

Podoplanin

Krishnan, Harini; Rayes, Julie; Miyashita, Tomoyuki; Ishii, Genichiro; Retzbach, Edward P.; Sheehan, Stephanie A.; Takemoto, Ai; Chang, Yao Wen; Yoneda, Kazue; Asai, Jun; Jensen, Lasse; Chalise, Lushun; Natsume, Atsushi; Goldberg, Gary S.

DOI:

[10.1111/cas.13580](https://doi.org/10.1111/cas.13580)

License:

Creative Commons: Attribution-NonCommercial (CC BY-NC)

Document Version

Publisher's PDF, also known as Version of record

Citation for published version (Harvard):

Krishnan, H, Rayes, J, Miyashita, T, Ishii, G, Retzbach, EP, Sheehan, SA, Takemoto, A, Chang, YW, Yoneda, K, Asai, J, Jensen, L, Chalise, L, Natsume, A & Goldberg, GS 2018, 'Podoplanin: an emerging cancer biomarker and therapeutic target', *Cancer Science*, vol. 109, no. 5, pp. 1292-1299. <https://doi.org/10.1111/cas.13580>

[Link to publication on Research at Birmingham portal](#)

General rights

Unless a licence is specified above, all rights (including copyright and moral rights) in this document are retained by the authors and/or the copyright holders. The express permission of the copyright holder must be obtained for any use of this material other than for purposes permitted by law.

- Users may freely distribute the URL that is used to identify this publication.
- Users may download and/or print one copy of the publication from the University of Birmingham research portal for the purpose of private study or non-commercial research.
- User may use extracts from the document in line with the concept of 'fair dealing' under the Copyright, Designs and Patents Act 1988 (?)
- Users may not further distribute the material nor use it for the purposes of commercial gain.

Where a licence is displayed above, please note the terms and conditions of the licence govern your use of this document.



When citing, please reference the published version.

Take down policy

While the University of Birmingham exercises care and attention in making items available there are rare occasions when an item has been uploaded in error or has been deemed to be commercially or otherwise sensitive.

If you believe that this is the case for this document, please contact UBIRA@lists.bham.ac.uk providing details and we will remove access to the work immediately and investigate.

Podoplanin: An emerging cancer biomarker and therapeutic target

Harini Krishnan¹ | Julie Rayes² | Tomoyuki Miyashita^{3,4} | Genichiro Ishii^{3,4} |
Edward P. Retzbach⁵ | Stephanie A. Sheehan⁵ | Ai Takemoto⁶ | Yao-Wen Chang⁷ |
Kazue Yoneda⁸ | Jun Asai⁹ | Lasse Jensen¹⁰ | Lushun Chalise¹¹ |
Atsushi Natsume¹¹  | Gary S. Goldberg⁵ 

¹Department of Physiology and Biophysics, Stony Brook University, Stony Brook, NY, USA

²Institute of Cardiovascular Science, College of Medical and Dental Sciences, University of Birmingham, Edgbaston, Birmingham, UK

³Division of Pathology, Exploratory Oncology Research and Clinical Trial Center, National Cancer Center, Kashiwa, Chiba, Japan

⁴Laboratory of Cancer Biology, Department of Integrated Biosciences, Graduate School of Frontier Sciences, The University of Tokyo, Kashiwa, Chiba, Japan

⁵Graduate School of Biomedical Sciences and Department of Molecular Biology, Rowan University School of Osteopathic Medicine, Stratford, NJ, USA

⁶Division of Experimental Chemotherapy, The Cancer Chemotherapy Center, Japanese Foundation for Cancer Research, Tokyo, Japan

⁷Graduate Institute of Biomedical Sciences, College of Medicine, Chang Gung University, Taoyuan, Taiwan, China

⁸Second Department of Surgery (Chest Surgery), University of Occupational and Environmental Health, Kitakyushu, Fukuoka, Japan

⁹Department of Dermatology, Kyoto Prefectural University of Medicine Graduate School of Medical Science, Kyoto, Japan

¹⁰Division of Cardiovascular Medicine, Department of Medical and Health Sciences, Linköping University, Linköping, Sweden

¹¹Department of Neurosurgery, Nagoya University School of Medicine, Nagoya, Japan

Correspondence

Gary S. Goldberg, Molecular Biology, Rowan University, Stratford, NJ, USA.

Email: gary.goldberg@rowan.edu
and

Atsushi Natsume, Department of Neurosurgery, Nagoya University School of Medicine, Nagoya, Japan.

Email: anatsume@med.nagoya-u.ac.jp

Funding information

This work was presented at the first International Meeting on PDPN Research hosted at Nagoya University with support from Proteintech (Founding Sponsor), Fox Rothschild, VWR, Sentrimed, and Rowan University. This work was supported in part with funding from the Osteopathic Heritage Foundation and New Jersey Health Foundation to GSG, the JSPS KAKENHI (Grant Number 25461674) to JA, the National Cancer Center Research and Development Fund (23-A-12), the Foundation for the Promotion of Cancer Research, the 3rd Term Comprehensive 10-

Podoplanin (PDPN) is a transmembrane receptor glycoprotein that is upregulated on transformed cells, cancer associated fibroblasts and inflammatory macrophages that contribute to cancer progression. In particular, PDPN increases tumor cell clonal capacity, epithelial mesenchymal transition, migration, invasion, metastasis and inflammation. Antibodies, CAR-T cells, biologics and synthetic compounds that target PDPN can inhibit cancer progression and septic inflammation in preclinical models. This review describes recent advances in how PDPN may be used as a biomarker and therapeutic target for many types of cancer, including glioma, squamous cell carcinoma, mesothelioma and melanoma.

KEYWORDS

cancer, chemotherapy, c-type lectin-like receptor 2, podoplanin

Krishnan, Rayes, Natsume and Goldberg contributed equally to this work.

This is an open access article under the terms of the Creative Commons Attribution-NonCommercial License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited and is not used for commercial purposes.

