

RESEARCH HIGHLIGHT

Podoplanin promotes malignancy through a diversity of strategies

Miguel Quintanilla¹, Patricia Carrasco-Ramírez, Lucía Montero-Montero¹, Andrés Ramírez-López¹, Istéfani L. da Silva^{1,2}, Jaime Renart¹, Ester Martín-Villar^{1,3}

¹*Instituto de Investigaciones Biomédicas Alberto Sols, Consejo Superior de Investigaciones Científicas (CSIC) - Universidad Autónoma de Madrid (UAM), 28029 Madrid, Spain*

²*Departamento de Patología, Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais, 31.270-901 Belo Horizonte, Brasil*

³*Departamento de Biotecnología, Facultad de Ciencias Biosanitarias, Universidad Francisco de Vitoria, 28223 Madrid, Spain*

Correspondence: Miguel Quintanilla

E-mail: mquintanilla@iib.uam.es

Received: June 21, 2016

Published online: August 08, 2016

Podoplanin (PDPN) is a small mucin-like glycoprotein upregulated in a variety of cancers where it can be expressed in tumor as well as in stromal cells, such as cancer associated fibroblasts (CAFs). In most cancers, especially in squamous cell carcinomas (SCC) and glioblastomas, PDPN expression is associated with increased risk of metastasis to lymph nodes and reduced overall survival, although the opposite has been found in other tumor types. Studies from different laboratories, including our own, suggest that PDPN is involved in different steps of the metastatic cascade. Thus, PDPN, which is connected to the actin cytoskeleton through the binding to ezrin and/or moesin, stimulates collective tumor cell migration/invasion, and induces an epithelial-mesenchymal transition allowing a directional migration of individual cells through its interaction with the hyaluronan receptor CD44. PDPN is a component of the invadopodium contributing to its stability and promoting an efficient invasion through the extracellular matrix. In addition, PDPN favors the survival of cancer cells in the bloodstream aiding to their metastatic dissemination by inducing platelet aggregation/activation through its binding to the platelet receptor CLEC-2. More recently, we have reported that PDPN is a component of microvesicles and exosomes released by tumor cells and that PDPN-containing exosomes enhance *in vitro* lymphangiogenesis. In this short review, we discuss the role of PDPN in all these processes that foster malignant progression.

Keywords: podoplanin; EMT; CD44; CLEC-2; ERM; invadopodia; exosomes; metastasis; lymphangiogenesis

To cite this article: Miguel Quintanilla, et al. Podoplanin promotes malignancy through a diversity of strategies. Can Cell Microenviron 2016; 3: e1384. doi: 10.14800/ccm.1384.

Copyright: © 2016 The Authors. Licensed under a *Creative Commons Attribution 4.0 International License* which allows users including authors of articles to copy and redistribute the material in any medium or format, in addition to remix, transform, and build upon the material for any purpose, even commercially, as long as the author and original source are properly cited or credited.

Podoplanin (PDPN), also known as PA2.26 antigen, Aggrus, T1 α and M2A, is a mucin-like transmembrane glycoprotein of 38-40 kDa that is expressed in a number of normal tissues and cell types, including glomerular

podocytes (hence its name), lung alveolar type I cells, osteoblasts, mesothelia, choroid plexuses and some types of neurons. In addition, PDPN is present in lymphatic endothelial cells, fibroblastic reticular cells, follicular

dendritic cells, a subset of macrophages, and effector T cells during acute inflammation^[1-3]. In fact, the only function assigned to PDPN in normal adult tissues is related to lymphoid organs and the immune response^[3-5]. PDPN null mice die immediately after birth due to respiratory problems. Studies carried out in these mice indicate a crucial role for this glycoprotein in organogenesis, as these mice show defects in the formation of alveoli, heart and the lymphatic vascular system^[1, 2].

The expression of PDPN is upregulated in a wide variety of tumor types, such as chondrosarcomas, osteosarcomas, angiosarcomas, malignant mesotheliomas, germ cell tumors, astrocytomas, glioblastomas and squamous cell carcinomas (SCCs) of diverse origins, including the oral cavity, pharynx, tongue, skin, esophagus and lung^[1]. Although some reports suggest that PDPN expression may be a favorable prognostic factor in uterine cervical carcinomas and lung SCCs, an overwhelming amount of clinical and experimental data point to PDPN expression in tumors as a bad prognosis factor, particularly in head & neck, esophagus and skin SCCs and glioblastomas^[1, 2]. PDPN staining is often found at the invasive front of SCCs^[6, 7]. In most tumors, PDPN is co-expressed with E-cadherin at the surface of SCC invasive cells^[7]; however, in some areas of oral SCCs, PDPN expression correlated with reduced or virtually absence of E-cadherin staining at cell-cell contacts^[6]. Indeed, studies from our laboratory indicate that PDPN induces a full epithelial-mesenchymal transition (EMT) when expressed in cultured epithelial cells^[1]. In addition, it has been reported that PDPN is a marker of tumor-initiating cells with chemoresistant and stem characteristics in a SCC cell line (A431) and two glioblastoma cell lines (SJ-1 and U87)^[8-10].

