Podoplanin promotes progression of malignant pleural mesothelioma by regulating motility and focus formation

Shinji Takeuchi,¹ Koji Fukuda,¹ Tadaaki Yamada,¹ Sachiko Arai,¹ Satoshi Takagi,² Genichiro Ishii,³ Atsushi Ochiai,³ Shotaro Iwakiri,⁴ Kazumi Itoi,⁴ Hisanori Uehara,⁵ Hiroshi Nishihara,⁵ Naoya Fujita² and Seiji Yano¹

¹Division of Medical Oncology, Cancer Research Institute, Kanazawa University, Kanazawa; ²Cancer Chemotherapy Center, Japanese Foundation for Cancer Research, Tokyo; ³Division of Pathology, Research Center for Innovative Oncology, National Cancer Center Hospital East, Kashiwa; ⁴Department of Respiratory Surgery, Hyogo Prefectural Amagasaki General Medical Center, Amagasaki; ⁵Department of Pathology and Laboratory Medicine, Institute of Biomedical Sciences, Tokushima University Graduate School, Tokushima; ⁶Department of Translational Pathology, Hokkaido University Graduate School of Medicine, Sapporo, Japan

Key words
focus formation, mesothelioma, motility, podoplanin, YAP1

Correspondence
Shinji Takeuchi and Seiji Yano, Division of Medical Oncology, Cancer Research Institute, Kanazawa University, 13-1 Takara-machi, Kanazawa, Ishikawa 920-0934, Japan.
Tel: +81-76-265-2794; Fax: +81-76-234-4524;
E-mails: takeuchi@staff.kanazawa-u.ac.jp and syano@staff.kanazawa-u.ac.jp

Funding Information
Program to Identify and Support Advanced Research on Drugs and Medical Devices of the National Institute of Biomedical Innovation (NIBIO), (Grant/Award Number:). Grant-in-Aids for Scientific Research in Innovative Areas, (Grant/Award Number:). Network for Integrative Research on Cancer Microenvironments of the Ministry of Education, Culture, Sports, Science, and Technology of Japan, JSPS KAKENHI Grant Number 16H05308

Received October 26, 2016; Revised January 13, 2017; Accepted January 23, 2017

Cancer Sci (2017)
doi: 10.1111/cas.13190

Malignant pleural mesothelioma (MPM) is a tumor that originates in the visceral pleura surrounding the lungs. This tumor then spreads to the lungs or into the thoracic cavity. The incidence of MPM is closely associated with asbestos exposure, and MPM can develop following a latent period of 20–40 years.¹ Early detection of MPM is difficult, so curative resection is also difficult. Moreover, MPM has limited sensitivity to radiation therapy and cytotoxic chemotherapy and a very poor prognosis, so effective MPM treatments need to be developed.

We previously identified a type-I transmembrane sialoglycoprotein, podoplanin (PDPN, also known as Aggrus), as a platelet aggregating factor in highly metastatic tumor cells.² PDPN binds to C-type lectin-like receptor 2 (CLEC2) expressed on platelets and causes platelets to aggregate; this aggregation depends upon Syk and Src family kinases and phospholipaseCγ2.³ As a result of its binding to CLEC2 on platelets, PDPN induces platelet aggregation and thereby promotes hematogenous metastasis.⁴ In addition, PDPN is known to form a complex with members of the ezrin-radixin-moesin (ERM) protein family, activate RhoA, and thus increase cell motility.⁵ PDPN is expressed by some non-cancer cells such as lymphendothelial cells and cancer-associated fibroblasts (CAF),⁶ though PDPN is frequently upregulated in several tumors, including squamous cell carcinoma, pleural mesothelioma, Kaposi’s sarcoma, testicular germ cell tumors, and brain tumors.⁷–⁹ PDPN is often expressed in MPM in particular, and the D2/40 antibody that recognizes PDPN is used as a marker of epithelial MPM.¹⁰

In the present study, we examined whether PDPN, a diagnostic marker for MPM, plays a critical role in disease progression.

Materials and Methods

Cell lines. The human mesothelioma cell lines MSTO-211H, H226, and H2452 were purchased from ATCC (Rockville,
MD, USA). YMESO-14 cells were kindly donated by Dr. Y. Sekido (Aichi Cancer Research Center Institute, Nagoya, Japan) and EHMES-1 cells were kindly donated by Dr. H. Hamada (Hiroshima University, Hiroshima, Japan). NCI-H290 was provided by Dr. Adi F. Gazdar (University of Texas Southwestern Medical Center, Dallas, TX, USA). Cells were cultured in RPMI-1640 medium supplemented with 10% FBS (Life Technologies, Grand Island, NY, USA). All cell lines were tested and authenticated by the Japanese Cancer Association on behalf of Japanese Cancer Association.

