Wnt Signaling Pathway in Non–Small Cell Lung Cancer

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Wnt/β-catenin alterations are prominent in human malignancies. In non–small cell lung cancer (NSCLC), β-catenin and APC mutations are uncommon, but Wnt signaling is important in NSCLC cell lines, and Wnt inhibition reduces proliferation. Overexpression of Wnt-1, -2, -3, and -5a and of Wnt-pathway components Frizzled-8, Dishevelled, Porcupine, and TCF-4 is common in resected NSCLC and is associated with poor prognosis. Conversely, noncanonical Wnt-7a suppresses NSCLC development and is often downregulated. Although β-catenin is often expressed in NSCLCs, it was paradoxically associated with improved prognosis in some series, possibly because of E-cadherin interactions. Downregulation of Wnt inhibitors (eg, by hypermethylation) is common in NSCLC tumor cell lines and resected samples; may be associated with high stage, dedifferentiation, and poor prognosis; and has been reported for AXIN, sFRPs 1–5, WIF-1, Dkk-1, Dkk-3, HDPR1, RUNX3, APC, CDX2, DACT2, TMEM88, Chibby, NKD1, EMX2, ING4, and mir-487b. AXIN is also destabilized by tankyrases, and GSK3β may be inactivated through phosphorylation by EGFR. Preclinically, restoration of Wnt inhibitor function is associated with reduced Wnt signaling, decreased cell proliferation, and increased apoptosis. Wnt signaling may also augment resistance to cisplatin, docetaxel, and radiotherapy, and Wnt inhibitors may restore sensitivity. Overall, available data indicate that Wnt signaling substantially impacts NSCLC tumorigenesis, prognosis, and resistance to therapy, with loss of Wnt signaling inhibitors by promoter hypermethylation or other mechanisms appearing to be particularly important. Wnt pathway antagonists warrant exploration clinically in NSCLC. Agents blocking selected specific β-catenin interactions and approaches to increase expression of downregulated Wnt inhibitors may be of particular interest.


Lung cancer (of which 80% to 85% is non–small cell lung cancer [NSCLC]) is the world’s leading cause of cancer death (1). In both the United States and Canada, not only is lung cancer by far the leading cause of cancer death (2,3), but also calculations from published data (2,3) reveal that it is the second overall leading cause of death from any cause after heart disease. The 5-year relative survival rate for lung cancer is only 17% (2,3) because in a high proportion of patients the disease is already metastatic at diagnosis or else it recurs after initial surgery or radiotherapy. Metastatic NSCLC is generally incurable because it will either have intrinsic resistance to chemotherapy or else will develop acquired resistance after an initial response (4). Cancer stem cells may be highly resistant to chemotherapy and may play an important role in the incurability of advanced NSCLC (4). The Notch, Hedgehog, and Wingless-type protein (Wnt) signaling pathways may each play a role in maintaining cancer stem cell populations and, as such, may play an important role in chemotherapy resistance (4). In this review, we will focus on the role of the Wnt pathway in NSCLC, concentrating primarily on Wnt pathway components that have been reported to be increased, decreased, or associated with cell line growth, cell survival, or patient prognosis in NSCLC.

