBM is about 1%, according to TCGA data set analysis.

Epigenetic silencing of negative effectors of WNT pathways can activate WNT signaling and contribute to malignant behavior in GBM. Soluble Frizzled-related proteins (FRPs) are soluble proteins that bind to WNT and interfere with

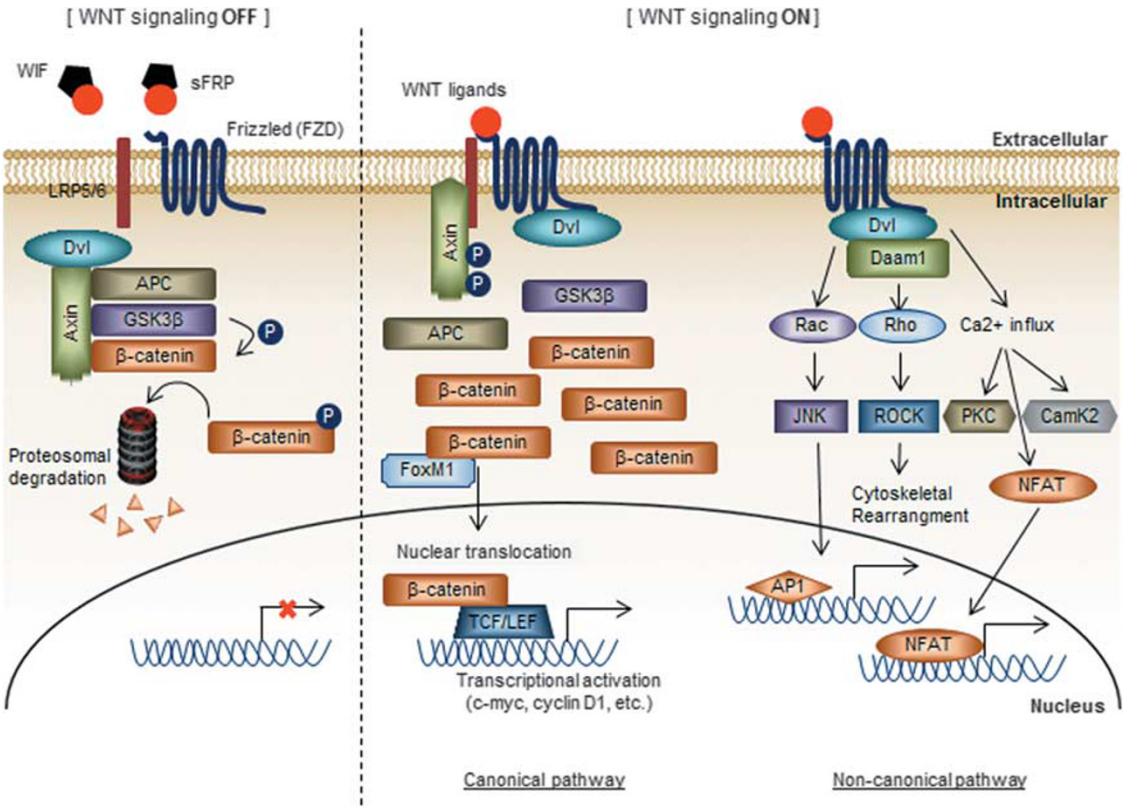

Figure 1 Overview of WNT signaling pathway. The WNT signaling pathway has crucial roles in cancer cells, which is shown as follows. (Left) WNT signaling is inactivated in the absence of WNT ligands. Under these conditions, β-catenin forms a complex with Dishevelled, AXIN, APC, and GSK3β. β-Catenin is phosphorylated by GSK3β and then degraded by the proteasome. (Middle) Canonical WNT signaling is depicted, ie, signaling dependent on β-catenin. Unphosphorylated β-catenin is shuttled into the nucleus, leading to transcriptional activation of WNT signaling-target genes. (Right) Non-canonical WNT signaling consists of the planar cell polarity (PCP) and Ca^{2+} pathways. The PCP signaling pathway has relevance for cell survival and skeletal rearrangement. The nuclear factor of activated T-cell-mediated Ca^{2+} signaling pathway is concerned with intracellular Ca^{2+} release and cell fate regulation.

WNT signaling. Dickkopf (DKK) acts as an antagonist of WNT signaling via binding to its co-receptor LRP.[57] Indeed, epigenetic silencing of WNT pathway inhibitor genes frequently occurs in gliomas, including promoter hypermethylation of sFRPs (sFRP1, sFRP2, sFRP4, sFRP5), Dickkopf (DKK1, DKK3) and Naked (NKD1, NKD2). In GBM, promoter hypermethylation of sFRP1, sFRP2 and NKD2 occurred in more than 40% of primary GBM specimens.[58] *Roth et al* reported the role of sFRP in the proliferation and migration of glioma cells.[58] In this study, ectopic expression of sFRP reduced glioma cell motility by decreasing MMP2. DKK1 promoter hypermethylation was identified in 50% of secondary GBM.[59,60] Collectively, these studies indicate that epigenetic alterations but not genomic mutations of WNT signaling components have major roles in WNT activation in GBM.

WNT SIGNALING IN GBM STEMNESS

WNT Signaling in Stem Cells

It is well-established that WNT signaling regulates stemness and stem cell niches in normal cells.[61,62] For instance, intestinal stem cells harboring a TCF4 mutation could not sustain self-renewal of stem cells, resulting in the regression of intestinal tissues.[61] In the hair follicle system, ectopic expression of DKK caused a deficit of hair follicles and mammary gland, indicating the role of WNT signaling in stem cell niches.[62] In contrast, activation of WNT signaling by forced expression of a mutant β-catenin increased stem cell pools in the hair follicle.[63]

The cancer stem cells (CSCs) model posits a cellular hierarchy, in which CSCs mainly drive tumor initiation and propagation. Some tumors may not follow the CSC model and there are ongoing controversies regarding the CSC immuno-phenotype, the reversibility of the stem cell state, and cell-of-origin.[17,64] Characteristics that are often associated with CSCs include the capacity for self-renewal, similarity with normal stem cells, and tumorigenicity *in vitro/vivo*.[65] Several studies have shown that inhibition of WNT signaling via modulation of β-catenin, LEF and TCF impeded the clonogenic growth of various cancer cells.[66–70] In addition, WNT inhibitory factor 1 (WIF1) induced cellular senescence, thereby impeding stemness and tumor growth.[67] As two

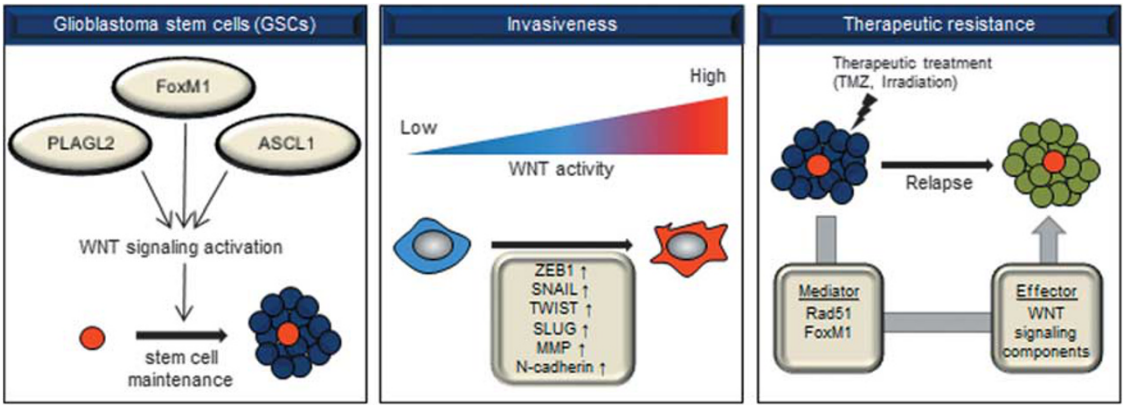

Figure 2 Multiple roles of WNT signaling. The role of WNT signaling in GBM is summarized. The roles of WNT signaling in GBM are categorized as follows: (1) maintenance of glioblastoma stem cells, (2) enhancement migration and invasion, and (3) induction of multi-drug resistance. WNT signaling regulators (such as PLAGL2, FoxM1, Evi/Gpr177, and ASCL1) lead to WNT signaling activation and thus increased self-renewal capacity (left). WNT signaling activation upregulates the expression of EMT-related genes (ie, ZEB1, SNAIL, TWIST, SLUG, MMP, and N-cadherin), resulting in enhanced the migration and invasion of GBM cells (middle). Despite chemo- and radiotherapy, upregulation of WNT signaling by a mediator (eg, DNA repair genes) promotes tumor regrowth and recurrence (right).

recent papers have provided excellent reviews of WNT signaling and CSCs,[71,72] this review focuses on studies conducted in GBM.