Western blotting. Lysates were prepared using Cell Lysis Buffer (Cell Signaling). The procedure for Western blotting was as previously described. The primary antibodies (Ab) used were anti-PDPN Ab (Angiopix, Inc.), anti-E-cadherin Ab (Cell Signaling), anti-N-cadherin Ab (Cell Signaling), anti-Vimentin Ab (Cell Signaling), anti-GAPDH Ab (Trevenigen), and anti-β-actin Ab (Cell Signaling).

Cell viability assay. Cell viability was measured by the MTT dye reduction method. Tumor cells were plated onto 96-well plates at 5 × 10^4 cells/well, and 2 × 10^3/100 μL per well in RPMI 1640 plus 10% FBS and cells were incubated for 24 h. Drugs were then added to each well, and incubation was continued for another 72 h. Cell growth was measured with MTT solution (2 mg/mL; Sigma, St. Louis, MO, USA), as described previously.

Wound healing assay. Cells were plated onto 6-well plates at 500,000 cells per well in RPMI 1640 plus 10% FBS and allowed to form a confluent monolayer. A wound was introduced by running a P200 pipette tip evenly across the monolayer. After incubation for 36 and 48 h, cells were observed under a microscope.

Transwell assay. Transwell assays were performed using the modified Boyden chamber method, with an 8-μm pore filter separating the upper and lower transwell chambers (BD Biosciences, NJ, USA). Tumor cells (10^4 cells/200 μL) were added to the upper chamber and incubated for 48 h. Cells that had not migrated were then removed from the upper surface of the filters with cotton swabs. Cells that had migrated to the lower surface of the filters were fixed, stained with H&E, and counted in six fields under a microscope at 200× magnification.

Transfection of the PDPN gene. Cells were seeded onto 6-well plates at a density of 1–2 × 10^5 cells/well. Twenty-four hours later, cells were transfected with a PDPN expression vector using Lipofectamine RNAiMAX (Invitrogen) according to the manufacturer’s instructions. After treatment with neomycin (Sigma-Aldrich), cells were cultured in the presence of neomycin and the D2-40 antibody to determine if they expressed PDPN. Ninety percent of the tumors from Japanese patients tested positive for PDPN (Table S1).

Immunohistochemistry. Formalin-fixed paraffin-embedded tumor sections were subjected to antigen retrieval and endogenous peroxidase blocking, and sections were incubated with primary antibody (Ab), anti-Ki-67 Ab (Dako), or anti-YAP1 Ab (Cell Signaling) at 4°C overnight. After incubating overnight, slides were rinsed and incubated with a peroxidase-labeled polymer. The tissue sections were then rinsed and stained with 3,3′-diaminobenzidine (DAB) substrate-chromogen and then counterstained with Hematoxylin (Sigma, St. Louis, MO, USA). Survival was analyzed by the Kaplan–Meier method. Differences between treatment and control groups were compared with the log-rank test. Differences at P < 0.05 were deemed significant.

Results

PDNP is highly expressed in pleural mesothelioma and promotes motility via RhoA/ROCK pathway activation. We first subjected tumors from 52 Japanese patients with MPM to immunostaining with the D2-40 antibody to determine if they expressed PDNP. Ninety percent of the tumors from Japanese patients with MPM tested positive for PDNP (Table S1, Fig. S1), so tumors from Japanese patients with MPM were found to express PDNP at high levels.

We then examined expression of PDNP in human MPM cell lines. High levels of expression were noted in three (H226, H2452, and EHMES-1) of six human MPM cell lines (Fig. 2a). In order to determine the role of PDNP in MPM cell lines, PDNP was knocked down with siRNA in 2 cell lines expressing high levels of PDNP (H226 and H2452). In H226 (Fig. 1b–d) and H2452 (Fig. S2) cells, knocking down PDNP did not alter cell viability and it did decrease cell motility. When PDNP was stably knocked down with shRNA in H226, motility was inhibited (Fig. 1e) but cell viability was not affected (Fig. S3c). The effects of shRNA were restored by transfection of shRNA-resistant PDNP mutants (Fig. S3a,b), so motility was definitely inhibited by knocking down PDNP.