Overview of the Wnt Canonical (β-Catenin) and Noncanonical Signaling Pathways

The Wnt signaling pathway helps maintain cancer stem cells, and putative stem cell markers such as LGR5/GPR49 (5), CD44 (5), CD24 (5), Epcam (5), and OCT-4 (6) are Wnt targets. The major (canonical) Wnt pathway signals through β-catenin. Wnt also signals through secondary (noncanonical) pathways, including the planar cell polarity pathway (5), the Wnt/Ca++ flux pathway (5,7), the protein kinase A pathway (5), c-Jun N-terminal kinase (JNK) (7), and the small GTPases Rho, Rac, and Cdc 42 (7). There are 19 Wnt proteins in mammalian cells (7). In the absence of Wnt, a β-catenin destruction complex consisting of Axis inhibition protein (AXIN), adenomatous polyposis coli (APC), and glycogen synthase kinase 3β (GSK3β) phosphorylates β-catenin, leading to its proteolytic degradation (5). If Wnt is present and available (Figure 1), it binds to members of the Frizzled (FZD) family of receptors (7), forming a stable receptor complex between Wnt, FZD, lipoprotein receptor–related protein (LRP), Dishevelled (Dvl), and AXIN (5). This phosphorylates Dvl, which then inhibits GSK-3β, thereby reducing phosphorylation/proteolytic destruction of β-catenin (5). Hence, cytoplasmic levels of β-catenin rise, and β-catenin migrates to the nucleus and complexes with members of the T-cell factor (TCF)/Lymphoid enhancer-binding factor (LEF) family of transcription factors (5). Basal transcription machinery and transcriptional coactivators are then recruited, including cAMP response element–binding protein (CREB)–binding protein (CBP) or its homolog p300 (5). This leads to expression of various target genes, including cyclin D1 (6,7) and c-Myc (7). The transmembrane receptor tyrosine kinase ROR2 (which plays a role in noncanonical Wnt signaling) may also be involved in canonical signaling through an interaction with FZD2 (8).
Overview of Wnt Pathway Inhibitors

There are also several Wnt inhibitors (Figure 2), including secreted frizzled-related proteins (sFRPs) (which compete with Wnt for binding to FZD) (7), Wnt inhibitory factor 1 (WIF-1) and Cerberus (which bind Wnt) (7), Disabled 2 (Dab2) (5), members of the Dickkopf (Dkk) family (secreted glycoproteins that inhibit Wnt signaling by binding to the LRP5/LRP6 component of the Wnt receptor complex) (7), and the Dvl antagonists Idax [coded by the CXXC4 gene (9)] and human homolog of Dapper (HDPR1) (10). Some members of the Wnt family itself, such as Wnt-5a and -5b,
may also block canonical Wnt signaling (11,12) by promoting the degradation of β-catenin through a GSK-3β-independent process (12). Anti-inflammatory agents, vitamins A and D, various plant-derived polyphenols (eg, curcumin), and some small molecule targeted agents may also inhibit the Wnt/β-catenin pathway (5). The Wnt pathway inhibitor sFRP-1 also downregulates the transforming growth factor β1 induction of epithelial–mesenchymal transition in NSCLC cell lines (13).

Wnt and Lung Cancer Tumorigenesis

In murine models, activation of Wnt signaling is associated with increasing tumor initiation potential (14), and there is growing evidence that the Wnt pathway is important in the development of NSCLC. In cultured respiratory epithelium, cigarette smoke components upregulated Wnt (15,16) and Hedgehog (15) signaling. Wnt pathway genes are also upregulated in the lungs of Kras transgenic mice (17), and Wnt pathway activation in Kras mutant mice markedly increases tumorigenesis (18). In Wnt-inducible mice, tumors regressed when Wnt induction was stopped, although tumors became Wnt independent in p53-deficient mice (19).

Wnt Signaling and Wnt Expression in NSCLC

Several Wnt pathway components (Figure 1) may be overexpressed in NSCLC (Table 1). A majority of NSCLC cell lines have active Wnt signaling (20) or Wnt-1 overexpression (6,21,22). Wnt signaling may be promoted in lung cancer cells by the sulfatases Sulf-1 and Sulf-2 (which are expressed in a high proportion of NSCLCs) (23), by miR21 (24), and by peristin (25). Of resected NSCLCs, 37% to 63% stained positively for Wnt-1 by immunohistochemistry (21,26–29), and Wnt-1 positivity was associated with a low apoptotic index (29), aberrant β-catenin expression (28,30), and increased expression of c-Myc (26,28,30), Cyclin D1 (28,30), VEGF-A (30), MMP-7 (30), Ki-67 (26,29,30), survivin (29), and the intratumoral microvessel density (30). Wnt pathway activation (31) and overexpression of Wnt-1 (26–30) are also associated with poor prognosis clinically. Downregulation of Wnt signaling by anti-Wnt monoclonal antibody (21) or small interfering RNA (siRNA) (21) or by other methods (22,32) induced apoptosis in cancer cells that expressed Wnt-1 (21), inhibited NSCLC cell line proliferation (22) and xenograft growth (21,32), reduced cell motility and invasion (32), and induced a more differentiated phenotype (22).
Table 1. Alteration of Wnt pathway components in non–small cell lung cancer*