WNT Signaling in GBM Stem Cells (GSCs)

Despite controversies in some tumor models, numerous studies support the theory that GSCs are the critical cell population that contributes to GBM malignancy, therapeutic resistance to standard therapies, and recurrence[18,73,74] (Figure 2). Regulatory connections between WNT signaling and GSCs have been elucidated in the following studies. A study from *Depinho* group found that PLAGL2 on chromosome 20q11.21 is amplified in primary GBM specimens and GBM cell lines.[75] PLAGL2 maintained the self-renewal ability of GBM cells, while restraining NSC differentiation. Overexpression of PLAGL2 in astrocytes and GBM cells led to upregulation of WNT signaling components, including WNT6, FZD9, and FZD2. Thus, PLAGL2 appears to be important for stem cell maintenance and gliomagenesis via activation of canonical WNT signaling.[75,76]

Another recent report showed the involvement of WNT signaling in GSCs via FoxM1.[33] In this study, the authors showed that FoxM1 promotes β-catenin nuclear translocation by directly binding to β-catenin. Accordingly, the expression level of nuclear FoxM1 correlated with that of nuclear β-catenin in GBM patient specimens. High levels of FoxM1 in GSCs have also been reported elsewhere, in which FoxM1 was shown to be phosphorylated by MELK, a GSC-enriched kinase, and to promote self-renewal and chemo-resistance of GBM cells.[77] In addition, FoxM1 appears to selectively bind to the promoter of Sox2, a master regulator of GSC self-renewal, and promotes stem cell transcription programs in GSCs.[78]

Rheinbay *et al* performed a comparative analysis of chromatin state in GSCs compared with the entire tumor and identified a set of developmental transcription factors unique to GSCs. They found that a human achaete-scute homolog (ASCL1) activates WNT signaling in GSCs by repressing the negative regulator DKK1.[79] Given that aberrant WNT activation in GBM is mediated by epigenetic regulation rather than genetic mutations, genome-wide epigenetic profiles will likely yield more insights in stem cell controlled mechanisms including the WNT pathway in GSCs. In addition, Bartscherer *et al* found that a conserved seven-pass transmembrane protein, Evi, is involved in the secretion of WNT ligands in *Drosophila* and human cells, affecting both canonical and non-canonical WNT signaling pathways.[80–82] Moreover, they showed that Evi was strongly expressed in gliomas and that Evi depletion in glioma cell lines impeded cellular proliferation, clonogenic growth, and invasion.[81]

Other studies have shown that WNT signaling components such as Frizzled and Dishevelled 2 (Dvl2) are overexpressed in GBM, and that these genes promoted clonogenic growth and stem-like characteristics of GBM cells.[3,68] Although most studies have addressed canonical WNT signaling, several studies have indicated the involvement of both canonical and non-canonical WNT signaling.[22,83]

WNT SIGNALING IN GBM INVASION

Tumor metastasis is a major factor contributing to tumor-associated death. Epithelial–mesenchymal transition (EMT) is a critical process that enables cancer cells of epithelial origin to metastasize to distal organs. Unsurprisingly, WNT signaling is involved in both tumor invasion and EMT. Several studies have shown that WNT signaling activation enhances the motility of bladder, breast, and pancreatic cancer cells.[84–87] Overexpression of positive WNT signaling

regulators was found to increase the expression of EMT-associated genes, such as ZEB1, SNAIL, TWIST, SLUG, and N-cadherin, indicating the role of WNT in EMT[88–94] (Figure 2). For example, ectopic expression of a constitutively active β-catenin induced the expression of ZEB1 in GBM cells and increased cell motility.[14] Conversely, inhibition of β-catenin suppressed cellular invasion in U87MG and LN229 GBM cells.[95]

In addition, WNT5A was shown to induce migration in GBM cells by activating a β-catenin-independent pathway. WNT5A knockdown in glioma cells significantly inhibited the migratory capacity of these cells without affecting proliferation kinetics.[96] Consistent with this, expression of a recombinant WNT5A protein stimulated migration in GBM cells via increase of MMP2 activity.[96] Similar observations have been made using other WNT regulators such as WNT2 and FZD2.[96,97]

In comparison with other solid tumors, GBM rarely metastasizes to other. However, GBM tumor cells disseminate widely into the neighboring brain parenchyma. The invasive and infiltrative growth pattern of GBM makes it almost impossible to perform radical, maximal tumor resection. The involvement of WNT signaling activation in GBM invasiveness was shown in a recent report.[68] In this study, the authors enriched highly invasive GBM cell populations through serial *in vivo* transplantation assays and analyzed mRNA expression profiles of these populations. FZD4, a positive WNT regulator, was identified and shown to be a causative effector for invasive phenotypes of GBM cells.[68] Together, these findings collectively indicate that WNT signaling has critical roles in GBM invasion and provide a rationale for targeting WNT signaling as a potentially effective anti-GBM therapeutic approach.

WNT SIGNALING IN THERAPEUTIC RESISTANCE

Most cancers develop resistance to radiotherapy and chemotherapy. Several studies have suggested that the activation of WNT signaling induces drug resistance in various cancers, including ovarian, colon and pancreatic cancer[98–100] (Figure 2). For example, WNT5A was upregulated in oxaliplatin-resistant ovarian carcinoma cell line.[98] Ectopic expression of WNT5A conferred greater resistance of ovarian cancer cells to paclitaxel, 5-fluorouracil, epirubicin, and etoposide.[100] WNT5A activated Akt signaling and rendered colon cells resistant to histone deacetylase inhibitors.[101] Conversely, inhibition of WNT5A led to increased drug-induced apoptosis in pancreatic cancer.[102] In GBM, Auger *et al* reported that WNT signaling promotes resistance to temozolomide, a standard chemotherapeutic agent for GBM patients. Activation of WNT signaling components such as FZD2 was also demonstrated in temozolomide-resistant subclones.[103]

WNT signaling also contributes to radioresistance of cancer cells.[104–106] In breast cell models, stabilized β-catenin selectively reinforced mammosphere formation and enhanced radioresistance in the $Sca1^+$ subpopulation compared with the corresponding $Sca1^-$ cells.[105,106] TCF4 was also shown to be required for radioresistance of colorectal cancer cell lines.[107] In GBM, *Bao et al* showed that $CD133^+$ GSC-enriched cells were more resistant to irradiation than $CD133^-$ cells; that was due in part to the enhanced DNA repair capacities of $CD133^+$ cells.[108] These results imply that $CD133^+$ tumor cell population confers radioresistance to GBM and most likely contributes to GBM recurrence. Similarly, *Zheng et al* showed that FoxM1 promotes GBM resistance via upregulation of Rad51, a critical component of the DNA damage repair process.[109] Using *in vivo* orthotopic xenograft tumor models combined with *in vivo* irradiation, *Kim et al* have obtained gene signatures that are highly enriched in radioresistant GBM cells compared with the parental tumor cells.[110] Radioresistant GBM cells expressed high levels of WNT signaling-related genes, such as WISP1, FZD1, LEF1, TCF4, WNT9B, and AXIN2. Inhibition of the WNT pathway by XAV939, a WNT signaling inhibitor, sensitized GBM cells to irradiation.

CROSS-TALK BETWEEN WNT AND OTHER SIGNALING PATHWAYS IN GBM

RTKs promote GBM survival, proliferation, and invasion. Hyperactivation of RTK signaling because of genomic amplification and/or activating mutations of RTKs occurs in more than 90% of GBMs.[13,111] Amplifications or somatic mutations in EGFR, platelet-derived growth factor receptor, FGFR, and MET often correlate with GBM subtypes.[13] In this section, we will address the potential relationships between WNT signaling and these RTK signaling pathways (Figure 3).

Cross-talk with EGFR Signaling Pathway

EGFR amplification and hyperactivation were observed in 60% of GBM patients.[112–115] Several activating mutations, most notably the EGFRvIII mutation, were also observed in GBM and are known to contribute to cancer development. Activation of EGFR induces downstream mitogenic signaling, such as the mitogen-activated protein kinase, phosphatidylinositol 3-kinase/Akt, and transducers and activators of transcription (STAT) pathways.[115,116]

Bioinformatic analysis with Search Tool for Retrieval of Interacting Genes/Proteins (STRING) indicated that β-catenin is associated with several genes, including Akt1, CCND1, JUN, tumor suppressors in the p53, and VEGFA.[95] Moreover, multiple signaling pathways, including the mitogen-activated protein kinase, insulin, focal adhesion and adherens junction and ErbB pathways, were proposed as β-catenin-related pathways. Based on these analyses, several studies attempted to find a relationship between the EGFR and WNT pathways. One study showed that β-catenin inhibition in GBM cell lines (U87MG and LN229) led to downregulation of EGFR, STAT3, Akt1, MMP2 and MMP9, FRA-1, and c-MYC. In another study, TCF4 downregulation reduced Akt1 expression by binding to the Akt1 promoter,

