Antagonism between Hedgehog and Wnt signaling pathways regulates tumorigenicity (Review)

MEI DING and XIN WANG

Department of Hematology, Shandong Provincial Hospital Affiliated to Shandong University, Jinan, Shandong 250021, P.R. China

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Abstract. The crosstalk of multiple cellular signaling pathways is crucial in animal development and tissue homeostasis, and its dysregulation may result in tumor formation and metastasis. The Hedgehog (Hh) and Wnt signaling pathways are both considered to be essential regulators of cell proliferation, differentiation and oncogenesis. Recent studies have indicated that the Hh and Wnt signaling pathways are closely associated and involved in regulating embryogenesis and cellular differentiation. Hh signaling acts upstream of the Wnt signaling pathway, and negative regulates Wnt activity via secreted frizzled-related protein 1 (SFRP1), and the Wnt/β-catenin pathway downregulates Hh activity through glioma-associated oncogene homolog 3 transcriptional regulation. This evidence suggests that the imbalance of Hh and Wnt regulation serves a crucial role in cancer-associated processes. The activation of SFRP1, which inhibits Wnt, has been demonstrated to be an important cross-point between the two signaling pathways. The present study reviews the complex interaction between the Hh and Wnt signaling pathways in embryogenesis and tumorigenicity, and the role of SFRP1 as an important mediator associated with the dysregulation of the Hh and Wnt signaling pathways.

Contents

Correspondence to: Mrs. Xin Wang, Department of Hematology, Shandong Provincial Hospital Affiliated to Shandong University, 324 Jingwu Road, Jinan, Shandong 250021, P.R. China
E-mail: xinw@sdu.edu.cn

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1. Introduction

Cancer is one of the predominant causes of human mortality worldwide. Despite several decades of unremitting efforts towards preventing and curing cancer, the translation of detailed molecular knowledge into more efficient cancer therapies remains a significant medical challenge. Cancer cells harbor a considerable number of genetic and epigenetic alterations; however, only a limited number of these alterations drive cancer progression. Tumor formation and metastasis is dependent on intracellular and intercellular signal transduction (1-5). Emerging data indicate that the crosstalk of multiple signaling pathways may account for malignant proliferation and metastasis (6-9). However, recent research has mainly focused on single pathways, ignoring the complexity of signaling networks. Exploration of the cross-regulation of signaling pathways may provide a more comprehensive understanding of the dissemination of information in such networks.

The most active field of research is that of the Hedgehog (Hh) and Wnt signaling pathways, which represent essential regulators of cell proliferation and differentiation during embryogenesis and tumorigenicity (10,11). Convergence of the two pathways involving secreted frizzled-related protein 1 (SFRP1) has been demonstrated (12,13). Nevertheless, studies regarding the interactions among signaling pathways are rare. The current review summarizes the most relevant literature regarding the cooperative interaction between the Hh and Wnt signaling pathways, and the role of SFRP1 as an important mediator of certain oncogenic and pro-metastatic activities that are associated with the Hh and Wnt signaling pathways. The targeted inhibition of this key point in the pathways has potential with regard to the development of therapies for cancer.

2. The Hh signaling pathway

The Hh signaling pathway is an important cascade for cellular growth and differentiation during the embryonic development.

The pathway was first identified in *Drosophila* fruit flies, and has been shown to be highly conserved in vertebrates and invertebrates (14-16). The Hh signaling pathway is complex and involves numerous regulatory proteins. In vertebrates, three Hh homologs have been identified: Sonic Hh (Shh), Indian Hh (Ihh), and desert Hh (Dhh) (17-19). Notably, the three Hh ligands activate the same signal transduction pathway, but regulate different organ systems: Shh is most widely expressed in the central nervous system, lungs, teeth, gut and hair follicles (20-24), while Ihh is involved in endochondral bone formation (25), and Dhh is expressed mostly in the gonads (26).

