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REVIEW ARTICLE

# Crosstalk of the Wnt/β-catenin pathway with other pathways in cancer cells

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**Abstract** Many cancers have similar aberrations in various signaling cascades with crucial roles in cellular proliferation, differentiation, and morphogenesis. Dysregulation of signal cascades that play integral roles during early cellular development is well known to be a central feature of many malignancies. One such signaling cascade is the Wnt/β-catenin pathway, which has a profound effect on stem cell proliferation, migration, and differentiation. This pathway is dysregulated in numerous cell types, underscoring its global oncogenetic potential. This review highlights regulators and downstream effectors of this receptor cascade and addresses the increasingly apparent crosstalk of Wnt with other tumorigenic signaling pathways. As understanding of the genetic and epigenetic changes unique to these malignancies increases, identifying the regulatory mechanisms unique to the Wnt/β-catenin pathway and similarly aberrant receptor pathways will be imperative.


## Introduction

Wnt signaling plays a central role in cell proliferation, differentiation, and morphogenesis.[1–3] It is a critical step in β-catenin signal transduction and is responsible for maintaining its own unphosphorylated state. In its dephosphorylated state, β-catenin is localized in the nucleus, where it activates transcription factors in the T-cell factor (TCF)/lymphoid enhancing factor (LEF) family.[4–6] In the absence of Wnt activity, β-catenin is phosphorylated by

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glycogen synthase kinase (GSK)-3b and marked for subsequent degradation by the Skp, Cullin, F-box containing complex (SCF)/ubiquitin/proteasome pathway.[7–12] In its active, dephosphorylated state, β-catenin has a profound effect on the regulation of stem cells.[13,14] Researchers have illustrated this with mammary stem cells in both native and ectopic locations.[15–18] When the Wnt pathway is upregulated, it consistently results in tumorigenesis in a variety of organs.[19–21]

In particular, aberrant activation of β-catenin–TCF/LEF signals secondary to adenomatous polyposis coli, axin, and β-catenin gain-of-function mutations are associated with the development of colon cancer, desmoid tumors, gastric cancer, hepatocellular carcinoma, medulloblastoma, melanoma, ovarian cancer, pancreatic cancer, and prostate cancer.[19–21] Studies have demonstrated that these mutations are not the sole sources of β-catenin hyperactivity, however. For instance, Ashihara et al[22] demonstrated nuclear accumulation of β-catenin in 60% of their endometrial tumor specimens. However, the CTNNB1 gene, which encodes β-catenin, was mutated in only 10% of the specimens. Investigators have found similar results in studies of melanoma, hepatocellular carcinoma, and glioma in which CTNNB1 and GSK-3b expression were largely normal in respective cancer cell lines.[23–28] A potential explanation for this phenomenon is the presence of additional pathways mediating the nuclear translocation of β-catenin. Increased epidermal growth factor and AKT-dependent β-catenin phosphorylation is associated with increased β-catenin nuclear localization.[29,30] Members of our laboratory have demonstrated that Forkhead box M1 (FoxM1), which is a downstream component of the Wnt signaling pathway, binds to cytosolic β-catenin and the two factors subsequently undergo nuclear translocation in glioma cells.[31]

Wnt signaling has a crucial role in neural development. Both in vitro and in vivo studies have demonstrated the necessity of Wnt signaling for neural stem cell (NSC) renewal and differentiation.[32] These findings were corroborated by a study using adult hippocampal progenitor cells in which β-catenin upregulation promoted hippocampal enlargement, whereas its downregulation led to small hippocampi.[33] Researchers have obtained similar findings for gain-of-function and loss-of-function mutations of β-catenin. Specifically, gain-of-function mutations increased NSC proliferation in the periventricular zone, causing brain enlargement, whereas loss-of-function mutations caused marked brain shrinkage.[34]

## Positive regulators of Wnt/β-catenin signaling

### Dishevelled and Frizzled 4 proteins promote nuclear localization of β-catenin

Various factors have been attributed to the dedifferentiating capacity of Wnt signaling. For instance, the scaffold protein, Dishevelled is activated by the Wnt pathway. The Dishevelled-Axin domain mediates the effects of the canonical Wnt pathway, which signals subsequent binding to Axin for disassembly of β-catenin from its destruction complex.[35] Glioblastoma (GBM) cells deficient in Dishevelled 2 exhibited unchecked proliferation.[36] Similarly, Wnt activation of the transmembrane protein Frizzled 4 (FZD4) can promotes nuclear translocation of β-catenin when overexpressed, whereas nuclear levels of it decrease when its gene is not present.[37]