© 2018 The Authors. *Cancer Science* published by John Wiley & Sons Australia, Ltd on behalf of Japanese Cancer Association.

Year Strategy for Cancer Control, and the Advanced Research for Medical Products Mining Programme of the National Institute of Biomedical Innovation (NIBIO) and JSPS KAKENHI (24659185 and 16H05311) to TM and GI, the British Heart Foundation (RG/13/18/30563) to JR, the Project for Cancer Research and Therapeutic Evolution (P-CREATE, No. 17cm0106205 h0002) and Medical Research and Development Programs Focused on Technology Transfer, Acceleration Transformative Research for Medical Innovation (ACT-MS, No. 17im0210607 h0002) from the Japan Agency for Medical Research and Development (AMED) to AT, and a Grant-in-Aid for Scientific Research on Innovative Areas "Chemistry for Multimolecular Crowding Biosystems" (JSPS KAKENHI 2617H06356) to AN.

1 | INTRODUCTION

Podoplanin (PDPN) is a unique transmembrane glycoprotein receptor. PDPN presents a heavily glycosylated amino terminal extracellular domain of approximately 130 amino acids, followed by a single transmembrane domain of approximately 25 amino acids, and a short intracellular domain of approximately 10 amino acids. PDPN does not contain known functional domains or enzymatic activities. It utilizes other proteins, including C-type lectin-like receptor-2 (CLEC-2), heat shock protein A9 (HSPA9), CD44, galectin 8, chemokine (C-C motif) ligand 21 (CCL21), ezrin, moesin, protein kinase A (PKA) and cyclin dependent kinase 5 (CDK5), to affect cell behavior as summarized schematically in Figure 1. These ligands and binding partners interact with PDPN to control tumor cell migration, invasion and metastasis.¹⁻⁴

Podoplanin expression is induced by tumor promoters including TPA, RAS and Src.⁵⁻⁷ For example, the Src tyrosine kinase utilizes the focal adhesion adaptor protein Cas/BCAR1 to induce PDPN expression to promote tumor cell motility.⁵ Src is a nonreceptor protein kinase that promotes nonanchored tumor cell growth and migration required for invasion and metastasis. Src is not mutated in most cancers. However, Src activity is associated with many types of human cancer, including tumors of the colon, breast, pancreas, brain and skin.^{8,9}

Cells transformed by a variety of chemicals, viral agents and oncogenes, including the Src tyrosine kinase, can be normalized by contact with nontransformed cells. This process, called "contact normalization" can force transformed cells to assume a normal morphology and reside in many organs, including breast, intestine and skin, for many years.¹⁰⁻¹² Comparisons between nontransformed cells, transformed cells and transformed cells undergoing contact normalization provide an extremely sensitive way to identify genes that control malignant and metastatic growth. However, Src kinase

activity alters the expression of approximately 3000 genes (approximately 10% of the transcriptome). However, fewer than 40 of these (approximately 0.1% of the transcriptome) are affected by contact normalization, with PDPN identified as a tumor promoter at the top of this list.^{5,12}

Podoplanin expression is induced by many tumor promoters and can be found in many types of cancer.^{1,3,12} High clonal expansion capacity is a characteristic feature of tumor initiating cells (TIC) and PDPN is a TIC marker for human squamous cell carcinoma.¹³ Using single-cell live imaging based on the fluorescent ubiquitination-based cell cycle indicator (Fucci) system, individual PDPN expressing A431 human squamous cell carcinoma cells were shown to create large colonies more often than single A431 cells that do not express PDPN.¹⁴ Although no significant differences in cell cycling were observed, cell death was significantly lower in the progenies derived from PDPN-positive single cells. RNA interference studies indicate that PDPN suppression increases cell death of single A431 cells, thus preventing them from forming larger colonies. Moreover, the frequency of large colony formation by PDPN-positive cells is decreased by treatment with a Rho-associated coiled-coil kinase (ROCK) inhibitor, whereas no difference was observed in single PDPN-negative cells.¹⁴ These data, summarized in Figures 1 and 2, point to a role for PDPN in the clonal expansion capacity of TIC populations.

2 | PODOPLANIN AS A CANCER BIOMARKER

Podoplanin is expressed in several types of cancer.^{1,3,12} Oral cancer exemplifies the utility of PDPN expression as a cancer biomarker. PDPN expression increases oral squamous cell carcinoma cell migration, which can lead to increased metastasis.¹⁵⁻¹⁷ Accordingly, PDPN