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<thead>
<tr>
<th>Increased expression in resected NSCLC</th>
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<tr>
<td>Wnt-1</td>
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<td>Destabilization by tankyrases</td>
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<td>Engagement of polycomb machinery</td>
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<td>Inactivation by phosphorylation by EGFR</td>
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<td>Nemo-like kinase</td>
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* APC = adenomatous polyposis coli; AXIN = Axis inhibition protein; CDX = caudal type homeobox; DACT = Dapper, antagonist of β-catenin, homolog; Dkk = Dickkopf; EMX = empty spiracles homeobox; GSK3β = glycogen synthase kinase-3β; HDRP1 = human homolog of Dapper; ING = inhibitor of growth family; NKD = naked cuticle homolog; NSCLC = non–small cell lung cancer; RUNX = Runx-related transcription factor 3; sFRP = secreted frizzled-related protein; TCF = Tcell factor; TMEM = transmembrane protein; WIFI = Wnt inhibitory factor; Wnt = Wingless-type.

NSCLCs may also overexpress Wnt-2 (33), Wnt-3 (34), Wnt-5a (25,34,35), and Wnt-11 (35). Wnt-2 expression correlated with FZD-8 expression in NSCLC tumor samples, and Wnt pathway activation required coexpression of Wnt-2 and FZD-8 in selected NSCLC cell lines (36). In NSCLC cell lines (33,36,37) and xenografts (36,37), inhibition of Wnt-2 by monoclonal antibody (33), siRNA (33), short hairpin RNA (shRNA) (37), or a dominant-negative construct (36) induced apoptosis (33,37), decreased colony formation (36), decreased xenograft growth (36,37), decreased expression of Wnt target genes (36), downregulated cytotoxic expression of β-catenin and survivin (33), and decreased TCF-dependent transcriptional activity (33).

Wnt-3 gene expression in resected NSCLC was statistically associated with high Ki67, low apoptosis, and high expression of c-Myc and survivin (34), and [as with Wnt-1 (26–30), overexpression was associated with poor prognosis (34). Similarly, Wnt-5a gene expression was associated with Ki67 expression in resected lung cancers (38). It was also associated with poor prognosis in one study (particularly in squamous carcinomas) (38), although it was not associated with Ki67 expression or survival in another study (29). Unlike Wnts-1, -2, and -3, Wnt-5a is a noncanonical Wnt that acts predominantly through Dvl-3 (39) and blocks canonical Wnt signaling (11) through activation of the Wnt calcium flux pathway (with calcium/calmodulin kinase [CaMK] inhibition of β-catenin/TCF) (40); it may also signal through the Wnt planar cell polarity/convergent expression pathway and various other pathways (40) (Figure 3). Wnt-5a overexpression was particularly common in squamous cell carcinomas (35) and in smokers (41), and this overexpression was associated with downregulation of canonical Wnt signaling (35). Cigarette smoke exposure leads to upregulation of expression of Wnt-5a in cell lines (41), and its expression in lung adenocarcinomas may be regulated in part by miR145 (42). Transfection of Wnt-5a into NSCLC cell lines stimulated cell proliferation, whereas Wnt-5a siRNA suppressed proliferation (43). Wnt-5a may also decrease cellular adhesion by reducing cadherin expression (35) and may protect cells from apoptosis by activation of protein kinase C and Akt (41). Overall, some Wnt-5a actions (such as blocking canonical Wnt signaling) might be expected to foster decreased tumor aggressiveness, whereas others might be expected to foster increased aggressiveness, but the net impact appears to be one of increasing aggressiveness.

Wnt-11 [another noncanonical Wnt that may signal through the planar cell polarity/convergent extension pathway (40) and that may promote activation of multiple kinases (44)] may also be overexpressed in NSCLC, particularly in squamous cell carcinomas, and this overexpression may be associated with downregulation of canonical Wnt signaling and with decreased cellular adhesion (by reduction of cadherin expression) (35).