To initiate the signaling pathway, the Hh ligand binds to its receptor, a 12-transmembrane Patched (PTCH) protein, which also has two known human homologs, PTCH1 and PTCH2. In the absence of Hh, PTCH forms an inactive complex with the downstream protein Smoothened (SMO), and works as a suppressor or inhibitory protein of SMO. When Hh is activated, binding of the Hh ligand to PTCH results in endocytosis of the PTCH-ligand complex, followed by migration of activated SMO to the cytoplasm and association with glioma-associated oncogene homolog (GLI) proteins. The GLI proteins subsequently migrate into the nucleus and promote the transcription of target genes, which are responsible for cellular growth and differentiation during embryonic development, and are involved in tissue repair and cancer occurrence and development in adults (Fig. 1) (7,27).

Dysregulation of the Hh signaling pathway has now been implicated in various types of human malignancy, including gastrointestinal, bladder and ovarian carcinomas, lung cancer, and hematological malignancies (28-35). Aberrant activation of the Hh signaling pathway in human cancers can occur in three ways. In the first, mutated component proteins can be secreted from cells and constantly activate Hh signaling pathway. An example of this is the inactivation of PTCH or oncogenic activation of SMO, which have been demonstrated to be common features in a high proportion of tumors. To date, this mode of Hh signaling is considered the most important for tumor development (36-40). The second mode of aberrant activation is autocrine: The Hh ligand is secreted by tumor cells and also affects the tumor cells themselves (41,42). In the third mode, which is paracrine activation, tumor cells secrete Hh ligands to act on peripheral stroma cells, which activates vascular endothelial growth factor, insulin-like growth factor and Wnt signaling pathways to promote self-proliferation (43,44). A paracrine pattern in which stromal cells secret Hh ligands, thus contributing to the activation of Hh signaling in the tumor cells, has also been described (45).

Based on the etiological study of the Hh signaling pathway, molecular targeted therapy is considered a promising therapeutic strategy for cancer. For example, methods for increasing the inhibitory action of PTCH or suppressing the activation of SMO may be utilized the treatment of tumors with a hyper-activated Hh pathway. A number of small molecule SMO antagonists have been evaluated in clinical trials and demonstrated promising therapeutic benefits (46,47). Vismodegib, a small 2-pyridyl amide molecule, blocks Hh signaling by selectively inhibiting SMO, and thus prevents the consequent induction of target genes (48). The therapeutic success of Hh inhibitors also depends on their appropriate combination with other drugs that target cooperative signaling pathways (49-51); therefore, the points of interaction between Hh and other signaling pathways in malignancies may be potential therapeutic targets.

3. The Wnt signaling pathway

The Wnt signaling pathway participates in the physiological processes of embryonic development, cellular proliferation and differentiation, and also plays an important role in the occurrence and development of various malignancies (52-55). Wnt signaling is conducted via three pathways, as follows. The canonical Wnt/β-catenin signaling pathway, which is considered the most important pathway, results in the accumulation of β-catenin in the nucleus and initiates the expression of target genes. In normal organisms, Wnt pathway is inactivated, and unconjugated β-catenin is scarce; the majority of the β-catenin molecules are combined with glycogen synthase kinase 3β (GSK-3β), adenomatous polyposis coli (APC) and Axin, which lead to the phosphorylation and degradation of β-catenin via the ubiquitin pathway. Conversely, activation of the Wnt signaling pathway inhibits the GSK-3β/APC/Axin complex, inducing the abnormal accumulation and translocation to the nucleus of β-catenin, and resulting in gene transcription (Fig. 2) (56-58). In another pathway, Wnt5a and Wnt11 activate cyclin-dependent kinase 2 and protein kinase C to increase cellular Ca^{2+} concentration, and promote nuclear factor of activated T-cells-induced gene transcription; this pathway is designated the Wnt/Ca^{2+} pathway (59). The third pathway, Wnt/planar cell polarity signaling pathway mainly participates in the regulation of cytoskeletal rearrangement during embryonic development (60). The present review primarily focuses on the functional interaction between the canonical Wnt/β-catenin signaling pathway and the Hh signaling pathway.

