

Wnt Pathway Activation in Mesothelioma: Evidence of Dishevelled Overexpression and Transcriptional Activity of β -Catenin¹

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ABSTRACT

Malignant pleural mesothelioma is a relatively uncommon and yet incurable tumor. The pathogenesis of mesothelioma remains poorly understood. This study evaluated the role of Wnt signaling in mesothelioma. Western blot analysis was conducted to confirm the expression of Dishevelled (Dvl) and cytosolic β -catenin in matched autologous tissue samples (tumor and normal pleura), malignant pleural effusions, and in established mesothelioma cell lines LRK1A, REN and H513. Thirteen of 15 mesotheliomas examined showed consistent overexpression of Dvl and increased cytosolic β -catenin levels as compared with controls. To evaluate T-cell factor (Tcf)-dependent transcriptional activity of β -catenin, luciferase assays were conducted. Fresh mesothelioma cells (effusion derived), as well as LRK1A, REN, and H513 cell lines showed a significant fold increase (1.5–2.4-fold, $P < 0.01$) in Tcf-dependent transcriptional activity of β -catenin. To evaluate the biological significance of Dvl function in mesothelioma, a PDZ domain deletion mutant (Δ PDZ-Dvl) was created and stably transfected into LRK1A, REN, and H513. The effect of Δ PDZ-Dvl on mesothelioma growth was assayed *in vitro* (colony formation assay in soft agar) and *in vivo* (s.c. implantation in athymic mice NCRNU-M). In mesothelioma cells tested, Δ PDZ-Dvl-mediated inhibition of Dvl decreased cytosolic β -catenin levels, diminished Tcf-mediated transcription, and suppressed tumorigenesis of LRK1A and REN *in vitro* and *in vivo*. Δ PDZ-Dvl also down-regulated expression of c-myc in REN and COX-2 in H513. Our data suggest that in malignant pleural mesothelioma, Wnt signaling is activated through Dvl overexpression and downstream signaling through β -catenin. Inhibition of this signaling leads to significant antitumor effects. These results demonstrate Dvl overexpression in human cancer and, specifically, that Wnt signaling plays a role in mesothelioma pathogenesis. These data offer possible new avenues for therapeutic intervention.

INTRODUCTION

Malignant pleural mesothelioma is an asbestos-related malignancy characterized by rapidly progressive and diffusely local growth, late metastases, and death. Approximately 3000 new patients are diagnosed in the United States annually. The incidence of this disease worldwide is rising and is expected to peak in the next two decades (1). The mechanism by which mesothelial cells undergo neoplastic transformation is largely unknown, although recent evidence suggests a multistep process involving both activation of oncogenes and inactivation of tumor suppressor genes (2). Unfortunately, the results of standard therapies (surgery, chemotherapy, and radiation) remain disappointing. Newer therapies based on an improved molecular understanding of mesothelioma are needed (3).

Previously, we have analyzed, by cDNA array, differential gene expression in early-stage, resected human mesothelioma. We found

consistent evidence of Wnt pathway activation; a pathway not previously known to be active in mesothelioma and, typically, only active in normal cells during development. Wnt signaling results in β -catenin accumulation and transcriptional activation of specific target genes during development. Recently, Matsuzawa and Reed (4) and Liu *et al.* (5) demonstrated that Siah-1 links the p53 pathway to β -catenin and promotes its degradation (4, 5). Most malignant pleural mesothelioma (>80%) have wild-type p53 but homozygous deletion of p14, resulting in functional inactivation of the p53 pathway. Inactivation of the p53 pathway may promote accumulation of β -catenin. Dysregulation of β -catenin signaling because of primary β -catenin mutation is an important event in the genesis of several human malignancies (6) but is not found in malignant pleural mesothelioma.

The Dvl³ family proteins (Dsh in *Drosophila*, Dvl in mammals) are membrane-proximal signaling intermediates in the Wnt pathway (7–11). Downstream of Dvl is the enzyme GSK3 β , a serine-threonine kinase that is a negative regulator of insulin and Wnt signaling (12). In the absence of a Wnt signal, GSK3 β phosphorylates β -catenin and induces its ubiquitination and proteolytic degradation (13, 14). The presence of a Wnt signal inhibits GSK3 β , which stabilizes β -catenin, allowing it to translocate to the nucleus, where it acts as an essential cofactor for Tcf/Lef-dependent transcription (15, 16). β -Catenin-Tcf/Lef induces transcription of important downstream target genes, *c-myc* (17), *cyclin D1* (18), among others, many of which have been implicated in cancer.