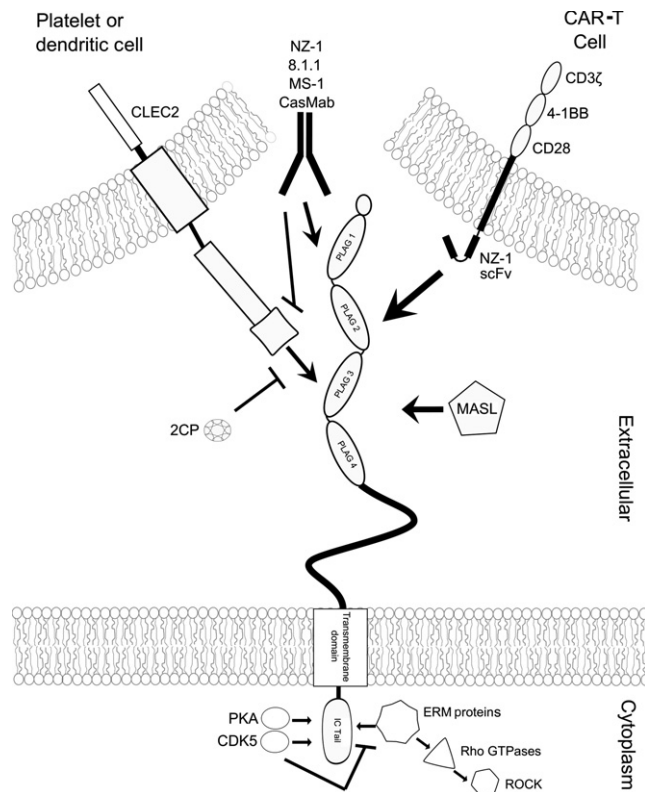


FIGURE 1 Podoplanin (PDPN) structure and targeting agents. PDPN contains an extracellular region, transmembrane domain, and intracellular (IC) tail. CLEC-2 interacts with PLAG domains in the extracellular region to induce inflammation and tumor progression, and this interaction can be blocked by antibodies, including 8.1.1, NZ-1, MS-1 and cancer-specific PDPN antibodies (CasMabs), as well as compounds exemplified by the small synthetic molecule 2CP. Antibodies can also target PDPN in order to inhibit transformed cell growth and motility directly, or can be incorporated into CAR-T cells. Lectins exemplified by MASL can also target PDPN to inhibit tumor progression and inflammation. protein kinase A (PKA) and cyclin dependent kinase 5 (CDK5) can phosphorylate serines on the intracellular tail to inhibit cell migration, presumably by blocking binding of ERM proteins that would otherwise lead to the activation of Rho GTPases and Rho-associated coiled-coil kinase (ROCK)

expression correlates with decreased 5-year survival rates of patients with these cancers.¹⁸ Moreover, PDPN expression in precancerous oral lesions (eg oral leukoplakias) correlates with a 3-fold increase in their transformation into malignancies compared to lesions without PDPN expression.¹⁹

In addition to cancer cells, PDPN expression can be found in cancer associated fibroblasts (CAF).^{1,3,12} For example, immunohistochemistry found PDPN expression in tumor cells from 38 out of 55 melanoma patients (69.1%). Podoplanin expression in CAF was observed in 25 of these patients (45.5%), including the 11 patients (44.0% with PDPN-positive CAF) with sentinel lymph node (SLN) metastasis. In contrast, only 4 of 30 (13.3%) patients without PDPN expression on CAF exhibited SLN metastasis. Furthermore, patients with PDPN-positive CAF experienced lower disease-free survival than those with PDPN-negative CAF ($P = .0148$).²⁰

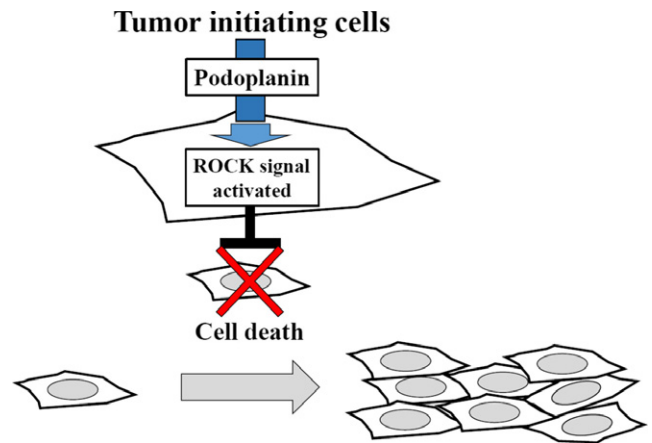


FIGURE 2 Podoplanin (PDPN) expression induces Rho-associated coiled-coil kinase (ROCK) activity to promote squamous cell carcinoma survival and colony expansion

In addition to histology and other standard techniques, a circulating tumor cell (CTC) chip is being developed as a blood-based marker to detect cancer. CTC are tumor cells shed from primary tumors, circulate in peripheral blood as surrogates of distant metastasis, and can be used to detect malignancies. CTC chips made of resin coated with PDPN antibodies are being developed as a microfluidic device to capture and detect CTC from metastatic cancers. For example, this technology has been used to capture and detect malignant pleural mesothelioma cells in preclinical models.

PDPN expression has been found in tumor cells as well as peritumoral basal keratinocytes which correlated with aggressive behavior in patients with extramammary Paget's disease (EMPD).²¹ PDPN expression in peritumoral basal keratinocytes was found in 25 out of 37 patients (67.6%) with EMPD. Half (50%) of in situ EMPD cases (9 in 18) exhibited PDPN-positive keratinocytes, whereas 84.2% (16 in 19) of invasive EMPD cases demonstrated positive staining for PDPN ($P < .05$). PDPN expression in peritumoral keratinocytes was also associated with tumor thickness ($P < .005$). By immunohistochemical analysis, PDPN-positive peritumoral keratinocytes were found to be negative for E-cadherin, one of the major adhesion molecules of keratinocytes, which might contribute to tumor invasion into the dermis through a crack in the basal cell layer induced by downregulation of cell adhesion therein.²¹

Model systems are being developed to delineate how PDPN and cadherins affect each other to control tumor invasion and other events that rely on cell motility. For example, downregulation of PDPN expression by siRNA inhibits the migration of normal human epidermal keratinocytes (NHEK). This is consistent with PDPN playing a key role in this keratinocyte motility and wound healing. Interestingly, PDPN downregulation caused an increase in E-Cadherin expression, suggesting that PDPN induces NHEK migration coupled with a loss of E-cadherin. Accordingly, platelets, which express the PDPN ligand CLEC-2, inhibit keratinocyte migration. Furthermore, CLEC-2 protein itself induces E-cadherin expression, downregulates RhoA GTPase and suppresses NHEK cell migration. Taken together, these data suggest that PDPN interacts with CLEC-2 to modulate E-

cadherin expression and RhoA activity to regulate keratinocyte migration during wound healing. These results also suggest that PDPN on keratinocytes associates with CLEC-2 on platelets and delays re-epithelialization until wound bed preparation is completed during wound healing.²²

3 | THE PODOPLANIN EXTRACELLULAR DOMAIN AS A THERAPEUTIC TARGET

Preclinical studies indicate that PDPN can be targeted to combat cancer. For example, CAR-T cells, antibodies and lectins that target PDPN can inhibit the growth and progression of glioma,^{23,24} oral squamous cell carcinoma,^{17,25} mesothelioma²⁶ and melanoma²⁷ in animal models. PDPN binds with CLEC-2 on platelets in the bloodstream to facilitate tumor embolism and hematogenous metastasis (Figure 1).²⁸⁻³⁴ Thus, PDPN-CLEC-2 interaction offers a unique opportunity to develop anticancer strategies.³⁵⁻³⁷