In contrast, transfection of PDNP into MSTO-211H cells expressing low levels of PDNP (Fig. 2a) did not alter cell...
viability (data not shown) but it did enhance motility. Both a wound healing assay and a migration assay using a transwell system revealed enhanced motility as a result of overexpression of PDPN (Fig. 2b–d). These findings revealed that PDPN regulates the motility of MPM cells.

Podoplanin is known to bind to the ERM protein family and activate Rho.(5) Thus, we examined whether or not PDPN promotes the motility of MPM cells via the Rho/ROCK/Rac pathway. Transfection of PDPN into MSTO-211H cells resulted in increased RhoA-GTP binding (Fig. 3a). Conversely, knocking down PDPN with specific siRNA in H226 cells resulted in decreased RhoA-GTP binding (Fig. 3b). We also explored the effects of compounds that inhibit ROCK downstream of Rho. Y-27632 is widely used as a ROCK inhibitor, and fasudil hydrochloride has been clinically approved for treatment of delayed cerebral vasospasms following a subarachnoid hemorrhage since it inhibits ROCK.(16) Neither Y-27632 nor fasudil hydrochloride altered the viability of MSTO-211H/PDPN cells (Fig 3c, Fig. S4a), but the two ROCK inhibitors did inhibit motility in a dose-dependent manner (Fig. 3d, Fig. S4b). These findings indicate that PDPN activates the RhoA/ROCK pathway, thus promoting the motility of MPM cells.

PDPN promotes the progression of mesothelioma in the orthotopic implantation model. The effects of PDPN on tumor progression were examined in a model of orthotopic intrathoracic implantation in SCID mice. In H226 cells, tumor progression (the intrathoracic tumor burden) was inhibited by the knockdown of PDPN with shRNA (Fig. 4a). In contrast, tumor progression was promoted by transfection of PDPN into MSTO-211H cells, and mice had a significantly reduced survival time (Fig. 4b,c). Similarly, tumors produced by MSTO-211H cells transfected with PDPN had an increased number of Ki-67-positive proliferating cells. In contrast, tumors produced by H226 cells when PDPN was knocked down with shRNA had a reduced number of Ki-67-positive proliferating cells (Fig. 4d, Fig. S5). In another cell line expressing low levels of PDPN (H290), transfection of the PDPN gene resulted in enhanced tumor progression in a model of orthotopic implantation and an increased number of Ki-67-positive proliferating cells (Fig. S6). However, PDPN expression did not affect the engraftment rate or the number of tumors produced by MPM cells. These findings revealed that PDPN sustains the growth of MPM cells in vivo and that it promotes tumor progression in the thoracic cavity.

PDPN promotes focus formation in vitro and induces YAP1 activation associated with a low level of E-cadherin expression in vivo. Promotion of MPM cell motility by PDPN may not be the only factor responsible for tumor enlargement in vivo. Therefore, we focused on contact inhibition as another mechanism. Loss of contact inhibition is a strong indicator of cell transformation(17) and facilitates tumor progression. We performed a focus formation assay to examine the effect of PDPN on contact inhibition in MPM cells. PDPN blocked contact inhibition and promoted the formation of foci in MSTO-211H (Fig. 5a) and H290 (Fig. S7) cells. In contrast, knockdown of PDPN enhanced contact inhibition in H226 cells (Fig. 5b) resulting in a remarkable decrease in the number of foci.

YAP1 is reported to block contact inhibition and promote tumor progression.(18) In order to determine the mechanisms by which PDPN blocks contact inhibition, YAP1 expression was examined in PDPN-transfected and PDPN-shRNA-treated cells. YAP1 was found to be activated in PDPN-transfected cells but not in PDPN-shRNA-treated cells. These findings suggest that PDPN activates YAP1 and promotes tumor progression through a low level of E-cadherin expression.