PDPN is also expressed in the stromal cells of tumors; i.e. cancer-associated fibroblasts (CAFs). We first observed the presence of PDPN in dermal fibroblasts during skin remodeling processes and in stromal fibroblast-like cells from chemically-induced mouse skin tumors^[11], and other laboratories have found the presence of this glycoprotein in fibroblasts of chronically inflamed tissues as well as in myofibroblast-like cells of different types of cancer^[12, 13]. Contradictory results have been reported about the prognostic value of PDPN expression in CAFs. Thus, in lung adenocarcinomas, invasive ductal breast carcinomas, intrahepatic cholangiocarcinomas and esophagus adenocarcinomas, the presence of PDPN in CAFs is associated with shorter overall survival and poor prognosis, whereas it is considered as a favorable prognostic factor in colorectal cancer and without significant prognostic value in lung SCCs and uterine cervical carcinomas^[1, 12, 13].

PDPN has a heavily O-glycosylated ectodomain, which

confers a negative net charge to this moiety due to sialic acid modifications α 2-3 to galactose and α 2-6 to N-acetyl-galactosamine. In addition, PDPN has a conventional hydrophobic transmembrane region and a short cytoplasmic tail of only nine amino acids that lacks any obvious enzymatic motif. Therefore, PDPN must exert its function(s) through protein-protein interactions^[1, 2]. In cultured cells, PDPN is concentrated at plasma membrane protrusions, such as microvilli, filopodia and ruffles, where interacts with ezrin and/or moesin that links PDPN to the actin cytoskeleton^[6, 14, 15]. Ezrin and moesin belong to the ERM (ezrin, radixin, moesin) protein family of connectors between the plasma membrane and the actin cytoskeleton that regulate cell adhesion and motility. Ectopic expression of PDPN in low motile epithelial cells recruits ezrin to plasma membrane extensions and cell-cell contacts, promoting the downregulation (or the aberrant localization) of E-cadherin and leading to increased motility^[6, 14]. Moreover, PDPN induces a full EMT in premalignant keratinocytes and Madin-Darby canine kidney (MDCK) cells associated with increased migration and invasiveness^[14, 15]. These actions require the binding of PDPN to ezrin/moesin through a cluster of basic amino acids within the cytoplasmic domain and activation of the small GTPase RhoA (likely by sequestering the Rho-GDP dissociation inhibitor, Rho-GDI), which in turn activates its downstream kinase ROCK, leading to a major rearrangement of the actin cytoskeleton^[15]. We also have found that the transmembrane domain of PDPN is a key structural element involved in PDPN-induced EMT and cell migration. This is due to the association of this domain with detergent-resistant membrane fractions (lipid raft micro-domains), which constitute dynamic platforms involved in membrane trafficking and signal transduction. The association of PDPN with lipid rafts is mediated by a transmembrane GXXXG motif (GIIVG in human PDPN) that also mediates PDPN self-assembly. Thus, a single G to L mutation within this motif that disrupts intramembrane helix-helix interaction inhibits PDPN association with lipid rafts and halts PDPN-induced EMT^[16].

An intriguing observation of these studies was that PDPN not only promoted cell migration and invasiveness, but also behaved as an oncogenic protein by inducing a full tumorigenic and metastatic phenotype^[17]. These data were confirmed by knockdown experiments in which downregulation of PDPN in human oral SCC cell lines drastically reduced tumor formation in nude mice^[18, 19]. An explanation for this behavior has come recently from proteomic profiling studies in which upregulation and downregulation of pro-oncogenic and tumor suppressor proteins, respectively, were observed upon PDPN expression in MDCK cells^[19].