Antibodies can be utilized to disrupt PDPN-CLEC-2 interaction.^{36,38} For example, the NZ-1 antibody, its derivatives (eg NZ-8, NZ-12) and other antibodies (eg MS-1) which bind to the ectopic PLAG domain of PDPN (Figure 1) can decrease tumor load in xenograft models of glioma,³⁶ mesothelioma³⁹ and lung cancer.^{39,40} Work with patient derived xenograft and metastasis models indicate that PDPN-CLEC-2 interaction induces platelet aggregation that promotes the extravasation step of metastasis.⁴¹ This process is enhanced by growth factors and cytokines released from activated platelets during hemostasis. These factors are exemplified by TGF β , which is released during platelet aggregation induced by PDPN on bladder squamous cell carcinoma (eg UM-UC-5) cells. Lung metastasis of these cells can be suppressed by intravenously injected administration of monoclonal antibodies specific for PDPN or TGF- β .

The generality of this pathway is confirmed by analysis of lung squamous cell carcinoma cells. Although PDPN expression may change over time in cell culture,³⁴ it can be found in over 60% of lung squamous cell carcinoma cells produced from fresh clinical samples. As with bladder carcinoma, xenograft models of these cells also show TGF β released during PDPN-induced platelet aggregation, with lung metastasis suppressed by the administration of antibodies specific for PDPN. In addition to TGF β signaling, some lung squamous cell carcinoma cells (eg PC-10) also implicate EGFR activation by platelet-derived growth factors induced by PDPN binding. This effect is suppressed by the administration of PDPN antibodies or the EGFR kinase blocker ertlotinib along with suppression of PC-10 tumor growth in vitro and in xenograft mouse models.⁴²

In addition to antibodies, synthetic compounds are being developed to block PDPN-CLEC-2 interactions. For example, a derivative of 4-O-benzoyl-3-methoxy-beta-nitrostyrene (BMNS), compound "2CP," effectively suppresses PDPN-mediated platelet aggregation and tumor cell-induced platelet activation.³³ 2CP specifically binds to CLEC-2 and interacts with critical positions (Asn105, Arg107, Phe116, Arg118 and Arg157) to inhibit its binding to PDPN, as shown in Figure 1.^{33,43} As the first defined CLEC-2 antagonist, 2CP

not only possesses anti-cancer metastatic activity but also enlarges the therapeutic efficacy of cisplatin while decreasing the risk of bleeding in experimental metastasis models.

Interactions between PDPN and CLEC-2 can also be blocked to modulate the inflammatory response in sepsis, which is often associated with cancer progression and treatments. Indeed, sepsis is a life-threatening, severe systemic inflammatory response associated with multiple organ failure and death, which affects over 19 million patients annually.^{44,45} Thrombocytopenia is common in sepsis and severe thrombocytopenia is associated with poor outcome in septic patients and mice.⁴⁶⁻⁴⁸ Platelets are now recognized as critical immunomodulators affecting immune cell recruitment, releasing cytokines and chemokines and trapping bacteria.⁴⁹ Platelet depletion or inhibition of platelet activation results in a decrease in survival from sepsis.^{49,50} Moreover, platelets maintain vascular integrity at the site of inflammation through the PDPN and collagen/fibrin receptors, CLEC-2 and glycoprotein VI (GPVI), respectively.⁵¹⁻⁵³ In sepsis, platelet interaction with inflammatory macrophages dampens macrophages pro-inflammatory phenotype and decrease the secretion of TNF- α .⁵⁰ Recent studies indicate that platelet CLEC-2 interaction with PDPN on inflammatory macrophages regulates the immune response in a mouse model of sepsis, cecal ligation and puncture (CLP). Platelet deletion of CLEC-2 or PDPN-deficient hematopoietic cells increased the clinical severity of sepsis associated with enhanced systemic inflammation and accelerated organ injury. Deletion of CLEC-2 from platelets or PDPN from macrophages potentiates the cytokine storm and reduces PDPN expressing inflammatory macrophage migration to the infected peritoneum. In addition, pharmacological inhibition of the CLEC-2-PDPN axis inhibits immune cells infiltrate at the site of infection and regulates their inflammatory phenotype.⁵⁴ These observations identify PDPN as a novel anti-inflammatory target regulating immune cell recruitment and activation in sepsis.

In addition to antibodies and synthetic molecules, lectins may be used to target PDPN on transformed cells. For example, *Maackia*

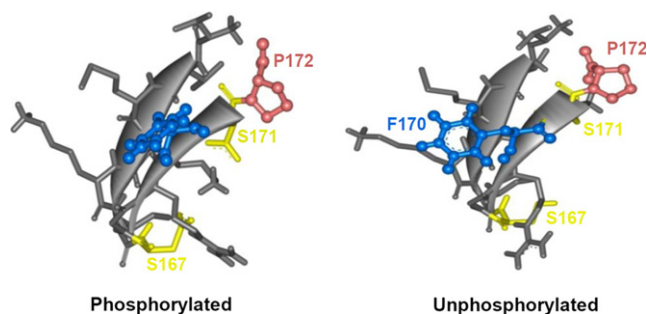


FIGURE 3 Predicted structural conformation of the intracellular domain of mouse podoplanin (PDPN) in the phosphorylated and unphosphorylated states. The intracellular domain of PDPN contains serine residues (yellow) that can be modified to affect cell motility. Least energy structural conformation calculated by PEP-FOLD predicts an alteration in the orientation of an intracellular phenylalanine residue (blue) that correlates with decreased cell migration

Amurensis seed lectin (MASL) binds to PDPN on melanoma and oral squamous cell carcinoma cells to inhibit their motility and growth in vitro and in syngeneic and xenograft mouse models (Figure 1). Interestingly, both MASL and NZ-1 antibody decrease tumor cell migration at nanomolar concentrations, apparently by inhibiting Cdc42 GTPase activity, and kill cells by nonapoptotic caspase independent necrosis at higher micromolar concentrations.^{17,27}