To confirm the activity of Wnt signaling in mesotheliomas, we performed Western blotting of Dvl and cytosolic β -catenin in fresh and cultured mesothelioma cells. In addition, we evaluated the transcriptional activity of β -catenin using a Tcf-dependent luciferase reporter gene. In this study, we demonstrate that mesothelioma cells overexpress Dvl. Furthermore, we show that overexpression of Dvl causes cytoplasmic accumulation of β -catenin, transcriptional activity, and tumor growth. Lastly, we demonstrate that activation of the Dvl- β -catenin signaling pathway contributes to tumorigenesis and that targeted inhibition of Dvl inhibits tumor growth.

MATERIALS AND METHODS

Cell Lines and Cell Culture. Mesothelioma cell lines were obtained as follows: LRK1A and REN as a generous gift from Dr. Steven Albelda (University of Pennsylvania, Philadelphia, PA) and NCI-H513 from NIH (Frederick, MD). All cell lines were cultured in RPMI 1640 complete media containing 10% fetal bovine serum. Fresh human malignant effusions and mesothelioma cells were obtained from patients undergoing resection of their tumors (Institutional Review Board approval H8714-15319-04) as described previously (19, 20).

Cellular Fractionation, Western Blot, and Immunohistochemistry. Whole cell lysates were obtained with M-Per mammalian protein extraction reagent (Pierce, Rockford, IL). Cytoplasmic fractions were prepared according to a protocol described previously (21). Briefly, the cell pellets were resuspended in ice-cold hypotonic buffer [20 mM Tris (pH 7.5), 25 mM sodium

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³ The abbreviations used are: Dvl, Dishevelled; GSK3 β , glycogen synthase kinase-3 β ; Tcf, T-cell factor; Lef, lymphocyte enhancer binding factor; Δ PDZ-Dvl, a PDZ domain deletion mutant; COX-2, cyclooxygenase-2.

fluoride, and 1 mM EDTA] containing a protease inhibitor mixture tablet (Roche, Indianapolis, IN). Cells were lysed by incubation on ice for 20 min, then subjected to ultracentrifugation at $100,000 \times g$ for 30 min at 4°C. The supernatant aliquots (20 μ g) containing the cytoplasmic fraction or the whole cell lysate aliquots (20 μ g) were separated on 4–15% gradient SDS-polyacrylamide gels and transferred to Immobilon-P (Millipore, Bedford, MA) membranes for Western blotting. Antigen-antibody complexes were detected by enhanced chemiluminescence blotting analysis system (Amersham Pharmacia Biotech, Piscataway, NJ). To compare the expression level, a gel densitometry (UN-SCAN-IT, Silk Scientific, Orem, UT) was used. The following primary antibodies were used: Dvl-1 (3F12) and Dvl-3 (4D3; all Santa Cruz Biotechnology, Santa Cruz, CA); β -actin (Sigma Chemical Co., St. Louis, MO); β -catenin and GSK-3 β (Transduction Laboratories, Lexington, KY); APC (Oncogene, Boston, MA); COX-2 (Cayman, Ann Arbor, MI); and Tcf-4 (Exalphan Biologicals, Inc., Boston, MA). Immunohistochemistry was done using snap-frozen, ornithine carbamyl transferase-embedded tumors that had been cut into 6- μ m sections. The primary antibody, β -catenin monoclonal antibody (Transduction Laboratories), was applied at 1:100 dilution, and tissues were incubated for 1 h at room temperature. Universal Labelled Streptavidin-Biotin+ system (Dako, Carpinteria, CA) and the secondary antibody were then applied. 3,3'-Diaminobenzidine was used as the chromogen, and the slides were counterstained with hematoxylin.

Sequencing Analysis. Genomic DNA from microdissected tissues was isolated using Puregene kit (Gentra Systems, Inc., Minneapolis, MN). Exon 3 of β -catenin was amplified using PCR. The primers design was based on the exon 3 sequence in GenBank, and the primer sequences are: forward, 5'-tcgtattatagctgatt-3' and reverse, 5'-taatactctaccagcta-3'. The PCR products were applied for sequencing using Applied Biosystems 377 DNA sequencer (Applied Biosystems, Foster City, CA). RNA was extracted from cell lines using an RNeasy mini kit (Qiagen, Inc., Valencia, CA) and reverse transcribed using Superscript II (Life Technologies, Inc., Rockville, MD). Whole coding region of β -catenin was amplified using LA *Taq* DNA polymerase (Panvera Corporation, Madison, WI). The primer sequences are forward, 5'-ataatagtcgacgtgattataaagatgatgatgataaaatggctactcaagctgattg-3' and reverse, 5'-ataatagcggccgcttattacagtcagatcaaacca-5'. The PCR products were cloned into *Sall/NorI* site of pSV-SPORT1 (Life Technologies, Inc.) vector to be applied for sequencing.