Fig. 1. Knocking down podoplanin (PDPN) inhibited the motility of human mesothelioma cells. (a) Expression of PDPN in human mesothelioma cell lines was determined using Western blotting. (b) H226 cells that expressed high levels of PDPN were treated with scrambled siRNA or PDPN-specific siRNA and expression of PDPN was determined using Western blotting. (c) The viability of the resulting cells was determined using an MTT assay. (d) The motility of the resulting cells was assessed with a wound healing assay. (e) The motility of H226 cells treated with PDPN-shRNA or Luc-shRNA (a negative control) was assessed using a wound healing assay. The data shown are representative of at least three independent experiments with similar results.
was examined in tumors obtained from an orthotopic implantation model. YAP1 is a transcription factor that facilitates the transcription of various genes upon nuclear translocation. In tumors produced by H226 cells expressing high levels of PDPN, YAP1 was detected in the nuclei of 50% or more tumor cells, indicating that YAP1 was activated. In tumors produced by H226 cells upon PDPN knockdown with shRNA, YAP1 was not detected in the nuclei of most tumor cells, indicating that YAP1 was inactive (Fig. 6a). In tumors produced by MSTO-211H or H290 cells expressing low levels of PDPN, YAP1 was not detected in the nuclei of most tumor cells. In tumors produced by MSTO-211H or H290 cells transfected with PDPN, YAP1 was detected in the nuclei of 60% or more tumor cells (Fig. 6b, Fig. S8). Moreover, PDPN knockdown in H226 cells resulted in increased E-cadherin expression, whereas transfection of PDPN into MSTO-211H cells resulted in decreased E-cadherin expression (Fig. 6c,d). These findings suggest that PDPN blocks contact inhibition via decreased expression of E-cadherin and YAP1 activation.

**Discussion**

The monoclonal antibody D2-40 recognizes PDPN, which is a well-established diagnostic marker for MPM. In the present
study, we demonstrated that PDPN stimulates motility of MPM cells via activation of the RhoA/ROCK pathway. Moreover, PDPN blocks contact inhibition and it promotes progression of MPM in the thoracic cavity. These findings clearly indicate that PDPN plays a major role in the progression of MPM.

Podoplanin increased the motility of MPM cells in both cells natively expressing high levels of PDPN and in cells that were

Fig. 4. Podoplanin (PDPN) promoted the progression of mesothelioma cells that were orthotopically implanted in SCID mice. (A) H226/ShLuc or H226/ShPDPN cells (1 × 10⁶) were orthotopically implanted in the thoracic cavity of SCID mice. Seventy days after tumor cell implantation, the mice were euthanized and tumor development was evaluated. (b) MSTO-211H/Vector or MSTO-211H/PDPN cells (1 × 10⁶) were orthotopically implanted in the thoracic cavity of SCID mice. Twenty-one days after tumor cell implantation, the mice were sacrificed and tumor development was evaluated. (c) MSTO-211H/Vector or MSTO-211H/PDPN cells (1 × 10⁶) were orthotopically implanted in the thoracic cavity of SCID mice. The survival of the mice was evaluated. (n = 20/group). (d) Ki-67-positive tumor cells were determined based on the immunohistochemistry of thoracic tumors. *P < 0.05.

Fig. 5. Podoplanin (PDPN) promoted focus formation in human mesothelioma cells. Confluent cultures of human mesothelioma cells, H. MSTO-211H/Vector or MSTO-211H/PDPN cells (a) and H226/ShLuc or H226/ShPDPN cells (b) in 35-mm dishes were incubated for additional 2 weeks and stained with crystal violet; the number of foci was counted under a microscope. Data are representative of three independent experiments with similar results. *P < 0.001.
increased by PDPN occurs via induction of an epithelial–
mesenchymal transition (EMT) or not. (20, 21) In the present study,
whether motility was increased due to PDPN expression was deter-
mined using western blotting (c) (d) in these thoracic tumors. *P < 0.001.

Podoplanin (PDPN) resulted in increased nuclear localization of YAP1 and decreased expression of E-cadherin in thoracic tumors produced by mesothelioma cells. Thoracic tumors produced by H226/ShLuc or H226/ShPDPN cells were harvested 70 days after inoculation, and which produced by H. MSTO-211H/Vector or MSTO-211H/
PDPN cells were harvested 21 days after inoculation. YAP1-positive tumor cells in nucleus were determined by immunohistochemistry (a) (b), and expression of EMT-related proteins was determined using western blotting (c) (d) in these thoracic tumors. *P < 0.001.