4 | TARGETING THE INTRACELLULAR PODOPLANIN DOMAIN

The intracellular domain of PDPN contains only 10 amino acids, including basic amino acids such as lysines and arginines. These basic amino acids act as binding sites for the ezrin family proteins. Upon binding to the intracellular domain of PDPN, the ezrin family proteins modulate Rho GTPases and reorganize the actin cytoskeleton to promote cell migration, as shown in Figure 1.⁵⁵

In addition to basic amino acids, the intracellular domain of PDPN also contains 2 conserved serine residues, which were long considered to be putative phosphorylation sites.^{15,56,57} The functional relevance of these serine residues was elucidated by mutagenesis and cell motility experiments. Interestingly, phosphorylation of serines inhibits PDPN-mediated cell migration. Furthermore, both serines need to be phosphorylated to inhibit cell migration.^{4,58} Phosphorylation can modify the structural conformation of amino acids in the PDPN intracellular domain, as shown in Figure 3.

The kinases that can phosphorylate PDPN cytoplasmic serine residues were identified as protein kinase A (PKA) and cyclin-dependent kinase 5 (CDK5), as shown in Figure 1. While PKA can phosphorylate either of the 2 serines (S167 or S171 in mouse PDPN), CDK5 preferably phosphorylates the C-terminal serine (S171 in mouse PDPN).⁴ These data suggest a scenario in which PKA and CDK5 work together to phosphorylate the intracellular serines of PDPN in order to inhibit cell motility. Reagents that can induce PDPN phosphorylation may be used to inhibit tumor motility. For

example, 8-br-cAMP, disulfiram and CARP-1 functional mimetics have been shown to induce PDPN phosphorylation and inhibit PDPN-mediated cell migration.^{4,59,60} Thus, PDPN may be targeted both on its intracellular domain as well as its extracellular domain to inhibit cell migration.

5 | PODOPLANIN CAR-T CELLS

CAR-T cells targeting PDPN are being developed to treat cancer. This is exemplified by recent work focused on glioblastoma. Glioblastoma (GBM) is the most common and lethal primary malignant brain tumor in adults, with a 5-year overall survival rate of less than 10%.⁶¹

Chimeric antigen receptors (CAR) consist of an extracellular domain derived from a single-chain variable fragment (scFv) taken from a tumor antigen-specific monoclonal antibody (mAb), a transmembrane domain, and a cytoplasmic signaling domain CD3 ζ chain (CD3 ζ) derived from the T-cell receptor complex.⁶² CAR-transduced T cells can recognize predefined tumor surface antigens independent of major histocompatibility complex (MHC) restriction, which is often downregulated in gliomas.⁶³ Third generation CAR, that include 2 costimulatory domains such as CD28 and 4-1BB (CD137), have been described and are highly likely to lyse tumor cells.⁶⁴

Several CAR have been generated against antigens expressed in GBM, including epidermal growth factor receptor variant III (EGFRvIII), human epidermal growth factor receptor 2 (HER2), interleukin-13 receptor alpha 2 (IL13R α 2), and, as described here, PDPN.²⁴ In particular, a lentiviral vector has been constructed with the EF1 α promoter followed by the leader sequence, NZ-1 PDPN antibody-based scFv, CD28, 4-1BB and CD3 ζ . The lentiviral vector was used to infect human T cells. A calcein-based nonradioisotope cytotoxic assay indicated that PDPN-positive LN319 cells and U87MG glioma cells were lysed by these NZ-1-CAR-T cells in an effector/target (E/T) ratio-dependent manner.²⁴ In contrast, specific lysis was not observed against PDPN-knockout (KO)-glioma cells.

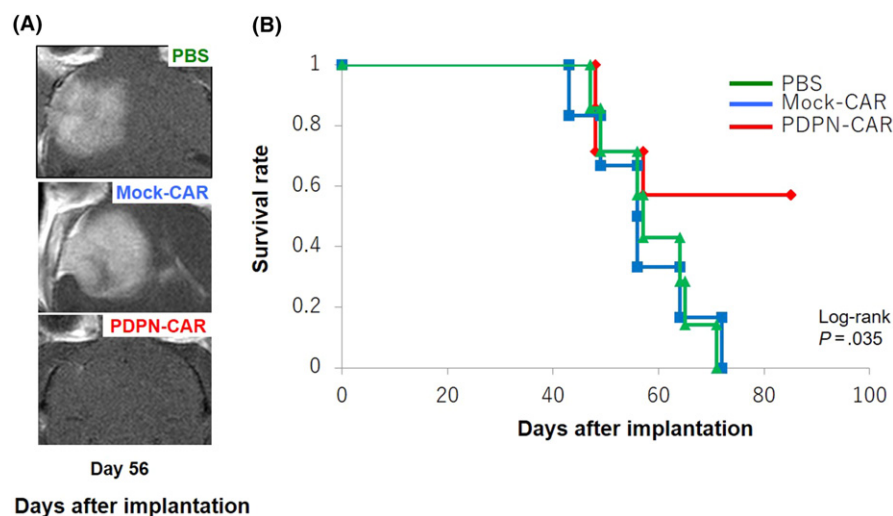


FIGURE 4 CAR-T cells targeting podoplanin (PDPN) inhibit glioblastoma progression in orthotopic xenograft mice. **(A)** Post-treatment MRI. **(B)** 60% of the mice treated with NZ-1 CAR-T were cured

In addition, NZ-1-CAR-T cells co-cultured with PDPN expressing glioma cells released significantly more IFN γ than mock-transduced T cells.²⁴

An intracranial glioma xenograft model was used to examine the distribution and anti-tumor effect of NZ-1-CAR-T cells.²⁴ To this end, glioma cells were stereotactically implanted into an immunodeficient mouse brain. Seven days after tumor implantation, NZ-1-CAR-T cells or mock-transduced T cells were infused intravenously via the tail vein. The non-treated mice were infused with PBS alone, and intracranial tumor growth was evaluated by 3T-MRI. In approximately 60% of the mice treated with NZ-1-CAR-T cells, tumors grew markedly slower and the mice survived significantly longer than control groups, as shown in Figure 4. Taken together, these data indicate that functionally active NZ-1-CAR-T cells recognize PDPN to inhibit glioma cell growth and tumor progression.