Wnt-7a

In normal tissues, Wnt-7a promotes neuronal differentiation (45). Unlike the upregulation of expression of some other Wnt family members, Wnt-7a is downregulated in most lung cancer cell lines and tumors (46). Additionally, decitabine-reversible methylation of the promoter of Wnt-7a is frequent in NSCLC (47), loss of Wnt-7a leads to increased cell growth in NSCLC cell lines (47), and NSCLC cell line transfection with Wnt-7a reversed cellular transformation through interaction with Fzd-9 (46). Combined expression of Wnt-7a and Fzd-9 in NSCLC cell lines led to ERK5 activation (which in turn led to increased PPARγ expression) (48), inhibited tumor growth (48), and (by activation of Sprouty-4) reversed epithelial–mesenchymal transition (Figure 4) (49). Wnt-7a and Fzd-9 do not activate β-catenin (46), but they do upregulate expression of miR-29b in NSCLC cell lines (which may suppress NSCLC cell growth by targeting MDM2) (50), and they activate JNKs (46,51). JNKs are mediators of Wnt-regulated epithelial cell programs, including planar cell polarity (51) [which may be particularly relevant in squamous cell lung cancers (52)] and convergent extension (51). The JNK pathway promotes an epithelial cell differentiation program in lung cancer cells (51), with induction of E-cadherin.
and Sprouty-4 expression (46). The induction of E-cadherin by Wnt-7a in lung cancer cell lines may be potentiated by histone deacetylase inhibition (53).

**β-Catenin**

β-Catenin was expressed in 94% of resected squamous cell lung cancer samples and in 51% of adenocarcinomas (54). Nuclear accumulation of β-catenin was associated with epidermal growth factor receptor (EGFR) mutations (55), and β-catenin overexpression was associated with NSCLC cell line resistance to gefitinib (56). Increasing extracellular matrix metalloproteinase inducer (EMMPRIN) levels in lung cancer cells upregulated the β-catenin signaling pathway, and silencing EMMPRIN inhibited β-catenin signaling, cell migration, proliferation, anchorage-independent growth, and xenograft growth (57). β-Catenin may

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**Figure 3.** Wingless-type protein 5a (Wnt-5a) noncanonical Wnt signaling. Wnt-5a can activate a number of different pathways via the Wnt–calcium flux pathway and the planar cell polarity/convergent extension pathway (which can also be activated by Wnt-11). Wnt-5a signaling inhibits the canonical Wnt signaling pathway but promotes tumor cell growth through some of its other actions. CaMK = calcium/calmodulin kinase; FZD = Frizzled; JNK = cJun N-terminal kinase; PKC = protein kinase C; Rac = Ras-related C3 botulinum toxin substrate; Rho = Ras homolog family; ROR = receptor tyrosine kinase-like orphan receptor; TCF = T-cell factor.

**Figure 4.** Wingless-type protein 7a (Wnt-7a) noncanonical Wnt signaling. Unlike other Wnts, Wnt-7a inhibits tumor growth. It antagonizes tumor cell growth through a variety of mechanisms, including by increasing tumor protein 53 (p53) and E-cadherin availability and by antagonizing epithelial–mesenchymal transition (EMT). ERK = extracellular-signal-regulated kinase; FZD = Frizzled; JNK = cJun N-terminal kinase; MDM2 = mouse double minute 2 homolog; PPARγ = peroxisome proliferator-activated receptor gamma.
also be dysregulated in lung cancer through interaction with Wolf–Hirschhorn syndrome candidate 1 (58). Sulindac suppressed β-catenin expression in lung cancer cells, downregulated transcriptional targets of β-catenin (c-Myc, cyclin D1, and cdk 4), and inhibited proliferation (59).

Paradoxically, despite these cell line observations, loss of β-catenin expression in NSCLC tumors was associated with increased tumor size, stage, and grade clinically (54), and β-catenin overexpression was associated with improved prognosis (rather than worsened prognosis) in some (54,60–62), but not all (28,63,64), studies, possibly because of a negative impact of β-catenin loss on E-cadherin function (54). Unlike in colon cancer, β-catenin mutations are uncommon in lung cancers (7,60) or lung cancer cell lines (65,66).