We and others have shown that PDPN is a novel component of invadopodia^[20, 21]. Invadopodia are ventral actin-rich plasma membrane projections with focalized proteolytic activity that mediate degradation of the extracellular matrix and are involved in local invasion of tumor cells through the basement membrane as well as entry into blood vessels. Recruitment of PDPN to invadopodia is mediated by binding to ERM proteins and lipid raft association^[21]. Gain-of-function and loss-of-function experiments suggest that PDPN is not involved in invadopodia formation but in the maturation and stability of these structures through activation of the RhoC/cofilin pathway, promoting an efficient degradation of the matrix^[21].

In early studies, we discovered PDPN as a cell-surface protein (named PA2.26 antigen) upregulated during wound healing and mouse skin chemical carcinogenesis^[11]. Interestingly, PDPN and the standard isoform of the hyaluronan receptor, CD44s, are coordinately regulated during progression of mouse skin carcinogenesis and EMT^[22]. Furthermore, PDPN binds CD44s at plasma membrane protrusions in individual cells actively migrating, but not in cells in contact, as demonstrated by fluorescence resonance energy transfer (FRET) monitored by fluorescence lifetime imaging microscopy (FLIM). PDPN-CD44 interaction appears to promote directional migration in SCC cells^[22]. Interestingly, CD44s has also been identified as a component of invadopodia^[23], where it plays an important role in the formation these structures and recruitment of MMP14 (MT1-MMP) metalloprotease. The implication of PDPN-CD44s interaction in invadopodia assembly and activity remains to be investigated.

Other authors have shown that PDPN binds the platelet C-type-lectin-like receptor 2 (CLEC-2) through three PLAG (platelet aggregation stimulating domain) tandem repeats within the extracellular domain of the glycoprotein^[24]. O-glycosylation of Thr-52 within the PLAG3 domain appears to be crucial for this binding. Besides platelets, CLEC-2 is present in neutrophils and dendritic cells, and PDPN-CLEC-2 interaction plays an important role in the immune response as well as in tumor metastasis^[2, 24]. PDPN on the surface of tumor cells bind CLEC-2 on platelets triggering a tyrosine kinase signaling pathway involving Syk, a member of the Src kinase family, which leads to platelet aggregation and activation^[25]. Platelet aggregation, besides protecting circulating tumor cells from mechanical stress, enable them to evade the immune system and favors embolization of the microvasculature and tumor cell extravasation. In addition, activated platelets secrete a wide variety of growth factors and cytokines that promote tumor growth and facilitate metastasis^[24]. CD9, a member of the

tetraspanin family identified as a suppressor of metastasis, reduces platelet aggregation and metastasis by interacting with PDPN^[26]. PDPN-CD9 interaction involves the transmembrane domains TM1 and TM2 of CD9 and localization of both proteins in low density membrane fractions, indicating that the transmembrane domain of PDPN is also involved in this interaction. Interestingly, binding of CD9 to PDPN does not interfere the interaction of the latter with CLEC-2, suggesting that CD9-mediated inhibition of platelet aggregation occurs by a mechanism downstream to the binding of PDPN to CLEC-2; i.e., preventing CLEC-2 multimerization, a condition necessary for platelet aggregation^[26].

In a recent article, we demonstrated that PDPN is secreted into the tumor microenvironment associated to the two main types of extracellular vesicles: exosomes and microvesicles^[19]. Exosomes are vesicles of endosomal origin that are released after fusion of multivesicular bodies containing intraluminal vesicles with the cell surface, whereas microvesicles are directly shed from the plasma membrane. Both types of vesicles transfer genetic information, including DNA, mRNAs and miRNAs, and proteins from tumors to recipient cells, and promote the dissemination of tumor cells by stimulating angiogenesis, remodeling the extracellular matrix, facilitating escape from the immune surveillance, and educating bone marrow progenitor cells towards a pro-metastatic phenotype^[27, 28]. Exosomes secreted by PDPN-expressing cells transport both PDPN mRNA and protein. Differential proteomic analysis of exosomes released by MDCK cells expressing PDPN revealed that PDPN induces a reprogramming of exosomal proteins. Most proteins highly downregulated in PDPN-containing exosomes are cell adhesion molecules and cytoskeletal keratins typical of epithelial cells, thus mimicking the pattern of EMT changes induced by PDPN in whole cells. Moreover, PDPN-exosomes were enriched in components of the semaphorin, ephrin and Src family kinase signaling pathways, integrins and other cell adhesion receptors, as well as proteins involved in actin dynamics and cytoskeletal remodeling. Strikingly, a large number of proteins enriched in PDPN-exosomes were implicated in the control of vesicle trafficking, including members of the Rab, Ral and Rap families of small GTPases, annexins, tetraspanins, components of the ESCRT and SNARE complexes that are required for the biogenesis and release of exosomes, respectively, and proteins involved in lipid raft formation. Interestingly, the levels of most of these proteins were unchanged in PDPN-expressing whole cells, suggesting a direct role of PDPN in promoting their incorporation into exosomes. Indeed, by measuring the amount of exosomes and microvesicles produced by the same number of cells in gain-of-function and loss-of-function experiments, we