6 | CONCLUSIONS AND FUTURE PERSPECTIVES

Cancer is extremely complex and heterogeneous, in which the underlying factors are often poorly understood at the level of individual patients. PDPN is expressed by many types of tumor cells and CAF. Moreover, high levels of PDPN expression is associated with reduced survival and cancer aggression. PDPN has clear potential as a cancer biomarker and therapeutic target. These therapies include a variety of compounds, biologics, antisera and CAR-T cells as summarized in Figure 1.

One concern with PDPN CAR-T therapy arises from nonspecific lysis of normal cells that express PDPN, including lymphatic endothelium and type I lung alveolar cells. Cancer-specific monoclonal antibodies (CasMabs) have been generated to address this concern. These PDPN CasMabs react with PDPN expressed by cancer cells, but not normal cells.⁶⁵ These should be extremely useful reagents to produce very specific CAR-T therapies that target PDPN to combat glioma and other cancers.

As with most other anticancer therapies, it is important to understand which patients are likely to benefit from anti-PDPN treatments, such that each patients' therapeutic program can be tailored to their specific disease. Histopathological examination, often supported by clinical imaging (MRI or CT) and findings during surgery can be used to classify PDPN in patient tumors. However, direct, functional assessment of drug responses on primary patient-derived tumor cells gives the most accurate information on whether the patient will respond to the tested drugs. For example, zebrafish tumor xenograft platforms allow human tumor samples to be grafted into zebrafish embryos, where their growth as primary tumors and their dissemination to distal regions can be determined in the presence or absence of drugs.⁶⁶⁻⁶⁸ This platform has been used to demonstrate efficacy of the anti-PDPN compounds, including MASL on oral squamous cell carcinoma and melanoma xenografts.^{17,27} This approach can be used to gather

critical information that can be reported back to oncologists in charge of treatment planning in less than 5 days after surgery.

CONFLICT OF INTEREST

Gary Goldberg has intellectual property and ownership in Sentrimed, which is developing agents that target PDPN to treat disease, including cancer, and received funding from the New Jersey Health Foundation to develop methods to target PDPN to treat cancer. The other authors have no conflicts of interest to declare.

ORCID

Atsushi Natsume  <http://orcid.org/0000-0002-9113-0470>
 Gary S. Goldberg  <http://orcid.org/0000-0001-5906-4111>

REFERENCES

- Renart J, Carrasco-Ramirez P, Fernandez-Munoz B, et al. New insights into the role of podoplanin in epithelial-mesenchymal transition. *Int Rev Cell Mol Biol*. 2015;317:185-239.
- Astarita JL, Acton SE, Turley SJ. Podoplanin: emerging functions in development, the immune system, and cancer. *Front Immunol*. 2012;3:283.
- Wicki A, Christofori G. The potential role of podoplanin in tumour invasion. *Br J Cancer*. 2007;96:1-5.
- Krishnan H, Retzbach EP, Ramirez MI, et al. PKA and CDK5 can phosphorylate specific serines on the intracellular domain of podoplanin (PDPN) to inhibit cell motility. *Exp Cell Res*. 2015;335:115-122.
- Shen Y, Chen CS, Ichikawa H, Goldberg GS. SRC induces podoplanin expression to promote cell migration. *J Biol Chem*. 2010;285:9649-9656.
- Gandarillas A, Scholl FG, Benito N, Gamallo C, Quintanilla M. Induction of PA2.26, a cell-surface antigen expressed by active fibroblasts, in mouse epidermal keratinocytes during carcinogenesis. *Mol Carcinog*. 1997;20:10-18.
- Nose K, Saito H, Kuroki T. Isolation of a gene sequence induced later by tumor-promoting 12-O-tetradecanoylphorbol-13-acetate in mouse osteoblastic cells (MC3T3-E1) and expressed constitutively in ras-transformed cells. *Cell Growth Differ*. 1990;1:511-518.
- Chatzizacharias NA, Kouraklis GP, Giaginis CT, Theocharis SE. Clinical significance of Src expression and activity in human neoplasia. *Histol Histopathol*. 2012;27:677-692.
- Krishnan H, Miller WT, Goldberg GS. SRC points the way to biomarkers and chemotherapeutic targets. *Genes Cancer*. 2012;3:426-435.
- Rubin H. Cell-cell contact interactions conditionally determine suppression and selection of the neoplastic phenotype. *Proc Natl Acad Sci USA*. 2008;105:6215-6221.
- Rubin H. Contact interactions between cells that suppress neoplastic development: can they also explain metastatic dormancy? *Adv Cancer Res*. 2008;100:159-202.
- Krishnan H, Goldberg GS. Contact normalization or escape from the matrix. In: Kandouz M, ed. *Intercellular Communication in Cancer*. Boston, MA: Springer; 2015:297-342.
- Atsumi N, Ishii G, Kojima M, Sanada M, Fujii S, Ochiai A. Podoplanin, a novel marker of tumor-initiating cells in human squamous cell carcinoma A431. *Biochem Biophys Res Commun*. 2008;373:36-41.
- Miyashita T, Higuchi Y, Kojima M, Ochiai A, Ishii G. Single cell time-lapse analysis reveals that podoplanin enhances cell survival and