Transfection and Luciferase Assays. Transfection was performed using Lipofectamine Plus reagent (Life Technologies, Inc.) according to the manufacturer's instructions. Cells were seeded into 24-well plates and then transiently transfected with 0.25 μ g of the TOPFLASH or FOPFLASH (kindly provided by Dr. Hans Clevers) reporter plasmids (22) or plus activator plas-

mid. The cells were incubated for 24 h at 37°C, washed once with PBS, and then lysed to measure luciferase reporter gene expression by dual-luciferase reporter assay system (Promega, Madison, WI). The luciferase activity was normalized to Renilla luciferase activity from cotransfected internal control plasmid pRL-TK. Tcf-mediated gene transcription was determined by the ratio of pTOPFLASH:pFOPFLASH luciferase activity, each corrected for luciferase activities of the pRL-TK reporter. All experiments were performed in duplicate, a minimum of three times. To analyze the effect of Dvl on β -catenin signaling, a pCS-mouse Dvl-1 and a Δ PDZ-Dvl construct (kindly provided by Dr. Patricia C. Salinas) were cotransfected with TOPFLASH or FOPFLASH for luciferase assays. Empty pCS2+ vector was added to control for the amount of plasmid DNA.

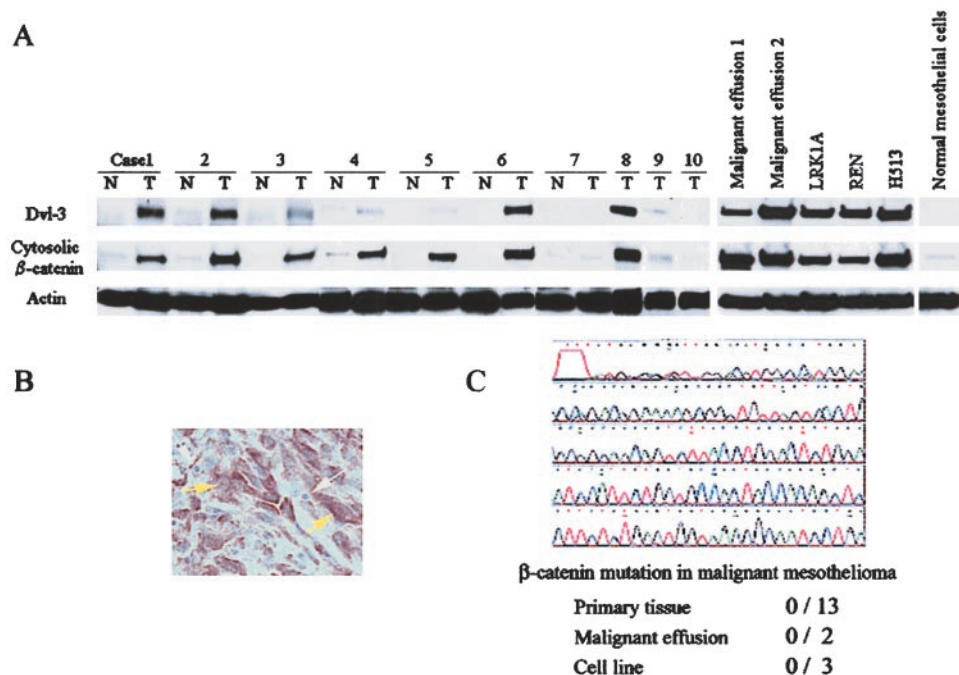
Creation of Cell Lines. To obtain cell lines expressing wild-type Δ PDZ-Dvl, the *XhoI/EcoRI* mouse Δ PDZ-Dvl-1 fragment of the pCS-mouse Δ PDZ-Dvl-1 was cloned into the *XhoI/EcoRI* site of pLXN. In the same manner, pLXN-mouse Dvl-1 was constructed from pCS-mouse Dvl-1. To prepare retroviral stocks, Phoenix A cells were transfected with pLXN-neo-Dvl-1, Δ PDZ-dvl-1, or pLXN-neo (empty vector) using Lipofectamine Plus reagent. Retroviral constructs were introduced into LRK1A, REN, and H513 and selected after culture in neomycin (1600 μ g/ml; 7 days).