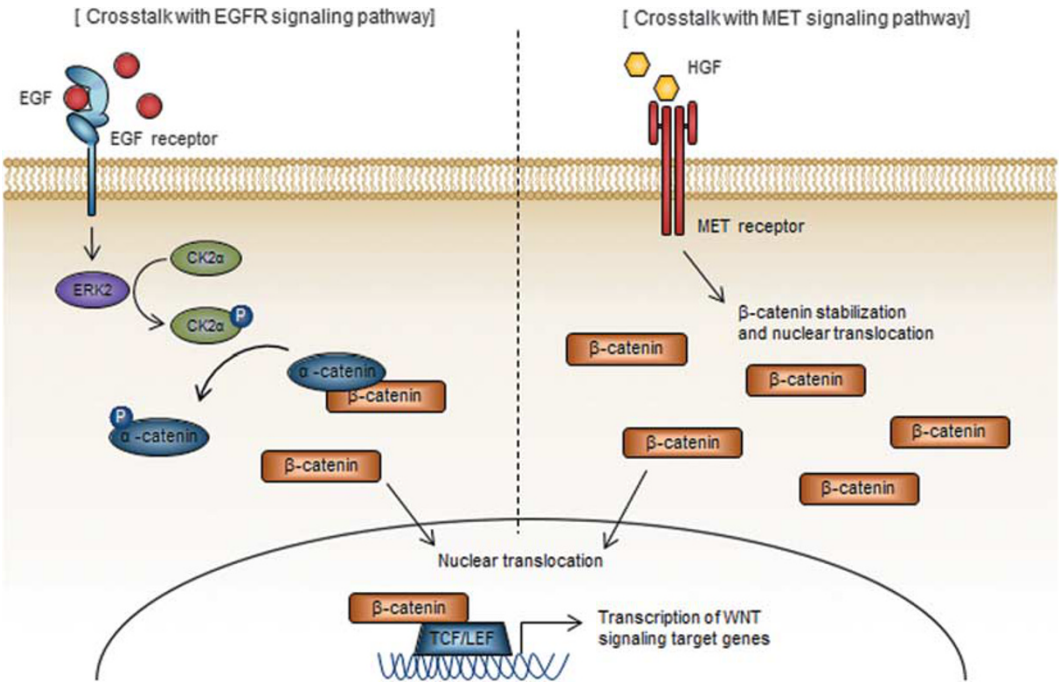

Figure 3 Cross-talk with other signaling pathways. Cross-talk with EGFR and MET signaling pathways are as follows. Ligand-mediated EGFR activation leads to CK2*α* phosphorylation, resulting in disassembly of *α*-catenin/*β*-catenin complex. (Left) Although the detailed mechanism is not precisely understood, it is known that MET activation increases the stability of *β*-catenin. The free cytoplasmic *β*-catenin is stabilized and translocates into the nucleus. Nuclear *β*-catenin binds to TCF/LEF transcription factor, which induces the expression of WNT signaling-target genes.

indicating a link between AKT signaling and the WNT pathway.[117]

Several reports have indicated that EGF signaling is an upstream regulator of the WNT pathway.[118,119] EGF-induced ERK2 upregulation resulted in phosphorylation of CK2a, and then CK2*α* with, and subsequent phosphorylation of, *α*-catenin at S641.[118] CK2*α*-mediated phosphorylation of *α*-catenin released *α*-catenin from binding to *β*-catenin, which led to shuttling of the latter into the nucleus where it formed a *β*-catenin/TCF/LEF complex. In addition, chronic EGF treatment resulted in downregulation of transcription of caveolin-1 and E-cadherin.[119] Loss of caveolin-1 induced *β*-catenin transactivation, whereas depletion of E-cadherin prevented cell–cell connection and induced EMT.

Cross-talk with MET Signaling Pathway

Hepatocyte growth factor receptor (MET) has crucial roles in cancer growth, stem cell maintenance, and metastasis.[120,121] In GBM, expression levels of MET correspond with poor patient survival and malignancy.[8,122] In addition, analyses of clinical GBM specimens revealed a positive association between MET expression and invasiveness-related genes (MMP2 and MMP9) and proto-oncogenes (c-MYC, KRAS, and JUN).[8]

Several lines of evidence suggest that the MET signaling pathway is connected to WNT signaling in cancer, although this cross-talk in GBM is not been yet fully understood. *Kim et al* showed that activation of MET signaling by the addition of HGF-induced nuclear translocation of *β*-catenin. Moreover, MET inhibition by small molecules led to blockade of *β*-catenin nuclear translocation and TCF/LEF promoter activity,[123] suggesting the possibility that MET signaling is an upstream regulator of WNT signaling.

Cross-talk with Sonic Hedgehog (SHH) Signaling Pathway

SHH signaling is a key pathway for cellular proliferation and tumorigenesis.[124–126] Molecular classification studies on medulloblastoma revealed that SHH and WNT are prominent signaling pathways that drive the formation of distinct tumor subgroups.[127,128] GLI1 in medulloblastoma cells physically interacted with *β*-catenin and led to its degradation, supporting the possibility that SHH and WNT may not be co-activated in these tumors.[129] Indeed, mutations in SHH signaling components (eg, PTCH1 and SUFU), which led to aberrant SHH signaling activation, were found in 30% of medulloblastoma patients.[130]

Although alterations of SHH signaling components and amplification of chromosome 12q region that contains GLI1 were rarely founded in gliomas,[131–135] activation of SHH pathways in GBM has been reported. For instance, blockade of SHH signaling with the chemical inhibitor *Vismodegib* induced cell cycle arrest and apoptosis, and downregulated GLI1 expression in patient-derived GBM cells.[136] Several studies have suggested that SHH signaling has a suppressive effect on WNT signaling.[129,137,138] For example, it was reported that GLI1 binds to the *sFRP1* promoter and increases

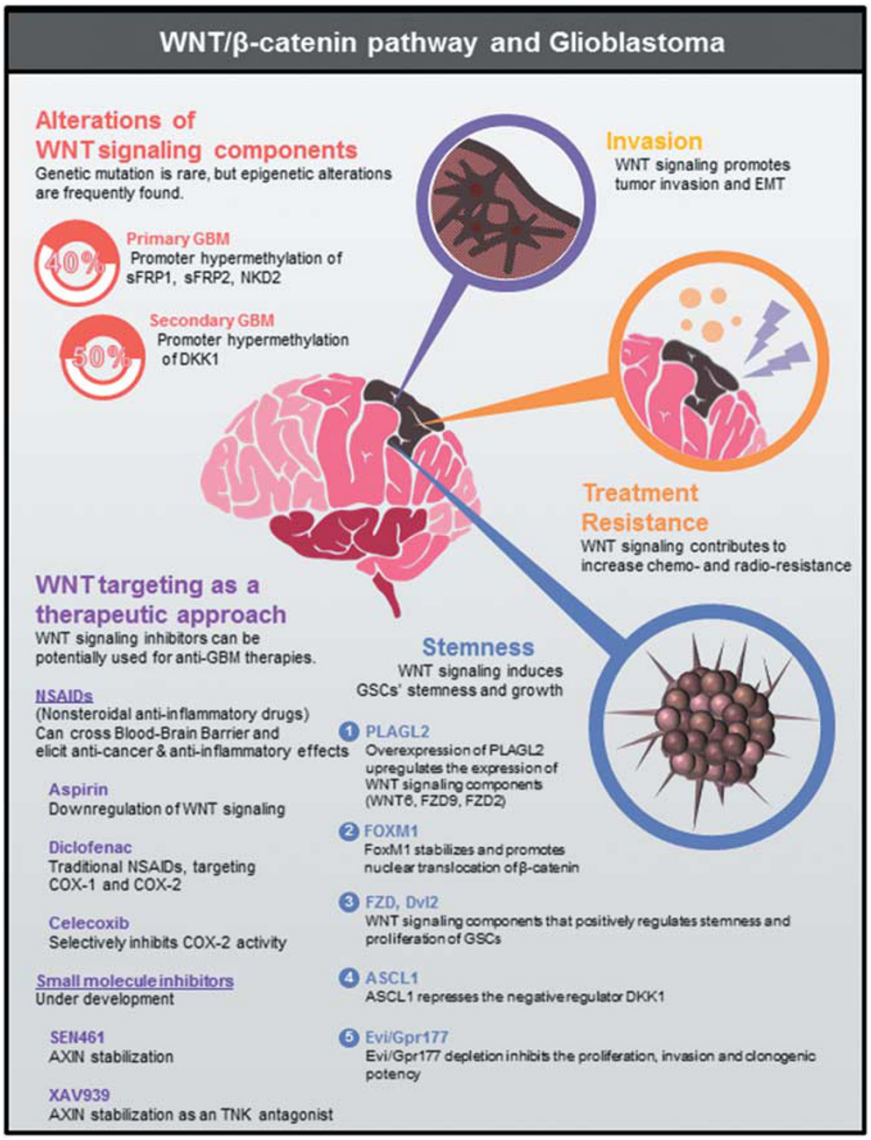

Figure 4 The multiple roles of WNT signaling in GBM. WNT signaling is one of the key signaling pathways in GBM. No genetic alterations of WNT signaling components were identified, whereas hypermethylation of WNT signaling repressors is observed in about 40–50% of GBM patients. WNT signaling has multiple roles during CNS development and gliomagenesis. The roles of WNT signaling are as follows: (1) stemness maintenance, (2) migration and invasion, and (3) induction of therapeutic resistance. Thus, therapeutic approaches that target WNT signaling will be important for eradication of GSCs and overcoming the resistance to standard therapies.

sFRP1 mRNA expression in GBM. Further studies are warranted to decipher the cross-talk between SHH signaling and WNT signaling in GBM.