### PLAGL2 contributes to a β-catenin-mediated undifferentiated state in NSCs

PLAGL2 is a zinc-finger protein localized to the nucleus that has been demonstrated to upregulate expression of Wnt pathway receptors and its associated proteins in NSCs. Investigators found that these PLAGL2 expressing NSCs were maintained in an undifferentiated state and that β-catenin expression was higher in the NSCs than in control cells. Moreover, the Wnt-promoting effects of PLAGL2 and the undifferentiated NSC state was reversible following transfection with the Wnt inhibitor Dickkopf-1 into the cells.[38]

### FoxM1 is a downstream component of Wnt signaling

Our laboratory personnel have contributed to elucidating the role of FoxM1 in β-catenin signaling. FoxM1 is a transcription factor with a pivotal role in glioma formation and has been shown to be overexpressed in GBM cells.[39,40] Using liquid chromatography-mass spectrometry, we confirmed that FoxM1 interacted with β-catenin. In the same study, we also revealed that FoxM1 and β-catenin were co-localized in both the cytoplasm and nucleus using various in vitro methods. Moreover, using Wnt3a-based treatment, we demonstrated nuclear accumulation of both FoxM1 and β-catenin in 293FT cells, which suggested that FoxM1 is a downstream component of the Wnt signaling pathway.[31]

### FoxM1 tumorigenicity is β-catenin-dependent

We confirmed that FoxM1 promoted nuclear translocation of β-catenin in glioma stem cells (GSCs) and showed that GSCs with knockdown of both FoxM1 and β-catenin expression had reduced numbers of neural colony-forming units.[31] Additionally, we found that FoxM1 markedly increased the formation of β-catenin–TCF/LEF transcriptional complexes, as well as the expression of its gene products Axin2, LEF-1, c-Myc, and cyclin D1. In another experiment, we found that FoxM1 overexpression maintained the undifferentiated state of GSCs and promoted neurosphere formation.[31] To clarify the downstream role of β-catenin in FoxM1-mediated tumorigenicity, we investigated β-catenin knockdown in GSCs overexpressing FoxM1. We found that the combination of β-catenin knockdown in lieu on FoxM1 overexpression still decreased tumor formation in mice. However, we observed increased tumor burdens in mice lacking β-catenin knockdown but possessing GSCs with FoxM1 overexpression.

We corroborated the finding that β-catenin and FoxM1 are overexpressed and colocalized to the nucleus in human GBM specimens. We proved that FoxM1 expression correlated directly with nuclear β-catenin expression. In addition, we showed that the expression of the stem cell marker nestin and the Wnt targets Axin2 and LEF-1 were elevated in direct correlation with FoxM1 expression. Finally, immunofluorescent staining of GBM specimens validated nuclear co-localization of β-catenin and FoxM1 and demonstrated increased Nestin and decreased glial fibrillary acidic protein staining in FoxM1-positive GBM specimens.[31] These findings demonstrated that the

tumorigenicity of FoxM1 is β-catenin-mediated and that FoxM1 expression promotes the undifferentiated state of stem cells.

## Negative regulators of Wnt/β-catenin signaling

### Paternally expressed gene 3 prevents Wnt activity via p53-mediated degradation

Paternally expressed gene 3 has been demonstrated to impair zebrafish embryogenesis by preventing Wnt-dependent tail development.[41,42] In addition, the absence of paternally expressed gene 3 expression was associated with increased glioma formation, whereas its overexpression resulted in decreased glioma tumorigenicity.[43] This paternally expressed gene 3-mediated inhibition of Wnt activity was found to be secondary to increased proteasomal degradation of β-catenin independent of GSK-3b. Similar to other studies demonstrating p53-mediated degradation of β-catenin, Jiang and colleagues showed that p53-associated decreases in β-catenin expression were lost with knockout of Siah1.[42] Furthermore, this group demonstrated that paternally expressed gene 3 knockdown is associated with increased β-catenin expression and that paternally expressed gene 3 expression inversely correlates glioma grade.[42,43]