cDNA Expression Array. For analyzing different gene expression in empty vector and Δ PDZ-dvl-1-introduced cells, Atlas human cancer 1.2 array (Clontech Laboratories, Inc., Palo Alto, CA) was used. Total RNA was extracted from the cell lines using an RNeasy mini kit (Qiagen, Inc.) according to the manufacturer's instructions. The materials provided with the kit were used, and the recommended protocol was followed in all steps. Five μ g of total RNA was converted into 33 P-labeled cDNAs for hybridization. The hybridized Atlas membranes were exposed to X-ray film for 3 days.

Soft Agar Assays. Cells (1×10^6) were placed in RPMI 1640 plus 15% FCS in 0.35% (w/v) low melting temperature agar between layers of 0.7% low melting temperature agar. After 4 weeks, colonies were stained with 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (Sigma Chemical Co.), and colonies containing >100 cells were scored. Colony assays were performed a minimum of six times, and results are reported as a mean of six experiments.

Tumorigenicity Assays. All animal experiments were performed in accordance with institutional guidelines. Athymic mice NCRNU-M (4–5 weeks old; Taconic, Germantown, NY) were maintained in pathogen-free conditions. For s.c. implantation, 4×10^6 cells were injected s.c. into anesthetized mice. Tumors were measured every 7 days, and tumor volumes were calculated using width (*a*) and length (*b*) measurements ($a^2 \times b / 2$, where $a < b$).

Fig. 1. Expression analysis of Dvl-3 and cytosolic β -catenin in mesothelioma. A, fresh malignant pleural mesothelioma cells were isolated from 10 freshly resected malignant pleural mesothelioma specimens, two mesothelioma patients' malignant effusions and the mesothelioma cell lines, LRK1A, REN, and H513. Except cases 7 and 10, all mesotheliomas revealed high expression of Dvl-3 and cytosolic β -catenin compared with that of matched normal pleural cells or irrelevant normal mesothelial cell controls. B, immunohistochemistry showing β -catenin staining in the normal pleural cells (white arrow) and the malignant pleural mesothelioma cells (yellow arrow) of case 1. In the normal pleural cells, β -catenin expression was strictly confined to cell membrane, as shown by a clear-cut linear pattern of immunostaining. Nuclear and cytoplasmic accumulation was evident in malignant mesothelioma cells. C, no mutation in exon 3 of β -catenin in mesothelioma. Genomic DNA from malignant lesion of case 1 was analyzed for mutation in exon 3 of β -catenin.



Statistical Methods. Results are expressed as means \pm SD. All statistical comparisons were made with a two-sided Student's *t* test. *P* of <0.05 was considered to be statistically significant.

RESULTS

Expression of Dvl-3 and Cytosolic β -Catenin in Mesothelioma.

Western blot analysis reveals that 8 of 10 fresh malignant mesothelioma tissues overexpress Dvl-3 protein and have increased cytosolic β -catenin compared with autologous matched normal pleural tissue controls (Fig. 1A). Furthermore, five additional malignant mesothelioma cells tested (two primary malignant pleural mesothelioma cultured cells and three cell lines, LRK1A, REN, and H513) had high levels of Dvl-3 and cytosolic β -catenin, compared with normal pleural controls. Immunohistochemical analysis of several of the tumor cells, demonstrated cytoplasmic, nuclear, and membrane bound β -catenin (Fig. 1B). We found no mutation in exon 3 of β -catenin in 13 mesothelioma tissues, including the cases tested by the Western blot analysis and two malignant effusions. Exon 3 was selected for mutational analysis because it encodes the NH₂-terminal regulatory domain of β -catenin previously found to contain activating mutations (Fig. 1C; Ref. 23). Furthermore, we detected no mutation in the whole coding region of β -catenin in three mesothelioma cell lines (LRK1A, REN, and H513).

Tcf-dependent Transcriptional Activity of β -Catenin in Mesothelioma. Western blot analysis was used to confirm APC, GSK-3 β , and Tcf4 expression in all tumors under studied (Fig. 2).