Inappropriate activation of the Wnt signaling pathway is associated with a variety of malignant diseases, such as gallbladder, lung and breast cancers (61-63); therefore, the development of drugs targeting this pathway is an area of interest with regard to cancer therapy research. Several Wnt inhibitors have been investigated in preclinical studies; for example, Lu *et al* (64) demonstrated that salinomycin is a potent inhibitor of the Wnt signaling pathway and acts by interfering with lipoprotein-related receptor 6 phosphorylation, and an anti-Frizzled antibody is currently being tested as potential cancer therapy (65,66). Although therapies targeting the Wnt signaling pathway are attractive in theory, in practice it has been difficult to create specific therapeutic agents, as numerous components of the Wnt signaling pathways are also involved in other cellular processes. The Wnt/β-catenin signaling pathway has been demonstrated to have crosstalk with other signaling pathways, such as the Hh, NOTCH, Hippo and mammalian target of rapamycin pathways (12,13,67-69). Elucidating the regulatory mechanisms and biological functions of these pathways may reveal potential therapeutic targets for the treatment of tumors.

4. Interaction between Hh and Wnt signaling promotes embryogenesis and cellular differentiation

The network of signaling pathways, including Hh, Wnt, signal transducer and transcription activator (STAT) and NOTCH,

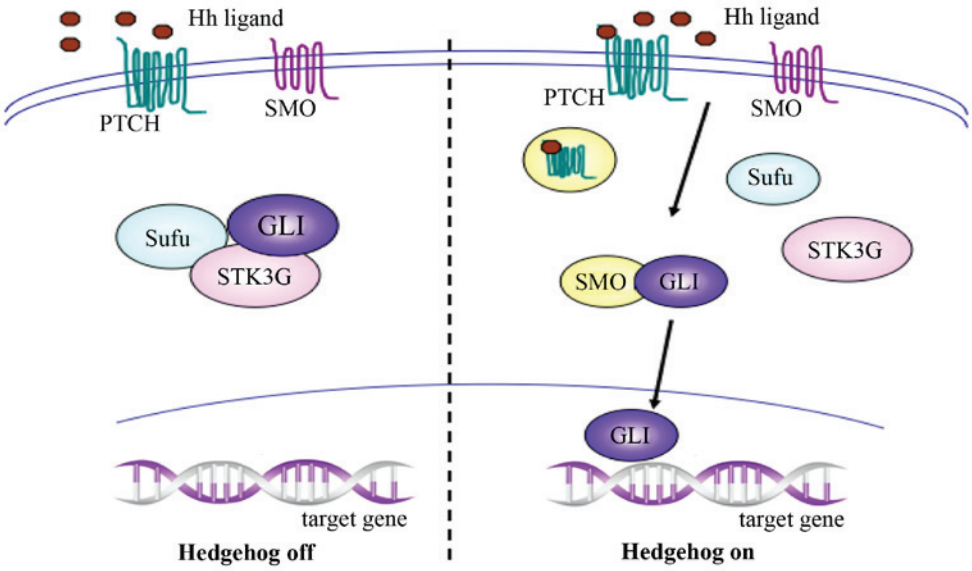

Figure 1. Activation of the Hh signaling pathway results in the activation of SMO and migration of GLI into nucleus. Hh, Hedgehog; SMO, Smoothened; GLI, glioma-associated oncogene homolog; PTCH, Patched; Sufu, Suppressor of fused; STK3, serine/threonine kinase 3.