TARGETING THE WNT SIGNALING PATHWAY IN GBM

Expression levels of WNT pathway genes have been found by multiple research groups to be associated with a poor prognosis in glioma patients. Through RT-PCR and immunohistochemical staining, expression levels of WNT components were analyzed.[31] mRNA expression of β-catenin, Dvl3, and cyclin D1 were significantly higher in glioma specimens compared with non-tumor brain tissue. Moreover, protein levels of β-catenin, TCF4, LEF1, c-MYC, n-MYC, c-JUN, and cyclin D1 were correlated positively with the degree of glioma. Among these components, β-catenin had a significantly positive correlation with TCF4 and LEF1. In a different study, expression of WNT1, β-catenin, and cyclin D1 was associated with malignancy and clinical outcomes of GBM patients.[32] Recent genomic studies have identified genetic and molecular heterogeneity between tumors and within GBM tumors. LEF1, a key effector of WNT signaling, appeared to regulate intra-tumoral heterogeneity, indicating a widespread interplay between this WNT signaling-related transcription factor and GBM driver pathways.[79,139]

Table 1 Expression of WNT signaling-related genes

	Cell or tissue	Method	Genes	Regulation	Reference
1	PLAGL2-overexpressing astrocyte and GBM cells with PLAGL2 amplification	RT-PCR	*WNT6*	Upregulation	75
			FZD9		
			FZD2		
2	NHA with ASCL1 overexpression, compared with NT	RT-PCR	*TLE1*	Upregulation	79
			AXIN2		
			DKK1	Downregulation	
			FZD5		
			LRG5		
			TCF7		
			TF7L1		
	GBM cells, compared with NHA	Microarray	*ASCL1*	Upregulation	
			DKK1	Downregulation	
3	Highly invasive GBM cells (U87R4), compared with non-invasive GBM cells (U87L4)	Microarray	*RANK1*	Upregulation	68
			DISC1		
			CD44		
			FZD4		
			Caspase3	Downregulation	
			SMAD6		
			MAML3		
			PDCD4		
4	GBM cells and U87MG with constitutive β-catenin S33Y	RT-PCR	*ZEB1*	Upregulation	14
			TWIST		
			N-cadherin		
			SNAIL		
5	Radioresistant U373, compared with NT	Microarray	*WISP1*	Upregulation	110
			FZD1		
			APC		
			LEF1		
			TCF4		
			CTNNBIP1		
			WNT9B	Downregulation	
			AXIN2		
6	U87 and LN229 cells with β-catenin downregulation	STRING analysis and RT-PCR	*AKT1*	Downregulation	95
			CTNND1		
			JUN		
			VEGFA		
	LN229 xenograft tumor from β-catenin siRNA-treated mice	IHC	*EGFR*	Downregulation	
			AKT (phospho-, total)		
			STAT3		
			MMP2		
			MMP9		
7	Patient-derived GBM cells with high MET expression, compared with MET low population	Microarray	*CD44*	Upregulation	123
			CCND1		
			TCF7		
			MYC		
			LEF1		

Gene expressions by regulating EMT, stemness and WNT-related genes are altered as follows.

Table 2 Clinical trials of candidate NSAIDs in human cancer

Compound	Tumor type	Identifier	Phase	Detailed description
Aspirin	Esophageal cancer	NCT02326779	3	Effect of low-dose aspirin on survival of esophageal cancer patients
	Colorectal cancer	NCT00224679	3	Efficacy test of regular low-dose aspirin in reducing the recurrent tumor of colorectal adenomatous polyps
		NCT00002527	3	Examination of chemo-preventive effect to reduce risk of colorectal cancer
		NCT02394769		
		NCT02125409	3	
		NCT00983580	2	
		NCT00062023	2	Safety/efficacy test of NSAIDs in colorectal cancer
	Breast cancer	NCT00727948		Examination of anti-angiogenic effect in breast cancer patients
		NCT01431053	2	Efficacy and safety test of aspirin as the adjuvant treatment in breast cancer patients
		NCT01612247	2/3	Safety/efficacy study of cyclophosphamide and methotrexate in combination with aspirin treatment in breast cancer
	Lung cancer	NCT01058902	3	Efficacy study of aspirin on survival of NSCLC
	Prostate cancer	NCT02420652	2	Examination of anti-proliferation effect in prostate cancer patients
		NCT00316927	3	Examination of anti-proliferation effect of locally advanced or metastatic prostate cancer
Diclofenac	Basal cell carcinoma	NCT01358045		Efficacy test of NSAIDs in basal cell carcinoma
	Breast cancer	NCT01596647	1	Examination of dovitinib–drug interaction effect on the pharmacokinetics of a cocktail including caffeine, diclofenac, omeprazole, and midazolam
Celecoxib	Head and neck cancer and Lung cancer	NCT00058006	2	Examination of chemo-preventive effect to reduce risk of recurrent cancer
		NCT00052611		
		NCT00527982		Examination of toxicity/efficacy test as adjuvant therapy
	Head and neck cancer	NCT00400374	1	Examination of tumor prevention effect on Erlotinib (OSI-774, Tarceva)
		NCT00061906	2	Safety/anti-proliferation efficacy study of celecoxib in differentated thyroid cadinoma
		NCT00581971	1b/2	Radiosensitizing effect with radiation treatment
	Pancreatic cancer	NCT00177853	1	Safety/efficacy study of celecoxib and irinotecan combination effect concurrent radiation treatment
	Esophageal cancer	NCT00137852	2	Safety/efficacy study of celecoxib treatment combined with irradiation
		NCT00520091		
	Glioblastoma	NCT00112502	2	Examination of anti-proliferation effect with radiation treatment

Clinical information pertaining to aspirin, diclofenac, and celecoxib in various cancer types is available at the following website: https://clinicaltrials.gov.

The above studies collectively indicate that WNT targeting can be an effective therapeutic approach against GBM (Table 1). WNT signaling inhibitors have been identified and demonstrated therapeutic efficacy in various human cancers.[140–149] However, relatively little is known about clinically applicable WNT inhibitors for the treatment of GBM. In this section, we introduce a list of WNT signaling inhibitors that can be potentially used for anti-GBM therapy. Several drugs targeting WNT signaling have been or are being developed for clinical trials. These drugs can be largely classified into three groups: (1) non-steroidal anti-inflammatory drugs, (2) small-molecule chemical inhibitors, and (3) therapeutic antibodies that target various WNT pathway components (Figure 4).

Nonsteroidal anti-inflammatory drugs (NSAIDs) have been used to treat treating inflammation, pain, and fever. NSAIDs inhibit the activity of the prostaglandin biosynthetic enzymes, the cyclooxygenase isoforms (COX-1 and COX-2). However, NSAIDs have shown anti-cancer effects as well as anti-inflammatory effects, and cross the blood–brain barrier efficiently.[150,151] Therefore, NSAIDs have attracted much attention as potential anti-cancer agents. (Table 2) Aspirin is a fat-soluble small molecule that is used to relieve pain. Several studies have proposed that aspirin inhibits the

Table 3 Experimental molecules targeting WNT signaling

Developmental agents	Type	Target	Tumor type	Detailed description	Reference
SEN461	Small-molecule inhibitor	AXIN	Glioblastoma	SEN461 protected AXIN degradation, causing β-catenin loss	158
XAV939	Small-molecule inhibitor	Tankyrase	Glioblastoma	XAV939 inhibited TNK degradation, increasing AXIN stability	110
Thiazolidinedione	Small-molecule inhibitor	β-Catenin	Colon cancer	Thiazolidinedione inhibited cellular proliferation and metastasis	144
ICG-001	Small-molecule inhibitor	β-Catenin	Colon cancer	ICG-001 downregulated survivin and cyclin D1 expression by disrupting the interaction between β-catenin and CBP	148
Artificial F box protein	Small-molecule inhibitor	β-catenin	Colorectal cancer	A chimeric F box protein (CFP) lead to *in vitro/vivo* growth inhibition by nuclear β-catenin destruction	149
	Small-molecule inhibitor	β-catenin	Colon cancer	A chimeric F box fusion protein reduced the β-catenin, which downregulated TCF/LEF promoter activity	147
Inhibitors of WNT response	Small-molecule inhibitor	AXIN2	Colorectal cancer	IWRs stabilized AXIN2 via interacting with AXIN2 protein, which induced β-catenin degradation	146
Inhibitors of WNT production	Small-molecule inhibitor	Porcupine	Colorectal cancer	IWPs was as a Porcn inhibitor, which blocked palmitoylation of WNT ligands	
FJ9	Small-molecule inhibitor	FZD7	Lung cancer	FJ9 inhibits the canonical WNT signaling, interrupting the interaction between FZD7 and Dvl.	143
WNT monoclonal antibodies	Monoclonal antibody	WNT1	Lung cancer	Anti-Wnt-1 suppressed cellular growth of lung cancer cells *in vitro* and *in vivo*	161
	Monoclonal antibody	WNT1	Colorectal cancer	WNT1 monoclonal antibody reduced the clonogenic potential and TCF/LEF promoter activity	140
	Monoclonal antibody	WNT2	Melanoma	WNT2 monoclonal antibody inhibited *in vitro/vivo* proliferation and WNT signaling activation, whereas it induced cellular apoptosis	141
pAb5a-5	Polyclonal antibody	WNT5A	Gastric cancer	pAb5a-5 inhibited migration of gastric cancer cells and WNT5A-dependent Rac1 activation	162
SFRP2 Mab	Monoclonal Antibody	SFRP2	Angiosarcoma, Breast cancer	SFRP2 antibody inhibited tumor growth and migration	163
Foxy-5	Peptide	FZD5	Murine breast cancer	Foxy-5 inhibited metastasis capacity of mouse breast cell lines	164
MAb92-13	Murine monoclonal antibody	FZD10	Synovial sarcoma	MAb92-13 bound to FZD10 and exhibited anti-tumor effect	165
TT641	Polyclonal	FZD10	Synovial sarcoma	TT641 decreased *in vivo/vitro* proliferation	142