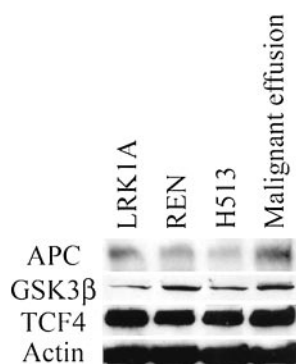


Fig. 2. APC, GSK3 β , and Tcf4 expression in mesothelioma. Mesothelioma cells tested have been confirmed to have expression of wild-type APC, GSK3 β , and Tcf4 by Western blot analysis.

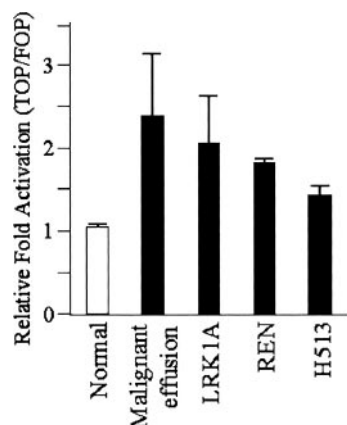


Fig. 3. Tcf-dependent transcriptional activity of β -catenin in mesothelioma. Constitutive transcriptional activation of the Tcf reporter construct pTOPFLASH exhibited >1.5 – 2.4 -fold higher than the mutant construct pFOPFLASH in malignant effusion, LRK1A, REN, and H513 ($P < 0.01$), compared with normal mesothelial cells. The results of all experiments were expressed as means \pm SD (experiments were performed in triplicate).

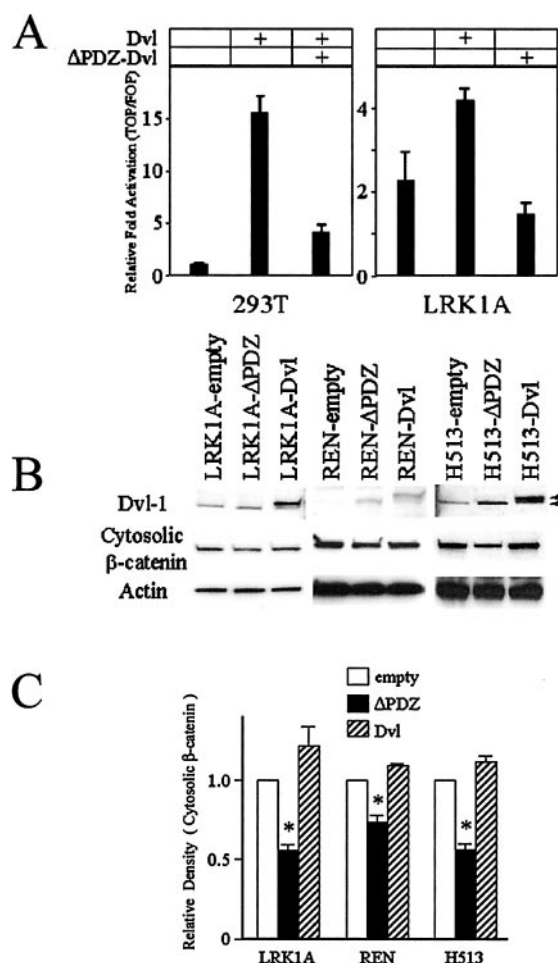


Fig. 4. Δ PDZ-Dvl inhibited the function of endogenous Dvl and the stability of cytosolic β -catenin in mesothelioma. A, pCS-Dvl-1 induced the transcriptional activities of Tcf-dependent transcriptional activity of β -catenin (pTOPFLASH/pFOPFLASH) in 293T. Cotransfection of pCS- Δ PDZ-Dvl-1 blocked the Dvl-1-induced Tcf-dependent transcriptional activity of β -catenin in 293T cells. Tcf-dependent transcriptional activity of β -catenin in malignant pleural mesothelioma LRK1A was increased after pCS-Dvl-1 transfection and reduced by pCS- Δ PDZ-Dvl-1. B, pLXN- Δ PDZ-Dvl-1 transfection decreased expression of cytosolic β -catenin in mesothelioma cells, LRK1A, REN, and H513, compared with control (cells transfected with empty vector or pLXN-Dvl-1). Exogenous Dvl-1 and Δ PDZ-Dvl-1 are indicated by a top and bottom arrow, respectively. C, quantitation of cytosolic β -catenin expression. Band densities were determined by densitometry. The relative density was calculated relative to the respective densities for cytosolic β -catenin of cells transfected with empty vector, after normalizing to actin. pLXN- Δ PDZ-Dvl-1 transfection significantly decreased expression of cytosolic β -catenin in mesothelioma cells, LRK1A, REN, and H513, compared with control (transfected with empty vector). The results of all experiments are expressed as mean \pm SD (experiments were performed three times). *, $P < 0.05$.