Other Factors Potentiating Wnt Pathway Signaling

FZD-8 is commonly overexpressed in both NSCLC cell lines and tumor samples, and FZD-8 inhibition by an shRNA decreased NSCLC cell line proliferation and xenograft growth (67). Similarly in immunohistochemistry assessments, Dvl expression was noted in 53.1% of resected NSCLCs (including 36.3% for Dvl-1, 36.3% for Dvl-2, and 41.6% for Dvl-3), whereas normal tissues were negative (68). Expression levels were higher in adenocarcinomas than in squamous carcinomas and were associated with poor differentiation (68). Dvl-1 and -2 were associated with higher stage, and Dvl-1 and -3 had higher expression in nodal metastases than in primary tumors (68). Expression of both Dvl-1 and -3 was associated with poor prognosis (69). Dvl-1 expression was associated with β-catenin expression (68,69), whereas Dvl-3 was associated with p120ctn expression (69). In lung cancer cell lines, exogenous expression of Dvl-1 and -3 enhanced invasiveness (68), Dvl-1 overexpression enhanced TCF-dependent transcriptional activity and β-catenin expression (69), and inhibition of Dvl-1, -2, and -3 inhibited growth (70). Unlike Dvl-1 and -2, Dvl-3 appeared to act primarily through p38 and JNK (noncanonical) Wnt pathways (69).

The porcupine gene and its human homolog, PPN/MG61, encode an endoplasmic reticulum membrane omicron-acetyltransferase involved in post-translational processing and secretion and function of Wnt signaling molecules (71–73), and PPN/MG61 is overexpressed in resected NSCLCs (72). PPN/MG61 expression correlated with expression of β-catenin, HIF-1α, and jun B (71), and PPN/MG61 siRNA induced apoptosis and reduced Wnt pathway activity in lung cancer cell lines (72).

Host genotype single nucleotide polymorphisms for the Wnt signaling mediator TCF-2 were associated with the risk of developing lung cancer (74), and tumor expression of TCF-4 was associated with NSCLC tumor type and with node metastases (75). Wnt/TCF activation was associated with relapse (76), development of brain metastases (64), and short survival (64) in lung adenocarcinomas, and TCF activity in lung adenocarcinoma cell lines was associated with their ability to form brain and bone metastases in mice (76). The Wnt/TCF target genes HOXB9 and LEFT1 are mediators of chemotactic invasion and colony outgrowth (76).

In resected NSCLC samples, increased immunohistochemical expression of the Wnt signaling transcriptional complex component Pygopus 2 was associated with poor differentiation, high stage, and poor prognosis, whereas Pygopus 2 downregulation in NSCLC cell lines was associated with decreased cell proliferation and increased apoptosis (77).

Wnt Pathway Inhibitors in NSCLC

Loss of Wnt inhibitors may play a major role in NSCLC (Table 1), although unlike in colon cancer, loss-of-function mutations in the Wnt pathway inhibitor APC are uncommon in NSCLC (7,78). AXIN is a negative regulator of Wnt/β-catenin signaling, and in resected NSCLC, AXIN expression was negatively associated with expression of the Wnt pathway transcription factor TCF-4 (75). Allelic loss and rearrangement is common in the chromosome area (17q24) containing AXIN2 (79), and the host genotype for codon 50 of AXIN2 was associated with the risk of developing lung adenocarcinomas (79). AXIN is destabilized by tankyrase-1 and -2 (poly-ADP-ribose polymerase enzymes that may be deregulated in NSCLC), and treatment of lung cancer cell lines with tankyrase inhibitors reduced cell growth (80), reduced tumor growth in xenograft models (80), increased AXIN1 levels (80), repressed expression of a Wnt-responsive luciferase construct (80), and increased the efficacy of EGFR inhibitors (81).

GSK3β represses the Wnt canonical signaling pathway and plays a role in regulating the balance between proliferation and apoptosis (82). EGFR may phosphorylate GSK3β into inactive p-GSK3β-ser9 (82). In NSCLC samples, p-GSK3β-ser9 expression was negatively associated with PTEN and Ki67 expression and positively associated with EGFR expression (82). P-GSK3β-ser9 and EGFR expression in NSCLC tumor samples was associated with short survival (82).