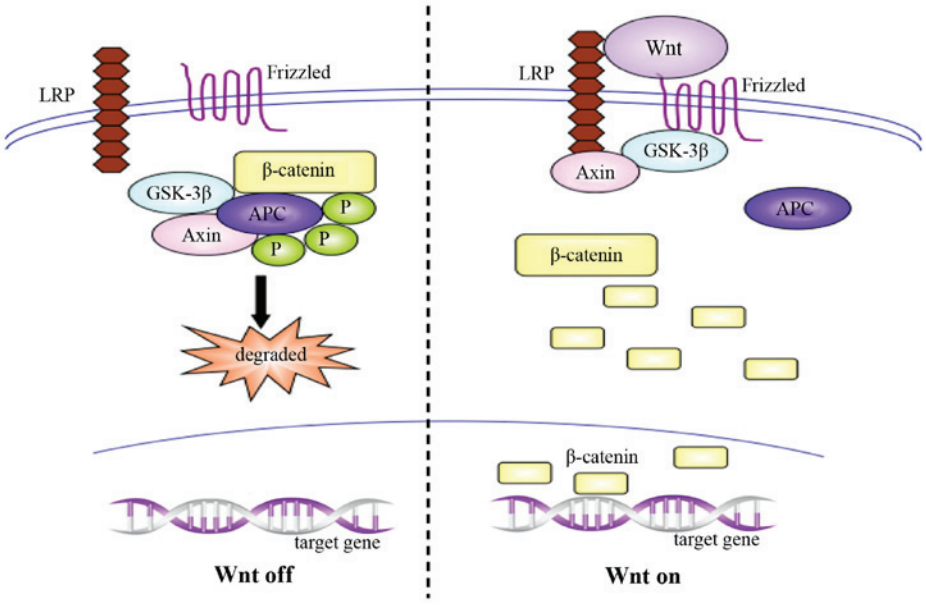

Figure 2. The Wnt signaling pathway leads to the accumulation of β-catenin in nucleus. LRP, lipoprotein receptor-related protein; GSK-3β, glycogen synthase kinase 3β; APC, adenomatous polyposis coli; P, phosphate.

contributes to cellular proliferation and differentiation, and to maintaining the stability of the internal environment (14-16,52-55). In invertebrates and lower vertebrates, activation of Wnt and Hh signaling pathways is crucial in embryogenesis and cellular differentiation (70,71). Previous evidence has indicated that the expression of myogenic basic helix-loop-helix genes in embryonic somites can be induced by the Wnt and Shh signaling pathways (65). Despite complete truncation, the limbs of amphibians show remarkable regeneration through wound healing, blastema formation and tissue differentiation (72-74). Pharmacological research has revealed the integration between Hh and Wnt signaling via active and inhibitory drugs. The Hh pathway acts upstream of Wnt to inhibit the activation of Wnt signaling; however, Wnt activation may rescue the suppressive signaling of Hh in regulating amphibian limb regeneration (75). The synergetic interaction was postulated by Day *et al* (76), who reported that Ihh signaling is activated at an early stage of osteoblast maturation during fracture repair, and that Wnt signaling is subsequently upregulated in differentiated osteoblasts. Further research has indicated that the deletion of the motor protein kinesin family member 3A in dental mesenchyme results in the suppression of Hh and activation of Wnt, affecting incisor and molar development (77). These findings may reveal as association between the two signaling pathways at the gene level. In addition, Oberhofer *et al* (78) investigated Hh and Wnt signaling in the head anlagen and growth zone of early insect embryos, and indicated that Wnt/β-catenin signaling