Small molecules and antibodies targeting WNT signaling are listed, along with their mechanisms of action.

proliferation of cancer cell lines that do not express COX-1 and COX-2.[152] Previous studies have suggested that aspirin downregulates WNT signaling in colorectal cancer cells.[153] It has been confirmed that daily aspirin treatment for 5 years or longer reduces the risk of colon cancer.[154–156] In GBM, aspirin inhibited proliferation and invasiveness and increased apoptosis via G0/G1 arrest in U87MG and A172 cells. These effects were driven by downregulation of WNT signaling. Following treatment with aspirin, TCF/LEF promoter activity and expression of WNT signaling-target genes (c-MYC, Cyclin D1, and FRA-1) were decreased in GBM cell lines.[157] Diclofenac is one of the traditional NSAIDs and functions through inhibition of COX-1 and COX-2, whereas Celecoxib is a newly generated drug and selectively inhibits COX-2 activity. Treatment with these drugs reduced proliferation, colony formation, and migration of glioma cells.[158]

Recent chemical screening efforts have identified several small-molecule inhibitors and antibodies targeted at WNT signaling (Table 3).[159] A random selection of 16 000 small

molecules were used in the screening. SEN461 was selected as a potent WNT signaling inhibitor and validated the molecular mechanism of action. SEN416 prevented proteosomal degradation of AXIN. Through the stabilization of AXIN, cytoplasmic level of phosphorylated β-catenin were increased, accompanied by a loss of total β-catenin. Experimentally, SEN461 was largely responsible for growth inhibition by suppressing WNT signaling in GBM cells. XAV939 is an antagonist of Tankyrase (TRF-1, TNK) by inhibiting its interaction with AXIN and regulating its stability. TNK enzyme activity mediated AXIN ubiquitination and proteosomal degradation. XAV939 controlled WNT signaling by increasing AXIN stabilization.[160] *Kim et al* have shown that XAV939 potently inhibited WNT signaling in radioresistant-U373 GBM cells.[110] However, no clinical progress of SEN461 or XAV939 has been reported to date.

Antibodies targeting WNT signaling are categorized as follows: anti-ligand antibodies that trap and neutralize WNT ligands (WNT1, 2, 5A, and sFRP2)[140,141,161–163] and anti-FZD antibodies (FZD5 and FZD10).[142,164,165] Most antibodies suppressed *in vitro/in vivo* proliferation and migration of lung, colorectal, gastric, and breast cancer cells. To increase the ability of therapeutic antibodies to penetrate the blood–brain barrier, new approaches (ie, nanoparticle conjugation and antibody engineering) are under investigation in the realm of therapeutic antibody development.[166,167]

CLOSING REMARKS

WNT signaling contributes to GBM pathology at multiple levels including tumor initiation, maintenance of stem cell status, invasion, and therapeutic resistance. Although GBMs do not harbor genetic alterations in WNT signaling components, aberrant activation of WNT signaling appears to be achieved mainly by epigenetic silencing of negative WNT regulators and overexpression of positive regulators. Although WNT pathways have proven difficult to target, recent progress has been made in generating multiple agents that can potently inhibit WNT activation in preclinical models. Accumulating further data to support the crucial roles of WNT in GBM may increase the feasibility of WNT inhibition as a therapeutic approach to treat GBM patients.

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DISCLOSURE/CONFLICT OF INTEREST

The authors declare no conflict of interest.

1. Kalani MY, Cheshier SH, Cord BJ *et al.* Wnt-mediated self-renewal of neural stem/progenitor cells. Proc Natl Acad Sci USA 2008;105:16970–16975.
2. Nusse R. Wnt signaling and stem cell control. Cell Res 2008;18:523–527.
3. Holland EC. Gliomagenesis: genetic alterations and mouse models. Nat Rev Genet 2001;2:120–129.
4. Pulvirenti T, Van Der Heijden M, Droms LA *et al.* Dishevelled 2 signaling promotes self-renewal and tumorigenicity in human gliomas. Cancer Res 2011;71:7280–7290.
5. Chenn A, Walsh CA. Regulation of cerebral cortical size by control of cell cycle exit in neural precursors. Science 2002;297:365–369.
6. Zechner D, Fujita Y, Hulsken J *et al.* Beta-catenin signals regulate cell growth and the balance between progenitor cell expansion and differentiation in the nervous system. Dev Biol 2003;258:406–418.
7. Kleihues P, Louis DN, Scheithauer BW *et al.* The WHO classification of tumors of the nervous system. J Neuropathol Exp Neurol 2002;61:215–225.
8. Louis DN. Molecular pathology of malignant gliomas. Annu Rev Pathol 2006;1:97–117.
9. Stupp R, Mason WP, van den Bent MJ *et al.* Radiotherapy plus concomitant and adjuvant temozolomide for glioblastoma. N Engl J Med 2005;352:987–996.
10. Cancer Genome Atlas Research N. Comprehensive genomic characterization defines human glioblastoma genes and core pathways. Nature 2008;455:1061–1068.
11. Holland EC. Glioblastoma multiforme: the terminator. Proc Natl Acad Sci USA 2000;97:6242–6244.
12. Zhu Y, Parada LF. The molecular and genetic basis of neurological tumours. Nat Rev Cancer 2002;2:616–626.
13. Verhaak RG, Hoadley KA, Purdom E *et al.* Integrated genomic analysis identifies clinically relevant subtypes of glioblastoma characterized by abnormalities in Pdgfra, Idh1, Egfr, and Nf1. Cancer Cell 2010;17:98–110.
14. Kahlert UD, Maciaczyk D, Doostkam S *et al.* Activation of canonical Wnt/Beta-catenin signaling enhances in vitro motility of glioblastoma cells by activation of Zeb1 and other activators of epithelial-to-mesenchymal transition. Cancer Lett 2012;325:42–53.
15. Zhang JX, Zhang J, Yan W *et al.* Unique genome-wide map of Tcf4 and Stat3 targets using Chip-Seq reveals their association with new molecular subtypes of glioblastoma. Neuro Oncol 2013;15:279–289.
16. Seymour T, Nowak A, Kakulas F. Targeting aggressive cancer stem cells in glioblastoma. Front Oncol 2015;5:159.
17. Suva ML, Rheinbay E, Gillespie SM *et al.* Reconstructing and reprogramming the tumor-propagating potential of glioblastoma stem-like cells. Cell 2014;157:580–594.
18. Wang J, Ma Y, Cooper MK. Cancer stem cells in glioma: challenges and opportunities. Transl Cancer Res 2013;2:429–441.
19. Auffinger B, Spencer D, Pytel P *et al.* The role of glioma stem cells in chemotherapy resistance and glioblastoma multiforme recurrence. Expert Rev Neurother 2015;15:741–752.
20. Brennan C, Momota H, Hambardzumyan D *et al.* Glioblastoma subclasses can be defined by activity among signal transduction pathways and associated genomic alterations. PLoS One 2009;4:e7752.
21. Yu CY, Liang GB, Du P *et al.* Lgr4 promotes glioma cell proliferation through activation of Wnt signaling. Asian Pac J Cancer Prev 2013;14:4907–4911.
22. Gong A, Huang S. Foxm1 and Wnt/Beta-catenin signaling in glioma stem cells. Cancer Res 2012;72:5658–5662.
23. Zhang K, Zhang J, Han L *et al.* Wnt/Beta-catenin signaling in glioma. J Neuroimmune Pharmacol 2012;7:740–749.
24. Trevino M, Stefanik DJ, Rodriguez R *et al.* Induction of canonical wnt signaling by alsterpaullone is sufficient for oral tissue fate during regeneration and embryogenesis in Nematostella Vectensis. Dev Dyn 2011;240:2673–2679.
25. Sumiyoshi E, Takahashi S, Obata H *et al.* The beta-catenin Hmp-2 functions downstream of Src in parallel with the Wnt pathway in early embryogenesis of *C. Elegans*. Dev Biol 2011;355:302–312.
26. Nakamura T, Akiyama T. Role of the Wnt signaling network in embryogenesis and tumorigenesis. Seikagaku 2005;77:5–19.
27. Klaus A, Birchmeier W. Wnt signalling and its impact on development and cancer. Nat Rev Cancer 2008;8:387–398.
28. Brennan KR, Brown AM. Wnt proteins in mammary development and cancer. J Mammary Gland Biol Neoplasia 2004;9:119–131.
29. Smalley MJ, Dale TC. Wnt signalling in mammalian development and cancer. Cancer Metastasis Rev 1999;18:215–230.