A high proportion of NSCLC cell lines (20,83–92) and resected lung cancers (10,20,55,84,87,89,90,93–107) have loss of gene heterozygosity (84,108), aberrant methylation (20,55,83–85,87–94,96,98,100,101,106), and/or downregulation of expression by unspecified mechanisms (10,20,84,86,87,93,95–97,99,102–105,107) of various Wnt antagonists, including AXIN (106), sFRP-1 (20,55,84,85,93,98), sFRP-2 (20,55,98), sFRP-4 (85), sFRP-5 (20,55,85,98,101), WIF-1 (20,55,83,92,95–98), Dkk-1 (20,100), Dkk-3 (20,55,86,87,98,99), HDPR1 (10), RUNX3 (20), APC (20,94,98,108), CDX2 (89), DACT2 (90), TMEM88 (102), Chibby (103), Naked1 (NKD1) (104), EMX2 (91), ING4 (which is decreased predominantly in lung adenocarcinomas) (105), nemo-like kinase (107), and miR-487b (88). Similarly, knockdown of HDPR1 enhanced the invasive ability of lung cancer cells (10). Exposure of NSCLC cell lines to tobacco smoke repressed the Wnt pathway inhibitor miR-487b (35) and engaged polycomb machinery to decrease expression of Dkk-1, activate Wnt signaling, and increase cell line tumorigenicity (109).

Aberrant methylation of Wnt antagonists was increased in the presence of Kras mutations (55). Clinically, WIF-1 methylation or downregulation was particularly common in squamous cell lung carcinomas (97) and in patients with chronic obstructive pulmonary disease (98), whereas methylation of sFRP-2 was most prevalent in women, nonsmokers, and adenocarcinomas (55). Promoter
hypermethylation of WIF-1 (98,110), AXIN (106), sFRP-1 (93,98), sFRP-5 (98,101), Dkk-3 (55,98), and DACT2 (90) decreased expression of NKD1 (104), HDPR1 (10), and nemo-like kinase (107), and loss of heterozygosity of APC (108) was associated with increased probability of node metastases (93,104,106), poor differentiation (90,104,106,107), poor prognosis (10,55,93,98,104,107,110), development of brain metastases (108), reduced benefit of EGFR tyrosine kinase inhibitors (101), and increased p120-catenin and β-catenin expression (10) in some NSCLC groups. Conversely, despite Dkk1 being a Wnt pathway inhibitor, serum Dkk1 levels were elevated in lung cancer patients, increased with stage and with metastases, and were negatively associated with survival (111). Dkk1 methylation was associated with a more favorable prognosis (100).

In lung cancer cell lines, miR-29 family members (which negatively regulate the DNA methyltransferases DNMT3A and DNMT3B) were associated with decreased promoter methylation and increased expression of WIF-1 (92), whereas methylation inhibitors demethylated WIF-1 (83,112), CDX2 (89), and DACT2 (90), restored or increased expression of WIF-1 expression (83,112) and DACT2 (90), reduced cytostatic β-catenin and TCF reporter activity (83), derepressed miR-487b (35), inhibited the canonical Wnt pathway (112), suppressed tumor proliferation (90), and induced apoptosis (112). Similarly, transfection with or restoration of function/expression of WIF-1 (113), ING4 (105), EMX2 (91), Dkk-3 (114), or AXIN (75) increased apoptosis (75,113), inhibited cell line proliferation (75,91,105,113), inhibited xenograft growth (114), decreased cell invasiveness (75,91), inactivated the canonical Wnt pathway (105), upregulated p27 (105), and downregulated cyclin D1, SKP2, and COX2 (105). Dkk-3 also acted as a proapoptotic protein in lung adenocarcinoma cells by decreasing intracellular levels of reactive oxygen species (115).

Wnt and Drug Resistance
Cancer cells expressing Wnt-1 are resistant to therapy-induced apoptosis (6,116), and Wnt/β-catenin signaling induces transcription of resistance factors such as MDR-1 (5), survivin (5), and Livin (117). In the A549 NSCLC cell line, Wnt pathway activation was associated with resistance to cisplatin (118,119). NSCLC heterotransplants that survived exposure to platinum-based chemotherapy had increased expression of Wnt pathway genes, suggesting a role for these genes in resistance (120). Exposure of A549 cells to the Wnt inhibitor GDK-100017 downregulated expression of Wnt pathway target genes and inhibited cell proliferation while also enhancing radiosensitivity (121). Inhibition of the Wnt ligand FZD-8 sensitized NSCLC cells to docetaxel (67), whereas restoration of expression of the Wnt antagonist EMX2 restored NSCLC cell line sensitivity to cisplatin (91), and restoration of expression of the Wnt antagonist ING4 enhanced sensitivity to both radiation and chemotherapy (105).