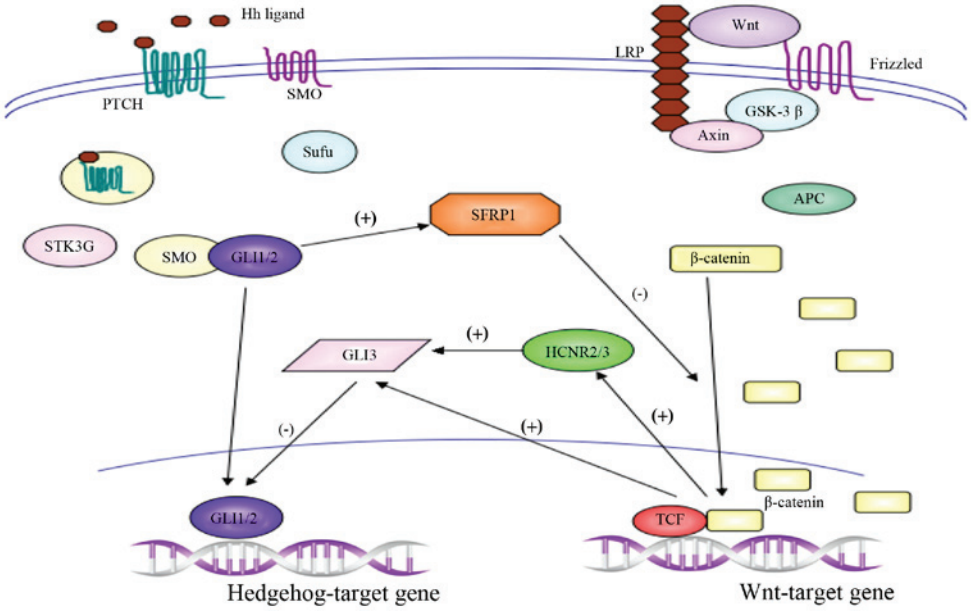

Figure 3. Crosstalk between the Hh and Wnt pathways forms regulatory loops. The Hh signaling pathway negative regulates Wnt activity via SFRP1, and Wnt/β-catenin pathway feedback regulates Hh activity via GLI3 transcriptional regulation. Hh, Hedgehog; SFRP1, secreted frizzled-related protein 1; GLI, glioma-associated oncogene homolog; PTCH, Patched; SMO, Smoothened; Sufu, Suppressor of fused; STK3, serine/threonine kinase 3; LRP, lipoprotein receptor-related protein; GSK-3β, glycogen synthase kinase 3β; APC, adenomatous polyposis coli; HCNR2/3, highly conserved non-coding DNA region 2/3; TCF, T cell factor.

acts upstream of Hh in the growth zone, yet downstream of Hh in the head, anlagen, suggesting the different roles of Hh and Wnt in these two regions (78).

Shin *et al* (79) reported a proliferative response to bacterial infection and chemical injury within the bladder in a mouse model, and demonstrated that the response is regulated by signal feedback between basal cells and stromal cells. In bacterial injury, Shh expression in the basal cells is activated and induces increased Wnt protein expression in the stromal cells. The increased activity of this signal circuit may help prevent the further spread of infection, and stimulate the restoration of urothelial and stromal cells (79). These findings demonstrate an interaction between the Hh and Wnt signaling pathways. Thus, there is evidence to suggest that organisms require a precise balance of these signaling pathways to control proliferation and differentiation.

5. The Hh signaling pathway attenuates Wnt activity through activated SFRP1

Although a number of studies have demonstrated that synergy between the two pathways promotes embryogenesis and cellular differentiation, conflicting data has also been reported that the Hh and Wnt signaling pathways are functionally antagonistic in vertebrates and invertebrates (80-84). The crosstalk protein SFRP1, which acts as an antagonist of Wnt signaling, was initially identified in 1998, and has subsequently been shown to be regulated by Hh in the developing spinal cord and gastric cancer cells (12,80-82). Borday *et al* (83) investigated the potential cross-regulation between the Wnt and Hh signaling pathways in neural stem/progenitor cells in the ciliary marginal zones using pharmacological tools. They detected Wnt activity and subsequently Hedgehog inhibition by 6-bromoindirubin-3'-oxime treatment, which worked as a selective activator of the canonical Wnt pathway. By contrast, Hedgehog signaling restricts Wnt activity, using Smoothened agonist purmorphamine, which was sufficient for activation of Hedgehog signaling. Furthermore, Hh signaling pathway negatively regulates Wnt activity via transcriptional regulation of SFRP1, and the Wnt/β-catenin pathway downregulates Hedgehog activity through Gli3 transcriptional regulation. The reciprocal inhibition between Hh and Wnt signaling pathways regulates a delicate balance between proliferation and differentiation of neural stem cells (83).

Although the Wnt and Hh signaling pathways participate in the physiological processes of cellular proliferation and differentiation, recent research has indicated that the two pathways also serve a crucial role in the pathological processes of various diseases, particularly malignancies. For example, enhanced Shh signaling restricts canonical Wnt signaling in the lambdoidal region by promoting the expression of genes encoding Wnt inhibitors, which is involved in the development of cleft lip (85). In addition, downregulation of Ihh expression may contribute to the activation of Wnt signaling via APC mutation, and subsequently lead to the development of colorectal tumors (86). Further studies have indicated that Wnt signaling may be a downstream pathway of Hh signaling, and that SFRP1 acts as an important cross-point to repress the canonical Wnt signaling pathway and restrict the expression of Wnt target genes. A gene chip assay of squamous cell carcinoma of the uterine cervix revealed that the expression of Hh signaling molecules was significantly increased in cervical intraepithelial neoplasia II/III and carcinoma, while SFRP1 gene expression was silent or low, which strongly suggests that the differential activation of the Wnt and Hh pathways may be involved in the development of uterine cervical carcinoma (87).