forced to express PDPN by gene transfection. These findings agree with the results of Yamaki et al., (19) which were obtained by forced expression of PDPN in MPM cells. Increased motility due to PDPN was noted in various types of cells, including breast cancer cells, pancreatic beta cell carcinoma, (20) and cells derived from the kidney. (21) This view is uncontested, but there is still debate as to whether motility increased by PDPN occurs via induction of an epithelial–mesenchymal transition (EMT) or not. (20, 21) In the present study, PDPN expression in MPM cells did cause a decrease in E-cadherin expression but it did not necessarily trigger an increase in vimentin (Fig. 6c,d). Moreover, PDPN expression did not induce a typical morphological change to mesenchymal-like spindle-shaped cells (data not shown). These findings suggest that PDPN may activate the RhoA/ROCK pathway and increase the motility of MPM cells, even if a classical EMT is not induced. Whether or not increased motility due to PDPN expression occurs via induction of a classical EMT (associated with a decrease in E-cadherin and an increase in vimentin) may differ depending on the type of cancer.

Loss of contact inhibition is a hallmark of cell transformation. (22) Recent studies have reported that loss of contact inhibition involves activation of YAP1. YAP1 is a transcription coactivator downstream of the Hippo pathway. Activation of the Hippo pathway inhibits cell growth and induces cell death. If the Hippo pathway is inactivated, however, YAPI is translocated to the nucleus, where it facilitates the transcription of various factors and promotes cell growth. (23) In addition to the role of YAP1 in the Hippo pathway, YAP1 activity is also regulated by E-cadherin. (24) E-cadherin is reported to regulate contact inhibition in proliferating breast cancer cells by directly controlling YAP localization. (25) The current study found that expression of PDPN in MPM cells caused decreased expression of E-cadherin, it promoted the nuclear translocation of YAP1, and it caused a loss of contact inhibition. In the future, analysis of the mechanisms by which PDPN inhibits expression of E-cadherin should prove crucial to revealing the full scope of the mechanisms by which PDPN blocks contact inhibition.

Malignant pleural mesothelioma usually originates in the visceral pleura and then spreads into the thoracic cavity, where it rapidly grows. In the present study, we found that PDPN blocks contact inhibition resulting in increased focus formation and increased motility in MPM cells. These findings suggest that PDPN may be a regulatory factor that plays a key role in facilitating the enlargement of the primary tumor, its dissemination, or the growth of implants. Moreover, PDPN is a potent platelet-aggregating factor. (2, 4) Recent studies have noted that platelets were present in tumor tissue and that various growth factors released by the aggregated platelets promoted the growth of cancer cells in tissue. (26) Thus, PDPN expressed on MPM cells may promote the growth of MPM both by blocking contact inhibition in tumor cells and by causing the aggregation of platelets that have leaked into the thoracic cavity and their release of platelet-derived growth factor.

Podoplanin is a diagnostic marker for MPM and it promotes the progression of MPM, so PDPN could potentially serve as a therapeutic target. Over the past few years, anti-PDPN antibodies that inhibit platelet aggregation and mediate antibody-dependent cellular cytotoxicity (ADCC) have been created to target PDPN. (27–33) Agents that target PDPN should prove effective in treating MPM.

In conclusion, we demonstrated that PDPN, a well-established diagnostic marker for MPM, plays a major role in MPM progression by stimulating cell motility via RhoA/ROCK pathway activation and by blocking contact inhibition associated with decreased E-cadherin expression and YAPI activation.
Collectively, our findings indicate that PDPN is an ideal target for treatment of patients with MPM.

Acknowledgments
The authors wish to thank Yoshitaka Sekido (Aichi Cancer Center) and Hamada (Hiroshima University) for providing the human mesothelioma cell lines used in this study. This study was supported in part by grants (to SY and NF) from the Program to Identify and Support Advanced Research on Drugs and Medical Devices of the National Institute of Biomedical Innovation (NIBIO), by Grant-in-Aids for Scientific Research in Innovative Areas (to SY and NF) as part of the “Network for Integrative Research on Cancer Microenvironments” of the Ministry of Education, Culture, Sports, Science, and Technology of Japan, JSPS KAKENHI Grant Number 16H05308 (to SY).