Hence, demonstration of expression of Wnt pathway components could help identify patients who were particularly likely to be resistant to chemotherapy and radiotherapy. However, with the limited exception of biomarkers that may help guide selection of targeted agents in advanced NSCLC [eg, presence of EGFR activating mutations as a guide to therapy using EGFR inhibitors (122)], to date, none of the numerous molecular biomarkers associated with therapy efficacy preclinically or clinically in NSCLC have proven useful in making patient management choices in other situations (4).

How Wnt Pathway Changes in NSCLC Compare With Those in Other Cancers
Wnt pathway alterations are common across a range of human cancers. Although some of the epigenetic changes affecting Wnt inhibitors in NSCLC are similar to those seen in some other malignancies, the Wnt pathway mutations seen in some other cancers are very uncommon in NSCLC (123). As recently reviewed by Polakis (123), mutations in the β-catenin gene CTNNB1 may be seen in hepatocellular carcinomas, Wilms' tumors, and medulloblastomas, APC mutations are present not only in colorectal carcinomas arising in patients with familial adenomatous polyposis but also in a high proportion of sporadic colorectal carcinomas, mutations in Axin1 and II are seen in hepatocellular carcinomas and colorectal carcinomas, CBP mutations have been reported in acute lymphoblastic leukemia and B-cell lymphomas, missplicing and in-frame deletions have been reported for GSK3β in chronic myelogenous leukemia and for LRP5 in breast and parathyroid tumors, TCF4 mutations are common in colorectal cancers with microsatellite instability, and WTX mutations have been noted in Wilms' tumors.

Wnt Pathway Components and NSCLC Patient Prognosis
As noted previously, Wnt pathway activation (31) and overexpression of various Wnt pathway components [eg, Wnt-1 (26–30), Wnt-3 (34), Wnt-5a (38), Dvl-1 (69), Dvl-3 (69), TCF-4 (64,75,76), and Pygopus 2 (77)] are associated with poor prognosis in NSCLC patients. [Of interest, unlike these factors, increased expression of β-catenin may be paradoxically associated with improved prognosis, rather than with poor prognosis (54,60–62), and this favorable association with prognosis may be mediated through its effect on E-cadherin function (54).] There is also an association with poor prognosis of promoter hypermethylation, decreased expression, loss of heterozygosity, and inactivation of various Wnt pathway inhibitors [eg, WIF-1 (98,110), AXIN (106), sFRP-1 (93,98), sFRP-5 (98,101), Dkk-3 (55,98), DACT2 (90), NKD1 (104), HDPR1 (10), APC (108), GSK3β (82), and nemo-like kinase (107)]. The association with clinical outcome of expression of Wnt pathway components and inactivation of Wnt inhibitors supports the concept that Wnt pathway signaling is important in NSCLC biology, although, as with most other NSCLC prognostic biomarkers, none of the Wnt pathway markers can currently predict outcome with sufficient certainty to permit them to guide approaches to patient management.

Wnt as a Therapeutic Target in NSCLC
Because the Wnt pathway is important biologically and prognostically in NSCLC, it could also potentially prove useful as a therapeutic target. As noted previously, inhibition of Wnt-1 (21,22,32), Wnt-2 (33,36,37), Wnt-5a (43), FZD-8 (67), and Dvl-1, -2, and -3
(70) was effective therapeutically in NSCLC cell lines and/or xenografts, as was restoration of expression or function of the Wnt pathway inhibitors Wnt-7a (47), AXIN (75,80), WIF-1 (83,112,113), DACT2 (90), ING4 (105), EMX2 (91), and Dkk-3 (114). It was initially felt that it would be difficult to block the Wnt pathway clinically because of the nature of the protein–protein interactions, because of the potential toxicity to gastrointestinal and bone marrow stem cells (124), and because of a possible negative impact on cell adhesion (125). However, a variety of Wnt pathway inhibitors have entered early clinical trials, including OMP-18R5 (a monoclonal antibody binding with FZD receptors that has activity against a variety of human tumor xenografts) (126,127), OMP-54F28 (an FZD-8/human immunoglobulin fusion protein that sequesters Wnt ligands) (127), LGK974 (a small molecule Porcupine inhibitor) (73,127), and PRI-724 (a small molecule that inhibits binding of β-catenin to CREB binding protein) (127,128).