Kim *et al* (84) investigated differentiation-associated signal interactions between the Wnt and Hh signaling pathways in

gastric cancer cells, and indicated that the expression levels of Shh signaling components were increased, whereas those of the Wnt signaling pathway were decreased. Further research indicated that ectopic expression of GLI1 increased the level of SFRP1 transcript, and that increased expression of GLI1 decreased nuclear β-catenin staining. By contrast, inhibition of GLI1 reduced SFRP1 expression. Thus, the Shh and Wnt pathways are differentially involved according to the differentiation of gastric cancer cells (84) (Fig. 3). Clinical studies have also demonstrated that the upregulation of Shh protein is associated with age, pathological status, tumor differentiation, depth of invasion, and nodal metastasis of gastric cancer, and Shh protein overexpression is considered a significant independent prognostic factor in gastric cancer (88).

6. Wnt/β-catenin pathway feedback regulates Hh activity through transcriptional regulation of GLI3

GLI3 is known to be a transcriptional repressor of the Hh signaling pathway in the absence of ligand stimulation (89). Alvarez-Medina *et al* (90) investigated dorsoventral neuron development, which is achieved by the combined activity of signaling pathways. In their study, the canonical Wnt signaling pathway was demonstrated to be important in dorsoventral patterning of the spinal cord, and this role was largely dependent on GLI activity. Furthermore, the study revealed that the expression of GLI3 within the dorsal neural tube is directly controlled by Wnt activity, as mice with mutated Wnt1 and Wnt3a exhibited diminished GLI3 expression, and gain and loss of β-catenin/T cell factor (β-catenin/Tcf) function in chick embryos also directly regulated GLI3 expression (90). Furthermore, previous studies characterized four highly conserved non-coding DNA regions (HCNRs) within the human GLI3 locus that work as potential enhancer modules; it was demonstrated that HCNR2 and HCNR3 contain sufficient information to direct the expression of GLI3 in the dorsal spinal cord, and that the activity of these two modules is dependent on β-catenin/Tcf transcriptional activity (90-92). Collectively, these data demonstrate that the Wnt/β-catenin pathway downregulates GLI3 expression, indicating an indirect mechanism initiated by Wnt signaling to repress Shh activity in the dorsal neural tube (90-92).

Borday *et al* (83) performed a pharmacological study using 6-bromoindirubin-3'-oxime (BIO) to selectively inhibit GSK-3, which resulted in a significant increase in GLI3 expression. In addition, treatment with IWR-1, which prevents Axin protein degradation, led to the opposite phenotype. Furthermore, morpholino-mediated GLI3 knockdown could rescue the decreased PTCH1 expression observed in BIO-treated tadpoles (83). This evidence suggests that GLI3 represents a key downstream effector of the Wnt pathway, which may account for its negative effect on Hh activity (82,93,94). Based on the research into the etiological roles of Wnt/β-catenin inhibition of Hh signaling, the preclinical study of Shh-dependent medulloblastoma is in progress, with the aim of developing novel therapeutic strategies for patients (95).

7. Conclusion

As a whole, the network of signaling pathways, such as Hh, Wnt, STAT and NOTCH, is crucial in cellular differentiation and tissue homeostasis, and its dysregulation may result in tumor occurrence and metastasis. Studies from numerous laboratories have made great efforts in exploring the complexity of the regulatory networks and the interaction between the Hh and Wnt signaling pathways. Inappropriate activation of Hh and Wnt signaling has been demonstrated in certain types of cancer. However, the mechanism of interaction between the two signaling pathways remains unclear, and several key questions remain to be addressed. Firstly, research has mainly been limited to cell and animal experiments, and the findings reviewed in the present study must be demonstrated in appropriate preclinical investigations. Secondly, some of the apparently conflicting reports regarding the interactions between the different signaling pathways must be studied and discussed in greater depth.

More than 20 years after the discovery of the Hh and Wnt pathways, we have entered an exciting era of research into these signaling pathways. A single pathway is susceptible to be affected by other pathways, and does not fully represent the entire signaling network. Therefore, further investigation of the crosstalk between different transcriptional signals could overcome this limitation of single pathways, and provide a more comprehensive understanding of the importance of these signaling pathways in the development of cancer. The therapeutic benefits of pathway antagonists are gradually being revealed in clinical studies, and the outcomes may have a far-reaching impact on the design of novel cancer therapies. In summary, the interactions between the Hh and Wnt signaling pathways in malignancies may provide a theoretical basis for potential cancer therapies.

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