References
1. Imai M, Hino O. Environmental carcinogenesis. Cancer Sci 2015; 106: 1483–5.
2. Kato Y, Fujita N, Kunita A et al. Molecular identification of Aggrus/T/alpha as a platelet aggregation-inducing factor expressed in colorectal tumors. J Biol Chem 2003; 278: 5159–605.
3. Suzuki-Inoue K, Kato Y, Inoue O et al. Involvement of the snake toxin receptor CLEC-2, in podoplanin-mediated platelet activation, by cancer cells. J Biol Chem 2007; 282: 25993–6001.
4. Fujita N, Takagi S. The impact of Aggrus/podoplanin on platelet aggregation and tumour metastasis. J Biochem 2012; 152: 407–13.
5. Martin-Villar E, Megias D, Castel S, Yurrita MM, Vilaro S, Quintanilla M. Podoplanin associates with CLEC2 in podoplanin-expressing cancer-associated fibroblasts predicts poor prognosis of lung adenocarcinoma. Int J Cancer 2008; 123: 1053–9.
6. Kawase A, Ishi G, Nagai K et al. Podoplanin expression by cancer-associated fibroblasts predicts poor prognosis of lung adenocarcinoma. Int J Cancer 2006; 119: 4541–53.
7. Kato Y, Sasaigawa I, Kaneko M, Osawa M, Fujita N, Tsuruo T. Aggrus: a diagnostic marker that distinguishes seminoma from embryonal carcinoma in testicular germ cell tumors. Urooncology 2004; 23: 8552–6.
8. Kato Y, Kaneko M, Sata M, Fujita N, Tsuruo T, Osawa M. Enhanced expression of Aggrus (T/alpha/podoplanin), a platelet-aggregation-inducing factor in lung squamous cell carcinoma. Tumour Biol 2005; 26: 195–200.
9. Mishima K, Kato Y, Kaneko MK et al. Podoplanin expression in primary central nervous system germ cell tumors: a useful histological marker for the diagnosis of germinoma. Acta Neuropathol 2006; 111: 563–8.
10. Kahn HJ, Bailey D, Marks A. Monoclonal antibody D2-40, a new marker of the lymphatic endothelium, reacts with Kaposi’s sarcoma and a subset of angiosarcomas. Mod Pathol 2002; 15: 434–40.
11. Takeuchi S, Fukuda K, Arai S et al. Hsp90 inhibition combined with zoledronic acid controls multiple organ metastases of chemotherapy-refractory multiple myeloma. Cancer Sci 2016; 107: 3934–46.
12. Albini A, Iwamoto Y, Kleiman HK et al. A rapid in vitro assay for quantifying the invasive potential of tumor cells. Cancer Res 1987; 47: 3239–45.
13. Takahashi A, Ishii G, Neri S et al. Podoplanin-expressing cancer-associated fibroblasts inhibit small cell lung cancer growth. Oncotarget 2015; 6: 9531–4051.
14. Li Q, Wang W, Machino Y et al. Therapeutic activity of glycoengineered anti-GM2 antibody against malignant pleural mesothelioma. Cancer Sci 2015; 106: 1027–8.
15. Sato M, Tanj, E, Fujikawa H, Kaibuchi K. Involvement of Rho-kinase-mediated phosphorylation of myosin light chain in enhancement of cerebral vasospasm. Circ Res 2000; 87: 195–200.
16. Zeng Q, Hong W. The emerging role of the hippo pathway in cell contact inhibition, organ size control, and cancer development in mammals. Cancer Cell 2008; 13: 188–92.

Disclosure Statement
The authors have no conflicts of interest in this study.

Abbreviations
CAF cancer-associated fibroblasts
CLEC2 C-type lectin-like receptor 2
ERM ezrin-radixin-moesin
MPM malignant pleural mesothelioma
PDPN podoplanin
SCID severe combined immune deficiency
Sh short hairpin
YAP1 Yes-associated protein 1

Supporting Information
Additional Supporting Information may be found online in the supporting information tab for this article:
Fig. S1. PDPN staining of MPM clinical specimens with anti D2-40 antibody.
Fig. S2. Knocking down PDPN inhibited the motility of H2452 cells.

Fig. S3. Transfection of shRNA-resistant PDPN genes restored the motility of mesothelioma cells treated with shRNA specific for PDPN.

Fig. S4. A ROCK inhibitor inhibited the motility, but not the viability, of MSTO-211H/PDPN cells.

Fig. S5. PDPN expression correlated with Ki-67-positive proliferating tumor cells in orthotopic tumors produced by mesothelioma cells.

Fig. S6. PDPN promoted the progression of H290 cells that were orthotopically implanted in SCID mice.

Fig. S7. PDPN promoted focus formation in H290 cells.

Fig. S8. PDPN resulted in increased nuclear localization of YAP1 in thoracic tumors produced by H290 cells.

Table S1. PDPN is highly expressed in MPM.