Additional Wnt pathway inhibitors are in preclinical development, including the tankyrase inhibitors XAV939 (127,129), JW55 (127,130), G007-LK (131), and G244-LM (131) and agents targeting β-catenin/TCF interactions (125), casein kinase 1α (125), and β-catenin (125). Because high β-catenin expression has been associated with good outcome rather than with poor outcome in NSCLC patients (54,60–62), it could potentially prove important to target specific downstream β-catenin functions (such as those mediated by binding to CREB binding protein and TCF) rather than using agents that could directly suppress β-catenin levels through upstream targeting of the Wnt pathway.

The difficulty of developing clinically useful Wnt inhibitors in NSCLC is increased by the fact that there have been no reliable biomarkers to predict sensitivity to Wnt inhibition (132). When the optimal predictive biomarkers are uncertain, the most effective way to identify them is to conduct detailed molecular/genetic comparisons of responders (if any) to nonresponders in early clinical trials. Evidence to date suggests that this is a much more efficient way to discover predictive biomarkers than use of survival endpoints (133).

With other types of targeted agents, activating mutations or gene amplification are particularly likely to confer sensitivity (133), but mutations and gene amplification in Wnt pathway components appear to be uncommon in NSCLC. In pancreatic cancer cell lines, presence of inactivating mutations of the Wnt inhibitor RNF43 have been associated with sensitivity to the Porcupine inhibitor LGK974 (132), but there are no data published on whether RNF43 mutations are also present in NSCLC, and the extent to which loss of other Wnt pathway inhibitors through hypermethylation and so on will be associated with sensitivity to therapeutic agents that inhibit the Wnt pathway remains unknown.

It is also unclear whether it will prove feasible to directly restore lost Wnt inhibitor function. Because AXIN loss in NSCLC is predominantly through promoter hypermethylation and allelic loss or rearrangement, it is uncertain whether the tankyrase inhibitors that may stabilize AXIN (80) will prove useful because AXIN stabilization alone may be insufficient if AXIN levels are too low. Because demethylating agents may increase expression of the Wnt pathway inhibitors WIF-1 (83,112), DACT2 (90), and miR-487b (35), they are also worth exploring. Demethylating agents have demonstrated clinical therapeutic activity in advanced NSCLC when used alone (134) or when combined with a histone deacetylase inhibitor (135).

The demethylating agent decitabine may increase the expression of a number of different factors in human solid tumors (136,137), including the tumor suppressor genes FHIT, WWOX, and TUSC2 (D.J. Stewart, unpublished data). It remains unknown whether demethylating agents will increase expression of Wnt pathway inhibitors clinically and whether any therapeutic impact is related to an effect on Wnt inhibitors, but this warrants further assessment.

Similarly, introduction of genes for Wnt inhibitors into deficient cells also has activity in preclinical systems (75,105,113,114). Previous methods using adenoviral vectors have not been able to achieve systemic delivery of genes for gene therapy clinically, but intravenous administration of the tumor suppressor gene TUSC2 in DOTAP nanoparticles successfully delivered the gene to metastatic tumor deposits (138) and might be explored as a mechanism to restore lost function of Wnt inhibitors.

**Conclusion**

Wnt signaling is complex, with potential interactions with several other pathways and with production of factors that may promote tumor aggressiveness as well as those that may inhibit it. Overall, the Wnt pathway appears to be important in NSCLC tumorigenesis and prognosis, and apart from the exception of Wnt-7a signaling, the overarching impact of Wnt appears to be to promote tumor aggressiveness and resistance to chemotherapy and radiation. Inhibition or loss of Wnt pathway inhibitors is a particularly prominent feature of NSCLC. The frequency of Wnt pathway alterations in NSCLC suggests that it is worth exploring the targeting of the Wnt pathway as a therapeutic option in advanced NSCLC.

**References**

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