Transcriptional activity mediated by Tcf- β -catenin protein complexes was assayed as a ratio to reporter gene activity in mesothelioma cell lines with significant overexpression of Dvl and cytosolic β -catenin (Fig. 3). Mesothelioma cells with high levels of cytosolic β -catenin, including cells from malignant pleural mesothelioma effusions, LRK1A, REN, and H513 cell lines showed a significant fold increase (1.5–2.4-fold, $P < 0.01$) in Tcf-mediated gene transcriptional activity of β -catenin (pTOPFLASH/pFOPFLASH). In contrast, normal mesothelial cells, which have minimal expression of cytosolic β -catenin, showed no difference.

Δ PDZ-Dvl Inhibits Endogenous Dvl and Stabilizes Cytosolic β -Catenin in Mesothelioma and Down-Regulated the Downstream Target Genes. Dishevelled proteins possess three conserved domains, a dix domain, present in the Wnt antagonizing protein Axin (24), a PDZ domain involved in protein-protein interactions (25), and a DEP domain found in proteins that regulate Rho GTPases (26).

Function of these three conserved domains is required for up-regulation of β -catenin and for stimulation of Lef-1-mediated transcription in mammalian cells (27). Transfection of pCS-mouse Dvl-1 to 293T cells resulted in a 15-fold increase in Tcf-mediated gene transcriptional activity of β -catenin confirming other investigators' findings (Fig. 4A; Refs. 27, 28). This activity was inhibited by a pCS-mouse Dvl-1 construct by cotransfection of pCS-cDNA-encoding Δ PDZ-Dvl-1. Furthermore, Tcf-dependent transcriptional activity of β -catenin in LRK1A was reduced by transfection of pCS- Δ PDZ-Dvl-1 (from 2.1- to 1.3-fold, $P < 0.05$), whereas transfection of pCS-Dvl-1 enhanced Tcf-dependent transcriptional activity of β -catenin (from 2.1- to 3.8-fold, $P < 0.05$), indicating that β -catenin Tcf-mediated transcription in these cells is regulated significantly by Dvl.

To examine additional Wnt pathway activation in malignant pleural mesothelioma, we transfected retrovirally, Δ PDZ-Dvl-1 and wild-type Dvl-1 into LRK1A, REN, and H513 cell lines, respectively (Fig. 4B). Retrovirus transfection of pLXN- Δ PDZ-Dvl-1 induced expression of Δ PDZ-Dvl-1 protein, which significantly reduced the expression of cytosolic β -catenin in all cells tested compared with controls ($P < 0.05$; Fig. 4C). These results demonstrate that Dvl regulates cytosolic β -catenin in mesothelioma cells.

Using Atlas human cancer 1.2 array, c-myc expression in REN was shown to be down-regulated by Δ PDZ-Dvl-1 transfection (Fig. 5A). On the other hand, COX-2, which has been confirmed to be one of target genes of Wnt/ β -catenin pathway, was down-regulated by Δ PDZ-Dvl-1 transfection using Western blot analysis (Fig. 5B).

Transfection of Δ PDZ-Dvl Inhibits Tumorigenicity of Mesothelioma Cell Lines in Soft Agar and in Athymic Mice. We examined the role of the Dvl/ β -catenin pathway in relationship to cell growth in malignant pleural mesothelioma cell lines. We induced expression of Δ PDZ-Dvl-1 in LRK1A, REN, and H513 through retroviral transfection using empty vector as control. After selection, cells were plated in 0.35% soft agar and colonies scored after 28 days. Colony formations of LRK1A and REN transfected with Δ PDZ-Dvl-1 decreased substantially compared with control ($P < 0.01$; Fig. 6, A and B). H513 was unable to grow in soft agar. In addition, the *in vivo* growth of both LRK1A and REN s.c. tumors in athymic mice was inhibited signifi-