- colony formation capacity of squamous cell carcinoma cells. *Sci Rep*. 2017;7:39971.
15. Scholl FG, Gamallo C, Vilaro S, Quintanilla M. Identification of PA2.26 antigen as a novel cell-surface mucin-type glycoprotein that induces plasma membrane extensions and increased motility in keratinocytes. *J Cell Sci*. 1999;112:4601-4613.
 16. Huber GF, Fritzsche FR, Zullig L, et al. Podoplanin expression correlates with sentinel lymph node metastasis in early squamous cell carcinomas of the oral cavity and oropharynx. *Int J Cancer*. 2011;129:1404-1409.
 17. Ochoa-Alvarez JA, Krishnan H, Pastorino JG, et al. Antibody and lectin target podoplanin to inhibit oral squamous carcinoma cell migration and viability by distinct mechanisms. *Oncotarget*. 2015;20:9045-9060.
 18. Kreppel M, Scheer M, Drebber U, Ritter L, Zoller JE. Impact of podoplanin expression in oral squamous cell carcinoma: clinical and histopathologic correlations. *Virchows Arch*. 2010;456:473-482.
 19. Swain N, Kumar SV, Routray S, Pathak J, Patel S. Podoplanin—A novel marker in oral carcinogenesis. *Tumour Biol*. 2014;35:8407-8413.
 20. Kan S, Konishi E, Arita T, et al. Podoplanin expression in cancer-associated fibroblasts predicts aggressive behavior in melanoma. *J Cutan Pathol*. 2014;41:561-567.
 21. Cho Z, Konishi E, Kanemaru M, et al. Podoplanin expression in peritumoral keratinocytes predicts aggressive behavior in extramammary Paget's disease. *J Dermatol Sci*. 2017;87:29-35.
 22. Asai J, Hirakawa S, Sakabe J, et al. Platelets regulate the migration of keratinocytes via podoplanin/CLEC-2 Signaling during cutaneous wound healing in mice. *Am J Pathol*. 2016;186:101-108.
 23. Chandramohan V, Bao X, Kato Kaneko M, et al. Recombinant anti-podoplanin (NZ-1) immunotoxin for the treatment of malignant brain tumors. *Int J Cancer*. 2013;132:2339-2348.
 24. Shiina S, Ohno M, Ohka F, et al. CAR T cells targeting podoplanin reduce orthotopic glioblastomas in mouse brains. *Cancer Immunol Res*. 2016;4:259-268.
 25. Kaneko MK, Nakamura T, Kunita A, et al. ChLpMab-23: cancer-specific human-mouse chimeric anti-podoplanin antibody exhibits anti-tumor activity via antibody-dependent cellular cytotoxicity. *Monoclon Antib Immunodiagn Immunother*. 2017;36:104-112.
 26. Abe S, Morita Y, Kaneko MK, et al. A novel targeting therapy of malignant mesothelioma using anti-podoplanin antibody. *J Immunol*. 2013;190:6239-6249.
 27. Ochoa-Alvarez JA, Krishnan H, Shen Y, et al. Plant lectin can target receptors containing sialic Acid, exemplified by podoplanin, to inhibit transformed cell growth and migration. *PLoS ONE*. 2012;7:e41845.
 28. Gupta GP, Massague J. Platelets and metastasis revisited: a novel fatty link. *J Clin Invest*. 2004;114:1691-1693.
 29. Kunita A, Kashima TG, Morishita Y, et al. The platelet aggregation-inducing factor aggrus/podoplanin promotes pulmonary metastasis. *Am J Pathol*. 2007;170:1337-1347.
 30. 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;282:25993-26001.
 31. Seki S, Fujiwara M, Matsuura M, et al. Prognostic value of podoplanin expression in oral squamous cell carcinoma—a regression model auxiliary to UICC classification. *Pathol Oncol Res*. 2014;20:521-528.
 32. Ordonez NG. Value of podoplanin as an immunohistochemical marker in tumor diagnosis: a review and update. *Appl Immunohistochem Mol Morphol*. 2014;22:331-347.
 33. Chang YW, Hsieh PW, Chang YT, et al. Identification of a novel platelet antagonist that binds to CLEC-2 and suppresses podoplanin-induced platelet aggregation and cancer metastasis. *Oncotarget*. 2015;6:42733-42748.
 34. Takemoto A, Miyata K, Fujita N. Platelet-activating factor podoplanin: from discovery to drug development. *Cancer Metastasis Rev*. 2017;36:225-234.
 35. Jurasz P, Alonso-Escolano D, Radomski MW. Platelet–cancer interactions: mechanisms and pharmacology of tumour cell-induced platelet aggregation. *Br J Pharmacol*. 2004;143:819-826.
 36. Kato Y, Kaneko MK, Kuno A, et al. Inhibition of tumor cell-induced platelet aggregation using a novel anti-podoplanin antibody reacting with its platelet-aggregation-stimulating domain. *Biochem Biophys Res Commun*. 2006;349:1301-1307.
 37. Wojtukiewicz MZ, Hempel D, Sierko E, Tucker SC, Honn KV. Anti-platelet agents for cancer treatment: a real perspective or just an echo from the past? *Cancer Metastasis Rev*. 2017;36:305-329.
 38. Sekiguchi T, Takemoto A, Takagi S, et al. Targeting a novel domain in podoplanin for inhibiting platelet-mediated tumor metastasis. *Oncotarget*. 2016;7:3934-3946.
 39. Abe S, Kaneko MK, Tsuchihashi Y, et al. Antitumor effect of novel anti-podoplanin antibody NZ-12 against malignant pleural mesothelioma in an orthotopic xenograft model. *Cancer Sci*. 2016;107:1198-1205.
 40. Takagi S, Sato S, Oh-hara T, et al. Platelets promote tumor growth and metastasis via direct interaction between Aggrus/podoplanin and CLEC-2. *PLoS ONE*. 2013;8:e73609.
 41. Takemoto A, Okitaka M, Takagi S, et al. A critical role of platelet TGF-beta release in podoplanin-mediated tumour invasion and metastasis. *Sci Rep*. 2017;7:42186.
 42. Miyata K, Takemoto A, Okumura S, Nishio M, Fujita N. Podoplanin enhances lung cancer cell growth in vivo by inducing platelet aggregation. *Sci Rep*. 2017;7:4059.
 43. Nagae M, Morita-Matsumoto K, Kato M, Kaneko MK, Kato Y, Yamaguchi Y. A platform of C-type lectin-like receptor CLEC-2 for binding O-glycosylated podoplanin and nonglycosylated rhodocytin. *Structure*. 2014;22:1711-1721.
 44. Adhikari NK, Fowler RA, Bhagwanjee S, Rubenfeld GD. Critical care and the global burden of critical illness in adults. *Lancet*. 2010;376:1339-1346.
 45. Angus DC, van der Poll T. Severe sepsis and septic shock. *N Engl J Med*. 2013;369:840-851.
 46. Venkata C, Kashyap R, Farmer JC, Afessa B. Thrombocytopenia in adult patients with sepsis: incidence, risk factors, and its association with clinical outcome. *J Intensive Care*. 2013;1:9.
 47. Wang M, Wang J, Wang T, Li J, Hui L, Ha X. Thrombocytopenia as a predictor of severe acute kidney injury in patients with Hantaan virus infections. *PLoS ONE*. 2013;8:e53236.
 48. Claushuis TA, van Vught LA, Scicluna BP, et al. Thrombocytopenia is associated with a dysregulated host response in critically ill sepsis patients. *Blood*. 2016;127:3062-3072.
 49. Semple JW, Italiano JE Jr, Freedman J. Platelets and the immune continuum. *Nat Rev Immunol*. 2011;11:264-274.
 50. Xiang B, Zhang G, Guo L, et al. Platelets protect from septic shock by inhibiting macrophage-dependent inflammation via the cyclooxygenase 1 signalling pathway. *Nat Commun*. 2013;4:2657.
 51. Goerge T, Ho-Tin-Noe B, Carbo C, et al. Inflammation induces hemorrhage in thrombocytopenia. *Blood*. 2008;111:4958-4964.
 52. Boulaftali Y, Hess PR, Getz TM, et al. Platelet ITAM signaling is critical for vascular integrity in inflammation. *J Clin Invest*. 2013;123:908-916.
 53. Gros A, Syvannarath V, Lamrani L, et al. Single platelets seal neutrophil-induced vascular breaches via GPVI during immune-complex-mediated inflammation in mice. *Blood*. 2015;126:1017-1026.
 54. Rayes J, Lax S, Wichaiyo S, et al. The podoplanin-CLEC-2 axis inhibits inflammation in sepsis. *Nat Commun*. 2017;8:2239.
 55. Martin-Villar E, Megias D, Castel S, Yurrita MM, Vilaro S, Quintanilla M. Podoplanin binds ERM proteins to activate RhoA and promote epithelial-mesenchymal transition. *J Cell Sci*. 2006;119:4541-4553.
 56. Martin-Villar E, Scholl FG, Amat CG, Quintanilla M. PA2.26 antigen (T1a/podoplanin): a small mucin-like transmembrane glycoprotein