30. 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;31:99–109.
31. Sareddy GR, Panigrahi M, Challa S *et al.* Activation of Wnt/Beta-Catenin/Tcf signaling pathway in human astrocytomas. Neurochem Int 2009;55:307–317.
32. Liu C, Tu Y, Sun X *et al.* Wnt/Beta-catenin pathway in human glioma: expression pattern and clinical/prognostic correlations. Clin Exp Med 2011;11:105–112.
33. Zhang N, Wei P, Gong A *et al.* Foxm1 promotes beta-catenin nuclear localization and controls Wnt target-gene expression and glioma tumorigenesis. Cancer Cell 2011;20:427–442.
34. Semenov MV, Habas R, Macdonald BT *et al.* Snapshot: noncanonical Wnt signaling pathways. Cell 2007;131:1378.
35. Cohen ED, Tian Y, Morrisey EE. Wnt signaling: an essential regulator of cardiovascular differentiation, morphogenesis and progenitor self-renewal. Development 2008;135:789–798.
36. Rao TP, Kuhl M. An updated overview on Wnt signaling pathways: a prelude for more. Circ Res 2010;106:1798–1806.
37. Simons M, Mlodzik M. Planar cell polarity signaling: from fly development to human disease. Annu Rev Genet 2008;42:517–540.
38. Kikuchi A, Yamamoto H, Sato A. Selective activation mechanisms of Wnt signaling pathways. Trends Cell Biol 2009;19:119–129.
39. Medyouf H, Ghysdael J. The Calcineurin/Nfat signaling pathway: a novel therapeutic target in leukemia and solid tumors. Cell Cycle 2008;7:297–303.
40. Hogan PG, Chen L, Nardone J *et al.* Transcriptional regulation by calcium, calcineurin, and Nfat. Genes Dev 2003;17:2205–2232.
41. Anastas JN, Moon RT. Wnt signalling pathways as therapeutic targets in cancer. Nat Rev Cancer 2013;13:11–26.
42. Kikuchi A. Tumor formation by genetic mutations in the components of the Wnt signaling pathway. Cancer Sci 2003;94:225–229.
43. Fattet S, Haberler C, Legoix P *et al.* Beta-catenin status in paediatric medulloblastomas: correlation of immunohistochemical expression with mutational status, genetic profiles, and clinical characteristics. J Pathol 2009;218:86–94.
44. Austinat M, Dunsch R, Wittekind C *et al.* Correlation between beta-catenin mutations and expression of wnt-signaling target genes in hepatocellular carcinoma. Mol Cancer 2008;7:21.
45. Kim YD, Park CH, Kim HS *et al.* Genetic alterations of wnt signaling pathway-associated genes in hepatocellular carcinoma. J Gastroenterol Hepatol 2008;23:110–118.
46. Sparks AB, Morin PJ, Vogelstein B *et al.* Mutational analysis of the Apc/Beta-Catenin/Tcf pathway in colorectal cancer. Cancer Res 1998;58:1130–1134.
47. Aust DE, Terdiman JP, Willenbucher RF *et al.* The Apc/Beta-Catenin pathway in ulcerative colitis-related colorectal carcinomas: a mutational analysis. Cancer 2002;94:1421–1427.
48. Sieber OM, Heinimann K, Gorman P *et al.* Analysis of chromosomal instability in human colorectal adenomas with two mutational hits at Apc. Proc Natl Acad Sci USA 2002;99:16910–16915.
49. Zurawel RH, Chiappa SA, Allen C *et al.* Sporadic medulloblastomas contain oncogenic beta-catenin mutations. Cancer Res 1998;58:896–899.
50. Eberhart CG, Tihan T, Burger PC. Nuclear localization and mutation of beta-catenin in medulloblastomas. J Neuropathol Exp Neurol 2000;59:333–337.
51. Yokota N, Nishizawa S, Ohta S *et al.* Role of Wnt pathway in medulloblastoma oncogenesis. Int J Cancer 2002;101:198–201.
52. Baeza N, Masuoka J, Kleihues P *et al.* Axin1 mutations but not deletions in cerebellar medulloblastomas. Oncogene 2003;22:632–636.
53. Silva R, Marie SK, Uno M *et al.* Ctnnb1, Axin1 and Apc expression analysis of different medulloblastoma variants. Clinics (Sao Paulo) 2013;68:167–172.
54. Polakis P. The many ways of Wnt in cancer. Curr Opin Genet Dev 2007;17:45–51.
55. Logan CY, Nusse R. The Wnt signaling pathway in development and disease. Annu Rev Cell Dev Biol 2004;20:781–810.
56. Reya T, Clevers H. Wnt signalling in stem cells and cancer. Nature 2005;434:843–850.
57. Shou J, Ali-Osman F, Multani AS *et al.* Human Dkk-1, a gene encoding a wnt antagonist, responds to dna damage and its overexpression sensitizes brain tumor cells to apoptosis following alkylation damage of DNA. Oncogene 2002;21:878–889.
58. Roth W, Wild-Bode C, Platten M *et al.* Secreted frizzled-related proteins inhibit motility and promote growth of human malignant glioma cells. Oncogene 2000;19:4210–4220.
59. Schiefer L, Visweswaran M, Perumal V *et al.* Epigenetic regulation of the secreted frizzled-related protein family in human glioblastoma multiforme. Cancer Gene Ther 2014;21:297–303.
60. Foltz G, Yoon JG, Lee H *et al.* Epigenetic regulation of wnt pathway antagonists in human glioblastoma Multiforme. Genes Cancer 2010;1:81–90.
61. Korinek V, Barker N, Moerer P *et al.* Depletion of epithelial stem-cell compartments in the small intestine of mice lacking Tcf-4. Nat Genet 1998;19:379–383.
62. Andl T, Reddy ST, Gaddapara T *et al.* Wnt signals are required for the initiation of hair follicle development. Dev Cell 2002;2:643–653.
63. Gat U, DasGupta R, Degenstein L *et al. De novo* hair follicle morphogenesis and hair tumors in mice expressing a truncated beta-catenin in skin. Cell 1998;95:605–614.
64. Facchino S, Abdouh M, Bernier G. Brain cancer stem cells: current status on glioblastoma multiforme. Cancers (Basel) 2011;3:1777–1797.
65. Gursel DB, Shin BJ, Burkhardt JK *et al.* Glioblastoma stem-like cells-biology and therapeutic implications. Cancers (Basel) 2011;3:2655–2666.
66. Kanwar SS, Yu Y, Nautiyal J *et al.* The Wnt/Beta-catenin pathway regulates growth and maintenance of colonospheres. Mol Cancer 2010;9:212.
67. Ramachandran I, Ganapathy V, Gillies E *et al.* Wnt inhibitory factor 1 suppresses cancer stemness and induces cellular senescence. Cell Death Dis 2014;5:e1246.
68. Jin X, Jeon HY, Joo KM *et al.* Frizzled 4 Regulates Stemness and Invasiveness of Migrating Glioma Cells Established by Serial Intracranial Transplantation. Cancer Res 2011;71:3066–3075.
69. Vermeulen L, De Sousa EMF, van der Heijden M *et al.* Wnt activity defines colon cancer stem cells and is regulated by the microenvironment. Nat Cell Biol 2010;12:468–476.
70. Fodde R, Brabletz T. Wnt/Beta-catenin signaling in cancer stemness and malignant behavior. Curr Opin Cell Biol 2007;19:150–158.
71. Clevers H, Loh KM, Nusse R. Stem cell signaling. An integral program for tissue renewal and regeneration: Wnt signaling and stem cell control. Science 2014;346:1248012.
72. Holland JD, Klaus A, Garratt AN *et al.* Wnt signaling in stem and cancer stem cells. Curr Opin Cell Biol 2013;25:254–264.
73. Ortensi B, Setti M, Osti D *et al.* Cancer stem cell contribution to glioblastoma invasiveness. Stem Cell Res Ther 2013;4:18.
74. Das S, Srikanth M, Kessler JA. Cancer stem cells and glioma. Nat Clin Pract Neurol 2008;4:427–435.
75. Zheng H, Ying H, Wiedemeyer R *et al.* Plagl2 regulates Wnt signaling to impede differentiation in neural stem cells and gliomas. Cancer Cell 2010;17:497–509.
76. Sekiya R, Maeda M, Yuan H *et al.* Plagl2 regulates actin cytoskeletal architecture and cell migration. Carcinogenesis 2014;35:1993–2001.
77. Joshi K, Banasavadi-Siddegowda Y, Mo X *et al.* Melk-dependent foxm1 phosphorylation is essential for proliferation of glioma stem cells. Stem Cells 2013;31:1051–1063.
78. Lee Y, Kim KH, Kim DG *et al.* Foxm1 promotes stemness and radioresistance of glioblastoma by regulating the master stem cell regulator Sox2. PLoS One 2015;10:e0137703.
79. Rheinbay E, Suva ML, Gillespie SM *et al.* An aberrant transcription factor network essential for wnt signaling and stem cell maintenance in glioblastoma. Cell Rep 2013;3:1567–1579.
80. Goodman RM, Thombre S, Firtina Z *et al.* Sprinter: a novel transmembrane protein required for Wg secretion and signaling. Development 2006;133:4901–4911.
81. Augustin I, Goidts V, Bongers A *et al.* The Wnt secretion protein Evi/Gpr177 promotes glioma tumourigenesis. EMBO Mol Med 2012;4:38–51.
82. Bartscherer K, Pelte N, Ingelfinger D *et al.* Secretion of Wnt ligands requires Evi, a conserved transmembrane protein. Cell 2006;125:523–533.
83. Gao X, Mi Y, Ma Y *et al.* Lef1 regulates glioblastoma cell proliferation, migration, invasion, and cancer stem-like cell self-renewal. Tumour Biol 2014;35:11505–11511.