### Wnt activation can be blocked by inhibiting its interactions with lipoprotein receptor-related protein 5 and 6 and FZD4

Dickkopf-1 (DKK1) is a well-described inhibitor of canonical Wnt activity in humans and has been demonstrated to have proapoptotic effects and increased expression in various human cancer cell lines.[44] DKK1 is a glycoprotein that binds to lipoprotein receptor-related protein 5 (LRP5) and LRP6 and the membrane protein Kremen, which promote endocytosis of the DKK1-LRP5/6-Kremen complex. This prevents the formation of a Wnt-LRP5/6-FZD4 complex that would activate the canonical pathway.[45] This effect has not been shown to decrease glioma tumorigenicity. On the other hand, inactivation of secreted FZD-related protein has been shown to be correlated with the development of various malignancies, including glioma.[47] Secreted FZD-related protein is a homolog of the FZD receptors that binds to extracellular Wnt and prevents it from interacting with ligands linked with β-catenin activation.[48-49] This was confirmed in in vitro and in vivo studies that demonstrated decreased β-catenin expression, reduced GSC viability, and decreased tumorigenicity in mice. Additionally, authors have reported that secreted FZD-related protein is a chemosensitizing agent.[37,50,51]

### a2-Macroglobulin/LRP1 binding downregulates Wnt activity

a2-Macroglobulin has been shown to suppress tumor invasion after binding to LRP1, which subsequently marks transforming growth factor-b, as well as various proteases involved in extracellular matrix destruction for endocytosis-related inactivation.[52,53] Linder et al found that the β-catenin-stabilizing effects of LiCl could be reversed by a2-macroglobulin administration in 1321N1 astrocytoma cells. Furthermore, LRP1 binding upregulated β-catenin, LRP1, E-cadherin, N-cadherin, and FZD expression while downregulating Wnt1, Wnt5a, and Wnt10b expression in 1321N1 cells.[54] These results were reproduced when using a polyclonal anti-LRP1 antibody to stimulate the a2-macroglobulin receptor in 1321N1 cells.

### YAP/TAZ-Axin1 interaction marks β-catenin for destruction

A central feature of β-catenin's ultimate fate is its interaction with the scaffold protein Axin1, which directs its subsequent interaction with other cytoplasmic proteins, such as GSK-3b, adenomatous polyposis coli, and casein kinase-1. GSK-3b-mediated presentation of β-catenin to the ubiquitin ligase b-transducin repeat-containing protein is an important step in β-catenin destruction.[55] In Wnt-off HEK293 cells, which contain a functioning β-catenin destruction complex, Azzolin et al showed that Axin1 possesses high levels of co-precipitation with β-catenin, GSK-3b, b-transducin repeat-containing protein, YAP, and TAZ.[56] Furthermore, Axin1/2 knockdown did not result in YAP/TAZ accumulation HEK293 cell with destruction complex components. This knockdown resulted in increased nuclear accumulation of YAP and TAZ, as well as increased transcriptional activity of the YAP/TAZ target gene CTGF.[56]

Similarly, treatment of HEK293 cells with Wnt3a resulted in nuclear localization of both YAP and TAZ, as well as upregulated expression of other YAP/TAZ target genes. In addition, this treatment decreased the association of YAP and TAZ with the destruction complex. Immunoprecipitation of these Wnt-treated cells demonstrated interaction with the LRP6 protein instead, suggesting that Wnt signaling inhibits β-catenin destruction by preventing YAP/TAZ interaction with the destruction complex. This was confirmed by loss of detectable co-precipitation of YAP and TAZ with destruction complex proteins in cells transfected with LRP6 gene-containing plasmids. Finally, Azzolin et al showed that the ubiquitin ligase b-transducin repeat-containing protein interacted with the destruction complex in both Wnt3a- and LRP6-treated cells, but this interaction was extinguished with increasing YAP expression in Wnt-on cells.[56]

## Downstream effects of Wnt signaling

### Wnt stimulation inhibits the mammalian target of rapamycin pathway of tuberous sclerosis complex 2 via a GSK-3β—AMP-activated protein kinase-dependent cascade

The canonical Wnt pathway has numerous downstream effects following its interaction with the TCF/LEF family of transcription factors. These effects are largely secondary to cyclin D1 and c-Myc activation.[4,57] The pathway vastly influences many other signal cascades attributed to derangements in cellular differentiation and growth. For example, the tuberous sclerosis complex 2 pathway is dependent on phosphorylation by various kinases, such as AMP-activated protein kinase (AMPK) for downstream activity. Wnt3a-treated cells have been shown to increase the phosphorylation of S6K, 4EBP1, and S6, which are markers of mammalian target of rapamycin (mTOR) function. These

effects were lost following administration of mTOR phosphorylation inhibitors, indicating that the effects of Wnt in part are mediated by this pathway.