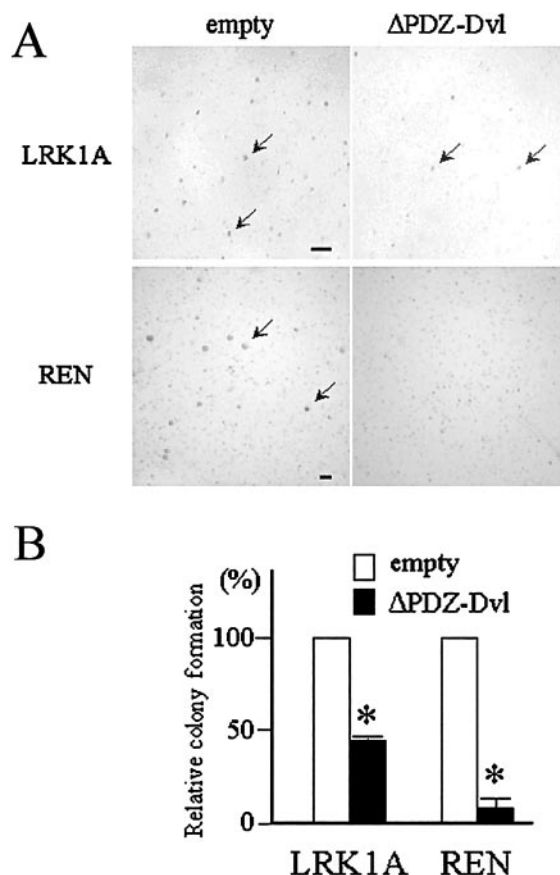


Fig. 6. Δ PDZ-Dvl reduces malignant pleural mesothelioma tumorigenicity *in vitro* colony assays. A, mesothelioma cells, LRK1A and REN, were transfected with pLXN or pLXN- Δ PDZ-Dvl-1 and selected with G418. Cells (1×10^6) were plated in soft agar, and colonies containing >100 cells were scored after 28 days. LRK1A and REN transfected with pLXN showed increased colony formation compared with those transfected with pLXN- Δ PDZ-Dvl-1. Scale bar, 0.25 mm. B, effect of Δ PDZ-Dvl on colony formation in soft agar. The bars indicate the percentage of the number of colonies relative to the control vector. The results of all experiments are expressed as mean \pm SD (experiments were performed a minimum of six times). *, $P < 0.01$.

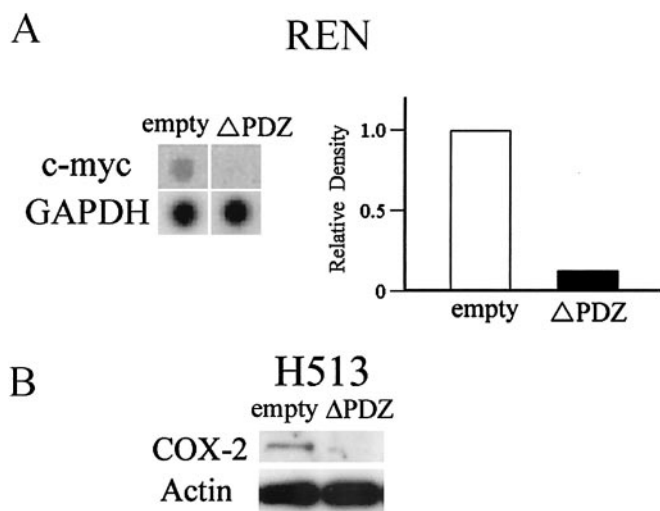


Fig. 5. C-Myc and COX-2 were down-regulated by Δ PDZ-Dvl transfection. A, Atlas human cancer 1.2 array showed down-regulation of c-myc expression in REN by Δ PDZ-Dvl transfection. RNAs from REN cells transfected with empty vector or Δ PDZ-Dvl were converted to cDNA and hybridized to Atlas membrane. Quantification using the densitometry showed down-regulation of c-myc expression, after normalizing to glyceraldehyde-3-phosphate dehydrogenase. B, COX-2 expression was down-regulated in H513 by Δ PDZ-Dvl transfection. Western blot analysis was achieved to compare the COX2 expression between H513 transfected with empty vector and Δ PDZ-Dvl.

cantly by transfection with a Δ PDZ-Dvl-1 mutant compared with control ($P < 0.05$ and $P < 0.005$, respectively; Fig. 7).