84. Xue Y, Li L, Zhang D *et al.* Twisted epithelial-to-mesenchymal transition promotes progression of surviving bladder cancer T24 cells with Htert-dysfunction. PLoS One 2011;6:e27748.
85. Sarrio D, Franklin CK, Mackay A *et al.* Epithelial and mesenchymal subpopulations within normal basal breast cell lines exhibit distinct stem cell/progenitor properties. Stem Cells 2012;30:292–303.
86. Joost S, Almada LL, Rohnalter V *et al.* Gli1 inhibition promotes epithelial-to-mesenchymal transition in pancreatic cancer cells. Cancer Res 2012;72:88–99.
87. Smit MA, Peeper DS. Zeb1 is required for trkb-induced epithelial-mesenchymal transition, anoikis resistance and metastasis. Oncogene 2011;30:3735–3744.
88. Han SP, Kim JH, Han ME *et al.* Snai1 is involved in the proliferation and migration of glioblastoma cells. Cell Mol Neurobiol 2011;31:489–496.
89. Wellner U, Schubert J, Burk UC *et al.* The Emt-activator Zeb1 promotes tumorigenicity by repressing stemness-inhibiting microRNAs. Nat Cell Biol 2009;11:1487–1495.
90. Mikheeva SA, Mikheev AM, Petit A *et al.* Twist1 promotes invasion through mesenchymal change in human glioblastoma. Mol Cancer 2010;9:194.
91. Yang HW, Menon LG, Black PM *et al.* Snai2/Slug promotes growth and invasion in human gliomas. BMC Cancer 2010;10:301.
92. Howe LR, Watanabe O, Leonard J *et al.* Twist is up-regulated in response to Wnt1 and inhibits mouse mammary cell differentiation. Cancer Res 2003;63:1906–1913.
93. Kemler R, Hierholzer A, Kanzler B *et al.* Stabilization of beta-catenin in the mouse zygote leads to premature epithelial-mesenchymal transition in the epiblast. Development 2004;131:5817–5824.
94. Scheel C, Eaton EN, Li SH *et al.* Paracrine and autocrine signals induce and maintain mesenchymal and stem cell states in the breast. Cell 2011;145:926–940.
95. Yue X, Lan F, Yang W *et al.* Interruption of beta-catenin suppresses the Egfr pathway by blocking multiple oncogenic targets in human glioma cells. Brain Res 2010;1366:27–37.
96. Kamino M, Kishida M, Kibe T *et al.* Wnt-5a signaling is correlated with infiltrative activity in human glioma by inducing cellular migration and Mmp-2. Cancer Sci 2011;102:540–548.
97. Pu P, Zhang Z, Kang C *et al.* Downregulation of Wnt2 and Beta-catenin by Sirna suppresses malignant glioma cell growth. Cancer Gene Ther 2009;16:351–361.
98. Varma RR, Hector SM, Clark K *et al.* Gene expression profiling of a clonal isolate of oxaliplatin-resistant ovarian carcinoma cell line A2780/C10. Oncol Rep 2005;14:925–932.
99. Anastas JN, Kulikauskas RM, Tamir T *et al.* Wnt5a enhances resistance of melanoma cells to targeted Braf inhibitors. J Clin Invest 2014;124:2877–2890.
100. Peng C, Zhang X, Yu H *et al.* Wnt5a as a predictor in poor clinical outcome of patients and a mediator in chemoresistance of ovarian cancer. Int J Gynecol Cancer 2011;21:280–288.
101. Bordonaro M, Tewari S, Cicco CE *et al.* A switch from canonical to noncanonical wnt signaling mediates drug resistance in colon cancer cells. PLoS One 2011;6:e27308.
102. Griesmann H, Ripka S, Pralle M *et al.* Wnt5a-Nfat signaling mediates resistance to apoptosis in pancreatic cancer. Neoplasia 2013;15:11–22.
103. Auger N, Thillet J, Wanherdrick K *et al.* Genetic alterations associated with acquired temozolomide resistance in Snb-19, a human glioma cell line. Mol Cancer Ther 2006;5:2182–2192.
104. Chen MS, Woodward WA, Behbod F *et al.* Wnt/Beta-catenin mediates radiation resistance of Sca1+ progenitors in an immortalized mammary gland cell line. J Cell Sci 2007;120:468–477.
105. Woodward WA, Chen MS, Behbod F *et al.* Wnt/Beta-catenin mediates radiation resistance of mouse mammary progenitor cells. Proc Natl Acad Sci USA 2007;104:618–623.
106. Zhang M, Atkinson RL, Rosen JM. Selective targeting of radiation-resistant tumor-initiating cells. Proc Natl Acad Sci USA 2010;107:3522–3527.
107. Kendziorra E, Ahlborn K, Spitzner M *et al.* Silencing of the Wnt transcription factor Tcf4 sensitizes colorectal cancer cells to (chemo-) radiotherapy. Carcinogenesis 2011;32:1824–1831.
108. Bao S, Wu Q, McLendon RE *et al.* Glioma stem cells promote radioresistance by preferential activation of the DNA damage response. Nature 2006;444:756–760.
109. Zhang N, Wu X, Yang L *et al.* Foxm1 inhibition sensitizes resistant glioblastoma cells to temozolomide by downregulating the expression of DNA-repair gene Rad51. Clin Cancer Res 2012;18:5961–5971.
110. Kim Y, Kim KH, Lee J *et al.* Wnt activation is implicated in glioblastoma radioresistance. Lab Invest 2012;92:466–473.
111. Noushmehr H, Weisenberger DJ, Diefes K *et al.* Identification of a Cpg island methylator phenotype that defines a distinct subgroup of glioma. Cancer Cell 2010;17:510–522.
112. Watanabe K, Tachibana O, Sata K *et al.* Overexpression of the Egf receptor and P53 mutations are mutually exclusive in the evolution of primary and secondary glioblastomas. Brain Pathol 1996;6:217–223.
113. Ekstrand AJ, Sugawa N, James CD *et al.* Amplified and rearranged epidermal growth factor receptor genes in human glioblastomas reveal deletions of sequences encoding portions of the N- and/or C-terminal tails. Proc Natl Acad Sci USA 1992;89:4309–4313.
114. Ohgaki H, Dessen P, Jourde B *et al.* Genetic pathways to glioblastoma: a population-based study. Cancer Res 2004;64:6892–6899.
115. Huang PH, Xu AM, White FM. Oncogenic EGFR signaling networks in glioma. Sci Signal 2009;2:re6.
116. Karpel-Massler G, Schmidt U, Unterberg A *et al.* Therapeutic inhibition of the epidermal growth factor receptor in high-grade gliomas: where do we stand? Mol Cancer Res 2009;7:1000–1012.
117. Chen L, Huang K, Han L *et al.* Beta-Catenin/Tcf-4 complex transcriptionally regulates Akt1 in glioma. Int J Oncol 2011;39:883–890.
118. Ji H, Wang J, Nika H *et al.* Egf-induced Erk activation promotes Ck2-mediated disassociation of alpha-catenin from beta-catenin and transactivation of beta-catenin. Mol Cell 2009;36:547–559.
119. Lu Z, Ghosh S, Wang Z *et al.* Downregulation of caveolin-1 function by Egf leads to the loss of E-cadherin, increased transcriptional activity of beta-catenin, and enhanced tumor cell invasion. Cancer Cell 2003;4:499–515.
120. Kong DS, Song SY, Kim DH *et al.* Prognostic significance of C-Met expression in glioblastomas. Cancer 2009;115:140–148.
121. Joo KM, Jin J, Kim E *et al.* Met signaling regulates glioblastoma stem cells. Cancer Res 2012;72:3828–3838.
122. Nabeshima K, Shimao Y, Sato S *et al.* Expression of C-Met correlates with grade of malignancy in human astrocytic tumours: an immunohistochemical study. Histopathology 1997;31:436–443.
123. Kim KH, Seol HJ, Kim EH *et al.* Wnt/Beta-catenin signaling is a key downstream mediator of met signaling in glioblastoma stem cells. Neuro Oncol 2013;15:161–171.
124. Pasca di Magliano M, Hebrok M. Hedgehog signalling in cancer formation and maintenance. Nat Rev Cancer 2003;3:903–911.
125. Dahmane N, Sanchez P, Gitton Y *et al.* The Sonic Hedgehog-Gli pathway regulates dorsal brain growth and tumorigenesis. Development 2001;128:5201–5212.
126. Takezaki T, Hide T, Takanaga H *et al.* Essential role of the Hedgehog signaling pathway in human glioma-initiating cells. Cancer Sci 2011;102:1306–1312.
127. Northcott PA, Korshunov A, Witt H *et al.* Medulloblastoma comprises four distinct molecular variants. J Clin Oncol 2011;29:1408–1414.
128. Taylor MD, Northcott PA, Korshunov A *et al.* Molecular subgroups of medulloblastoma: the current consensus. Acta Neuropathol 2012;123:465–472.
129. Zinke J, Schneider FT, Harter PN *et al.* Beta-Catenin-Gli1 Interaction regulates proliferation and tumor growth in medulloblastoma. Mol Cancer 2015;14:17.
130. Taylor MD, Liu L, Raffel C *et al.* Mutations in Sufu predispose to medulloblastoma. Nat Genet 2002;31:306–310.
131. Reifenberger G, Reifenberger J, Ichimura K *et al.* Amplification of multiple genes from chromosomal region 12q13-14 in human malignant gliomas: preliminary mapping of the amplicons shows preferential involvement of Cdk4, Sas, and Mdm2. Cancer Res 1994;54:4299–4303.
132. Collins VP. Gene amplification in human gliomas. Glia 1995;15:289–296.
133. Reifenberger G, Ichimura K, Reifenberger J *et al.* Refined mapping of 12q13-Q15 amplicons in human malignant gliomas suggests cdk4/sas and mdm2 as independent amplification targets. Cancer Res 1996;56:5141–5145.