- associated with cell migration and cancer. *Revista de Oncología*. 2003;5:491-499.
57. Martin-Villar E, Scholl FG, Gamallo C, et al. Characterization of human PA2.26 antigen (T1alpha-2, podoplanin), a small membrane mucin induced in oral squamous cell carcinomas. *Int J Cancer*. 2005;113:899-910.
58. Krishnan H, Ochoa-Alvarez JA, Shen Y, et al. Serines in the intracellular tail of podoplanin (PDPN) regulate cell motility. *J Biol Chem*. 2013;288:12215-12221.
59. Ashour AE, Jamal S, Cheryan VT, et al. CARP-1 functional mimetics: a novel class of small molecule inhibitors of medulloblastoma cell growth. *PLoS ONE*. 2013;8:e66733.
60. Cheryan VT, Wang Y, Muthu M, et al. Disulfiram suppresses growth of the malignant pleural mesothelioma cells in part by inducing apoptosis. *PLoS ONE*. 2014;9:e93711.
61. Stupp R, Mason WP, van den Bent MJ, et al. Radiotherapy plus concomitant and adjuvant temozolomide for glioblastoma. *N Engl J Med*. 2005;352:987-996.
62. Eshhar Z, Waks T, Gross G, Schindler DG. Specific activation and targeting of cytotoxic lymphocytes through chimeric single chains consisting of antibody-binding domains and the gamma or zeta subunits of the immunoglobulin and T-cell receptors. *Proc Natl Acad Sci USA*. 1993;90:720-724.
63. Yeung JT, Hamilton RL, Ohnishi K, et al. LOH in the HLA class I region at 6p21 is associated with shorter survival in newly diagnosed adult glioblastoma. *Clin Cancer Res*. 2013;19:1816-1826.
64. Carpenito C, Milone MC, Hassan R, et al. Control of large, established tumor xenografts with genetically retargeted human T cells containing CD28 and CD137 domains. *Proc Natl Acad Sci USA*. 2009;106:3360-3365.
65. Kaneko MK, Yamada S, Nakamura T, et al. Antitumor activity of chLpMab-2, a human-mouse chimeric cancer-specific antihuman podoplanin antibody, via antibody-dependent cellular cytotoxicity. *Cancer Med*. 2017;6:768-777.
66. Chen X, Wang J, Cao Z, et al. Invasiveness and metastasis of retinoblastoma in an orthotopic zebrafish tumor model. *Sci Rep*. 2015;5:10351.
67. Vazquez Rodriguez G, Abrahamsson A, Jensen LD, Dabrosin C. Estradiol promotes breast cancer cell migration via recruitment and activation of neutrophils. *Cancer Immunol Res*. 2017;5:234-247.
68. Liu C, Zhang Y, Lim S, et al. A zebrafish model discovers a novel mechanism of stromal fibroblast-mediated cancer metastasis. *Clin Cancer Res*. 2017;23:4769-4779.

How to cite this article: Krishnan H, Rayes J, Miyashita T, et al. Podoplanin: An emerging cancer biomarker and therapeutic target. *Cancer Sci*. 2018;109:1292-1299. <https://doi.org/10.1111/cas.13580>