DISCUSSION

In this article, we have shown that Wnt signaling appears to be active in malignant pleural mesothelioma tumors and cell lines. In particular, we have identified that Dvl is overexpressed, leading to downstream signaling through β -catenin pathways. In addition, we have attempted to clarify the significance of β -catenin in mesotheliomas. Mesotheliomas appear to have elevated levels of β -catenin. Dvl- β -catenin signaling leads to transcription, whereas inhibition of this pathway reduces growth. Furthermore, the positive transcriptional activity of β -catenin appears to correlate with the tumorigenesis of mesothelioma cells. To the best of our knowledge, this is the first study supporting a critical role of upstream Dvl activation and increased β -catenin signaling in mesothelioma, in particular, and in cancer, in general.

We sought to determine whether wild-type Dvl or Δ PDZ-Dvl could control the expression of cytosolic β -catenin and Tcf transcription in malignant pleural mesothelioma. Previously, it was shown that Dvl can activate Tcf-dependent transcriptional activity in mammalian cells (27, 28) and that the PDZ domain of Dvl appears critical for this activity (27). Our studies confirm that in malignant pleural mesothelioma, Dvl can enhance Tcf-dependent transcriptional activity of

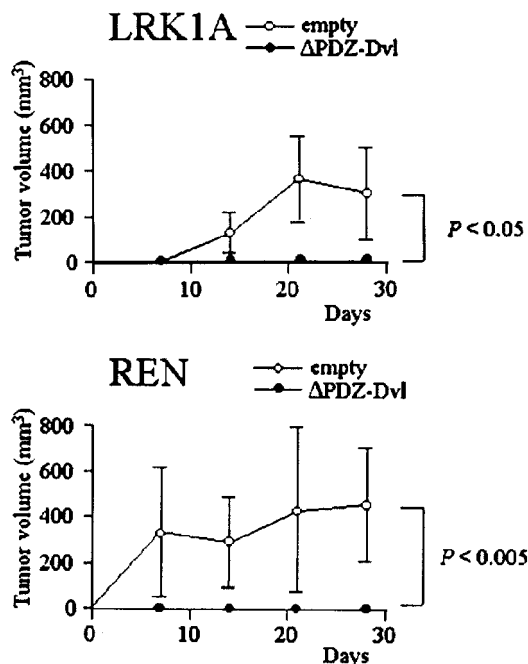


Fig. 7. Δ PDZ-Dvl inhibited the tumorigenesis of mesothelioma cells *in vivo*. Δ PDZ-Dvl transfected malignant pleural mesothelioma LRK1A and REN cells were unable to grow after s.c. injection in athymic mice compared with empty vector-transfected controls. Results are the means \pm SD (bars) for five animals in each group.

β -catenin and that, conversely, Δ PDZ-Dvl inhibits significantly this activity. Furthermore, the stable expression of Δ PDZ-Dvl reduces the expression of cytosolic β -catenin in malignant pleural mesothelioma with accompanied reduction of c-myc expression in REN and COX-2 in H513. C-Myc was the first target gene of the β -catenin signaling pathway to be demonstrated in humans, and overexpression of c-myc has been detected in malignant mesotheliomas (17, 29). COX-2 has been showed to be a downstream target of the Wnt signaling pathway, and high expression of COX-2 has been proposed to be correlated with poor survival in malignant mesothelioma (30–32). Finally, multiple malignant pleural mesothelioma analyzed have high expression of Dvl and cytosolic β -catenin suggesting activation of Wnt signaling.

We wondered whether these transcriptional activities had biological relevance and whether they affected cell growth or tumorigenicity in mesothelioma cells. Growth characteristics of malignant pleural mesothelioma cells transfected stably with either empty vector or Δ PDZ-Dvl correlated with the transcriptional activity of β -catenin and tumorigenesis. Colony formation and growth in athymic mice of malignant pleural mesothelioma cell lines, LRK1A and REN (which have high β -catenin potential transcriptional activity), were reduced significantly after transfection with Δ PDZ-Dvl. These results suggest that malignant pleural mesothelioma cells may require this critical Dvl- β -catenin pathway for tumor formation.

In conclusion, our data suggest that in malignant pleural mesothelioma, the Wnt pathway is activated through Dvl overexpression and downstream signaling through β -catenin. These malignant pleural mesothelioma tumors appear to be driven, in part, by the transcriptional regulation of translocated β -catenin. Additional investigation of Wnt signaling and Dvl- β -catenin activation in mesothelioma may offer new avenues for intervention and development of effective novel therapies.

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