134. Werner CA, Dohner H, Joos S *et al.* High-level DNA amplifications are common genetic aberrations in B-cell neoplasms. Am J Pathol 1997;151:335–342.
135. Rao PH, Houldsworth J, Dyomina K *et al.* Chromosomal and gene amplification in diffuse large B-cell lymphoma. Blood 1998;92: 234–240.
136. Chandra V, Das T, Gulati P *et al.* Hedgehog signaling pathway is active in Gbm with Gli1 Mrna expression showing a single continuous distribution rather than discrete high/low clusters. PLoS One 2015;10: e0116390.
137. Rossi M, Magnoni L, Miracco C *et al.* Beta-cCatenin and Gli1 are prognostic markers in glioblastoma. Cancer Biol Ther 2011;11: 753–761.
138. He J, Sheng T, Stelter AA *et al.* Suppressing Wnt signaling by the Hedgehog pathway through Sfrp-1. J Biol Chem 2006;281: 35598–35602.
139. Sottoriva A, Spiteri I, Piccirillo SG *et al.* Intratumor heterogeneity in human glioblastoma reflects cancer evolutionary dynamics. Proc Natl Acad Sci USA 2013;110:4009–4014.
140. He B, Reguart N, You L *et al.* Blockade of Wnt-1 signaling induces apoptosis in human colorectal cancer cells containing downstream mutations. Oncogene 2005;24:3054–3058.
141. You L, He B, Xu Z *et al.* An anti-Wnt-2 monoclonal antibody induces apoptosis in malignant melanoma cells and inhibits tumor growth. Cancer Res 2004;64:5385–5389.
142. Nagayama S, Fukukawa C, Katagiri T *et al.* Therapeutic potential of antibodies against Fzd 10, a cell-surface protein, for synovial sarcomas. Oncogene 2005;24:6201–6212.
143. Fujii N, You L, Xu Z *et al.* An antagonist of Dishevelled protein-protein interaction suppresses beta-catenin-dependent tumor cell growth. Cancer Res 2007;67:573–579.
144. Yoshizumi T, Ohta T, Ninomiya I *et al.* Thiazolidinedione, a peroxisome proliferator-activated receptor-gamma ligand, inhibits growth and metastasis of Ht-29 human colon cancer cells through differentiation-promoting effects. Int J Oncol 2004;25:631–639.
145. Emami KH, Nguyen C, Ma H *et al.* A small molecule inhibitor of beta-catenin/creb-binding protein transcription. Proc Natl Acad Sci USA 2004;101:12682–12687.
146. Chen B, Dodge ME, Tang W *et al.* Small molecule-mediated disruption of Wnt-dependent signaling in tissue regeneration and cancer. Nat Chem Biol 2009;5:100–107.
147. Liu J, Stevens J, Matsunami N *et al.* Targeted degradation of beta-catenin by chimeric F-Box fusion proteins. Biochem Biophys Res Commun 2004;313:1023–1029.
148. Lazarova DL, Chiaro C, Wong T *et al.* Cbp activity mediates effects of the histone deacetylase inhibitor butyrate on Wnt activity and apoptosis in colon cancer cells. J Cancer 2013;4: 481–490.
149. Su Y, Ishikawa S, Kojima M *et al.* Eradication of pathogenic beta-catenin by Skp1/Cullin/F Box ubiquitination machinery. Proc Natl Acad Sci USA 2003;100:12729–12734.
150. Parepally JM, Mandula H, Smith QR. Brain uptake of nonsteroidal anti-inflammatory drugs: ibuprofen, flurbiprofen, and indomethacin. Pharm Res 2006;23:873–881.
151. Courad JP, Besse D, Delchambre C *et al.* Acetaminophen distribution in the rat central nervous system. Life Sci 2001;69:1455–1464.
152. Zhang X, Morham SG, Langenbach R *et al.* Malignant transformation and antineoplastic actions of nonsteroidal antiinflammatory drugs (NSAIDs) on cyclooxygenase-null embryo fibroblasts. J Exp Med 1999;190:451–459.
153. Dihlmann S, Siermann A, von Knebel Doeberitz M. The nonsteroidal anti-inflammatory drugs aspirin and indomethacin attenuate Beta-Catenin/Tcf-4 signaling. Oncogene 2001;20:645–653.
154. Thun MJ, Namboodiri MM, Heath Jr CW. Aspirin use and reduced risk of fatal colon cancer. N Engl J Med 1991;325:1593–1596.
155. Sandler RS, Halabi S, Baron JA *et al.* A randomized trial of aspirin to prevent colorectal adenomas in patients with previous colorectal cancer. N Engl J Med 2003;348:883–890.
156. Baron JA, Cole BF, Sandler RS *et al.* A randomized trial of aspirin to prevent colorectal adenomas. N Engl J Med 2003;348:891–899.
157. Lan F, Yue X, Han L *et al.* Antitumor effect of aspirin in glioblastoma cells by modulation of beta-catenin/T-cell factor-mediated transcriptional activity. J Neurosurg 2011;115:780–788.
158. Sareddy GR, Kesanakurti D, Kirti PB *et al.* Nonsteroidal anti-inflammatory drugs diclofenac and celecoxib attenuates Wnt/Beta-Catenin/Tcf signaling pathway in human glioblastoma cells. Neurochem Res 2013;38:2313–2322.
159. De Robertis A, Valensin S, Rossi M *et al.* Identification and characterization of a small-molecule inhibitor of Wnt signaling in glioblastoma cells. Mol Cancer Ther 2013;12:1180–1189.
160. Huang SM, Mishina YM, Liu S *et al.* Tankyrase inhibition stabilizes axin and antagonizes wnt signalling. Nature 2009;461:614–620.
161. He B, You L, Uematsu K *et al.* A monoclonal antibody against Wnt-1 induces apoptosis in human cancer cells. Neoplasia 2004;6:7–14.
162. Hanaki H, Yamamoto H, Sakane H *et al.* An Anti-Wnt5a antibody suppresses metastasis of gastric cancer cells *in vivo* by inhibiting receptor-mediated endocytosis. Mol Cancer Ther 2012;11:298–307.
163. Fontenot E, Rossi E, Mumper R *et al.* A novel monoclonal antibody to secreted frizzled-related protein 2 inhibits tumor growth. Mol Cancer Ther 2013;12:685–695.
164. Safholm A, Tuomela J, Rosenkvist J *et al.* The Wnt-5a-derived hexapeptide Foxy-5 inhibits breast cancer metastasis *in vivo* by targeting cell motility. Clin Cancer Res 2008;14:6556–6563.
165. Fukukawa C, Hanaoka H, Nagayama S *et al.* Radioimmunotherapy of human synovial sarcoma using a monoclonal antibody against Fzd10. Cancer Sci 2008;99:432–440.
166. Hernandez-Pedro NY, Rangel-Lopez E, Vargas Felix G *et al.* An update in the use of antibodies to treat glioblastoma multiforme. Autoimmune Dis 2013;2013:716813.
167. Gabathuler R. Approaches to transport therapeutic drugs across the blood-brain barrier to treat brain diseases. Neurobiol Dis 2010;37: 48–57.