In addition, mice overexpressing Wnt10b had marked enlargement of calvarial tissue, as well as increased phosphorylation of S6.[58] S6 phosphorylation decreased when rapamycin, an mTOR inhibitor was administered to mice bearing mammary tumor cells overexpressing Wnt1. Similarly, mice with knockdown of LRP1 receptor expression had smaller than normal calvarias. Furthermore, investigation of Wnt-expressing hyperplastic mammary tissue and mammary tumor cells revealed elevated mTOR activity that could be reversed following rapamycin treatment, which also reduced the size of both cell types.

These effects were not influenced by β-catenin activity, as underexpression of both β-catenin and TCFs had no effect on S6 phosphorylation. However, in GSK-3b expressing HEK293 cells there was decreased S6 phosphorylation, and administration of the GSK-3b inhibitors LiCl and GSKi in HEK293 and mouse embryonic fibroblast cells increased S6 phosphorylation levels. This was an effect of the interdependence of AMPK and GSK-3b activities. When AMPK activators were introduced into Wnt-treated cells, S6 phosphorylation decreased. On the other hand, administration of LiCl diminished the mTOR-blocking ability of AMPK activators. Likewise, AMPK inhibition negated the inhibitory effects of GSK-3b in the HEK293 cell line.[58]

### Pygopus 2 mediates the downstream effects of Wnt involved in Notch signaling crosstalk

Wnt signaling has been shown to increase the maintenance of mammary stem cells in their undifferentiated state, whereas Notch signaling promotes the differentiation of these stem cells into a luminal cell type.[15–18,59–61] With this in mind, deletions of Pygopus 2 (Pygo2), which is a known transcriptional co-factor with Wnt signaling, has been demonstrated to impair mammary morphogenesis.[62] Pygo2-deficient mammary stem cells were shown to differentiate into more mature luminal/alveolar variants. These cells had upregulated expression of Notch signaling components, Notch3, Dll4, and Hes1. In another experiment, Wnt signaling was upregulated by treatment with a GSK-3b inhibitor or cloning of a stabilized β-catenin, both of which decreased luminal/alveolar differentiation. This effect was lost in cells with Pygo2 knockdown. Moreover, micro chromatin immunoprecipitation assays revealed that both β-catenin and Pygo2 had high affinity for the Notch3 locus as well as the Axin2 locus. Furthermore, β-catenin binding mammary stem cells was markedly reduced in Pygo2's absence, suggesting that Pygo2 is a key co-activator of Wnt signaling's downstream effects and in its crosstalk with the Notch pathway.[63]

### Wnt signaling has repressive effects on Sonic hedgehog signaling

Sonic hedgehog (SHH) has a critical role in embryogenesis and is responsible for morphogenesis of neural structures as well as the proliferation of granule neuron precursors in the cerebellum.[64,65] In its active state, SHH binds to Patch receptors and increases the activity of Smoothened receptors, thus allowing the transcription of glioma-associated oncogenes homologs (Gli1/2/3).[66] When dysregulated the SHH pathway can promote gastric cancer, medulloblastoma, skin cancer, and GBM formation. Despite an association of both Wnt and SHH overexpression with a propensity for tumor development, Wnt activity can impair SHH-mediated tumorigenesis.[67–71]

Nuclear staining for β-catenin was shown to be increased in poorly differentiated gastric adenocarcinomas (60%), whereas nuclear staining for Gli1 was decreased in these tumors (30%).[67] The inverse was observed in well-differentiated adenocarcinomas with respect to nuclear staining for Gli2 (100%) and β-catenin (70%). This was confirmed following treatment with NaBU, an agent that promotes cellular differentiation, which did not change levels of cytoplasmic β-catenin but decreased β-catenin nuclear expression in AGS and MKN-45 cells. These gastric cancer cells were later treated with a Gli-overexpressing vector or NaBU, both of which increased expression of the Wnt antagonist secreted FZD-related protein 1. This effect was lost following introduction of a Gli antagonist into the cells. Finally, chromatin immunoprecipitation verified that both Gli and secreted FZD-related protein 1 are bound β-catenin during its suppression.[66]

Wnt10b and nuclear β-catenin expression levels in the keratinocytes of skin tumor placodes in mice were measured and compared with the levels in fully developed skin tumors.[70] The keratinocytes had higher levels of the Wnt signaling components than did the tumors. In contrast, Patch1, Patch2, and Gli1 expression increased in the keratinocytes of the skin tumor placodes as the researches progressed from normal to skin tumor regions in mice. To clarify the roles of Wnt and SHH in tumor initiation and growth, the Wnt inhibitor exisulind (Aptosyn) and SHH inhibitor cyclopamine were injected into separate groups of mice. In comparison with transgenic mice, Aptosyn-treated animals had markedly lower new hair follicle growth and lower nuclear β-catenin levels expression. In comparison, cyclopamine-treated mice had no changes in the degree of new follicle outgrowth but did have substantially lower tumor growth than did the transgenic group.[70]

Similar findings were demonstrated by Pöschl et al.[68] In their study, the proliferation of granule neuron precursors with upregulation of Smoothened 2 activity was 2.6-fold higher than that of wild-type granule neuron precursors. Conversely, CTNNB expression reduced the proliferative capacity, as well as cellular proliferation in the granule neuron precursors with increased Smoothened 2 expression to near-wild-type levels. These results were reproduced in mice, which had much smaller cerebellums and cerebellar tumors when the Smoothened 2 and CTNNB genes were co-expressed than when Smoothened 2 was expressed alone. Further experimentation confirmed that both Axin2 and β-catenin expression were increased but Gli1 expression was decreased in mice with upregulation of expression of both genes, supporting Wnt-mediated inhibition of SHH signaling.[68]

In another study of the effects of Wnt signaling on SHH activity in medulloblastoma cells, LiCl administration caused an expected rise in nuclear β-catenin expression as well as decreased Patch2 and Gli1 expression.[69] This could be only partly reversed by proteasome inhibition, suggesting that another Wnt-dependent mechanism contributed to decreased Gli1 activity. Of note, many more

medulloblastoma cells were in a nonproliferative state following LiCl treatment as opposed to cells treated with NaCl. This was demonstrated by decreased Ki-67 and phosphor-histone H3 staining and increased senescence-associated nuclear b-galactosidase immunopositivity following LiCl treatment. Immunoprecipitation assays verified both nuclear and cytoplasmic co-precipitation of the two factors in LiCl-treated cells but not in control cells. In addition, the researchers observed that Gli1 knockdown did not inhibit Wnt signaling. Finally, mice injected with Patch-expressing medulloblastoma cells had markedly longer tumor formation intervals than did control mice when administered LiCl.[68]

Much more investigation must be done to clarify the role of Wnt/SHH crosstalk in GBM cells. Previous studies demonstrated a role for SHH in promoting migration of glioma cells.[72] In addition, gene network analysis demonstrated expression trends linking Wnt and SHH signaling through the Gli2 transcription factor. This connection links the destruction complex components, GSK-3b and casein kinase-1-a1 with the Wnt and SHH pathways, as both elements have roles in the inactivation of downstream signals.[71] Although the mechanisms of interplay between Wnt and SHH signaling remain unclear, the modes of crosstalk may be similar to those in previous gastric cancer, skin cancer, and medulloblastoma studies.

## Conclusions and future directions

Wnt signaling has a far-reaching influence with respect to its involvement in cellular differentiation and tumor initiation and progression. Many of its roles involve the interplay of separate signaling cascades. Fig. 1 illustrates the various regulators and downstream effectors of the Wnt pathway discussed in this review. Of interest are the roles of Wnt and SHH expression in various stages of glioma progression, as both pathways are associated with increased tumor invasiveness owing to upregulation of metalloproteinase activity.[73,74] However, SHH-mediated invasiveness cancer cell lines has been demonstrated only when Gli1 mutations have resulted in truncation of the Gli1

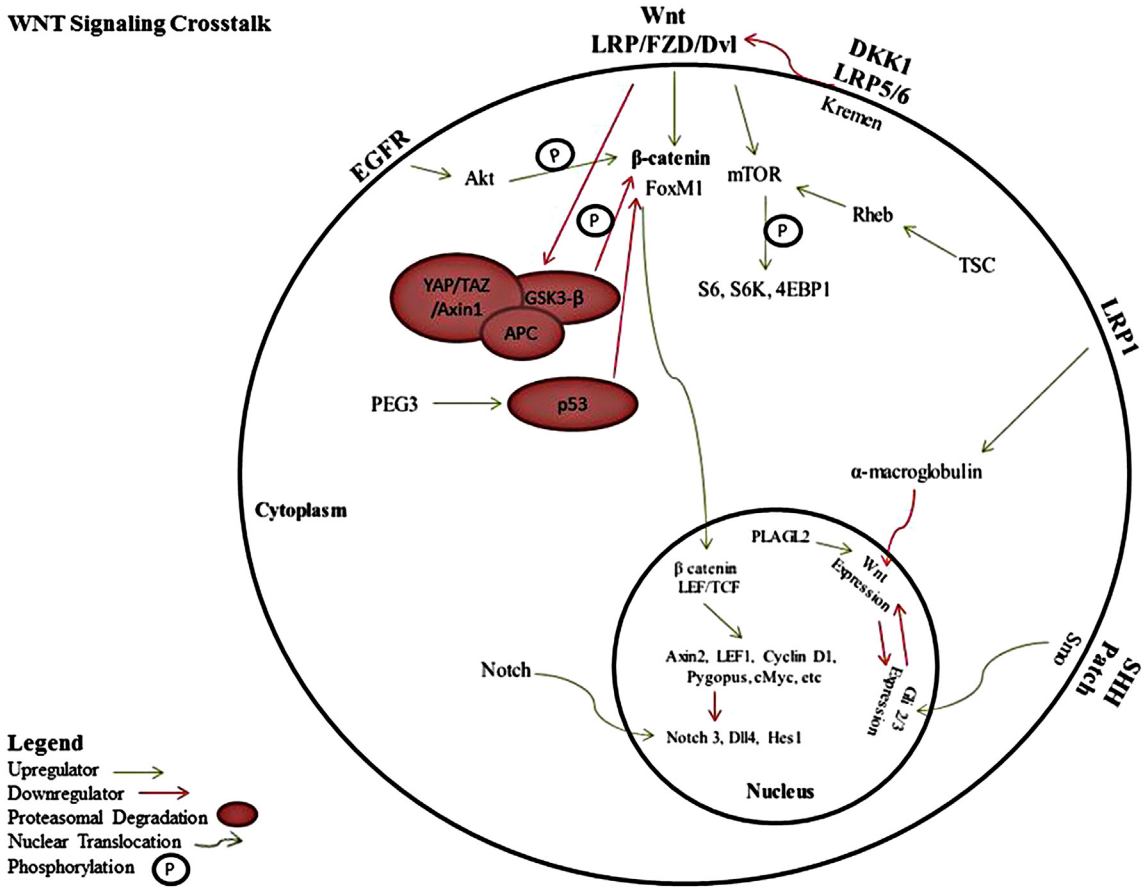


**Figure 1** The figure demonstrates the wide array of interactions present in the Wnt signaling cascade. Wnt signaling prevents β-catenin phosphorylation by the GSK-3/APC/YAP-TAZ destruction complex. In addition, Wnt has been shown to promote mTOR-mediated phosphorylation of its targets, S6, S6K, and 4EBP1. Once associated with FoxM1, β-catenin undergoes nuclear translocation to promote transcription of its various target genes. One of the β-catenin target genes, Pygopus has been shown to decrease the transcription of the Notch pathway target genes. EGFR signaling can inhibit β-catenin activity by AKT-mediated phosphorylation; on the other hand, PEG3 can facilitate p53-mediated proteasomal degradation of β-catenin. Further downstream, both Wnt and Sonic Hedgehog signaling decrease Gli 2/3 and Wnt expression, respectively. Similarly, LRP1 activation and subsequent α2-macroglobulin activity decrease the expression of Wnt.

transcription factor. Future studies are needed to identify targets of these pathways that can safely modulate their activity because of their integral roles in the normal processes in cells with high mitotic activity, such as skin, blood, and gut epithelial cells.

## Conflicts of interest

No potential conflicts of interest were declared.

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