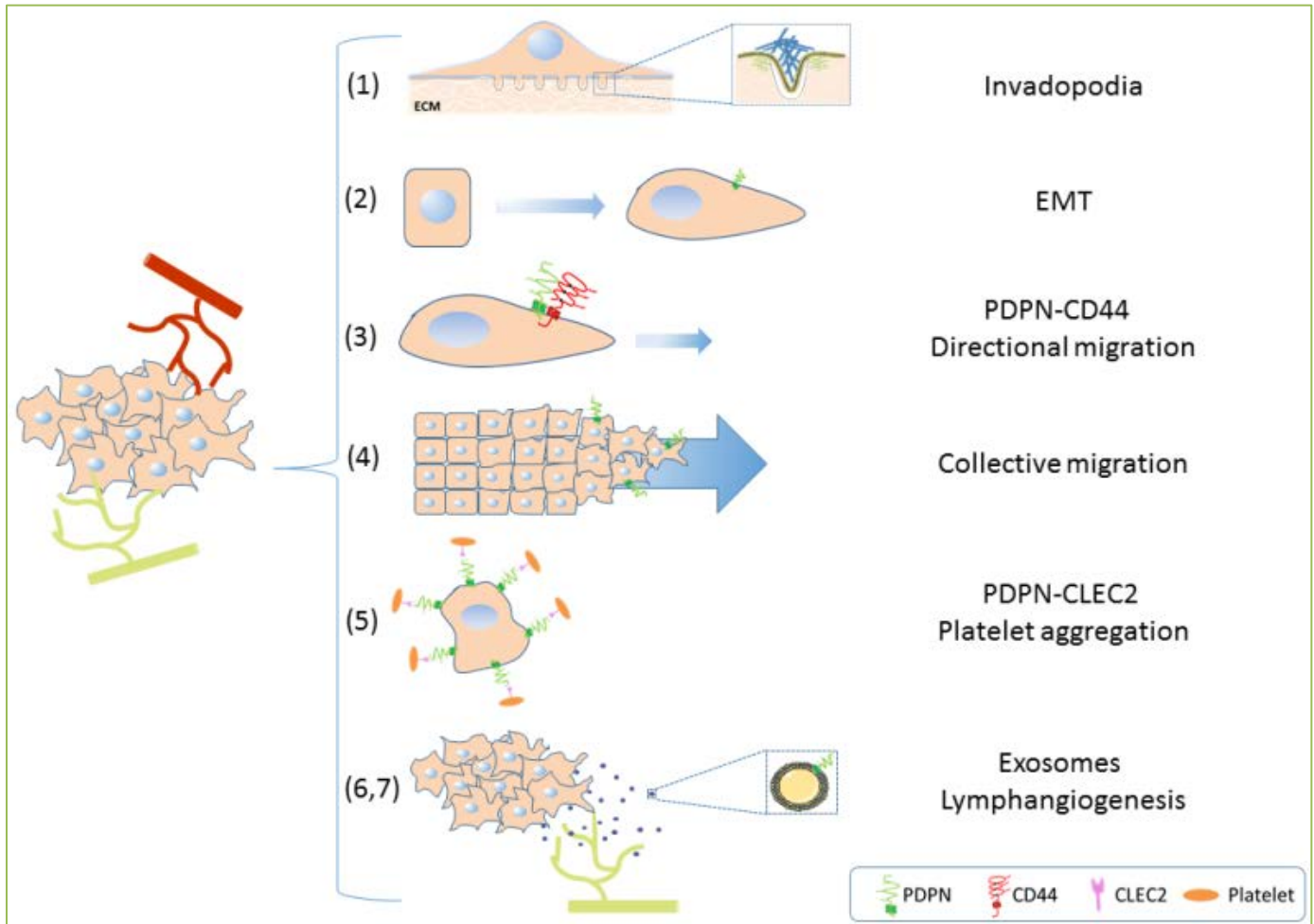


Figure 1. PDPN stimulates malignancy by different strategies. For additional details see the text.

demonstrated that PDPN stimulates the biogenesis and/or release of these vesicles^[19]. We speculate that PDPN may promote endocytosis and/or exocytosis, processes that require rearrangements of the actin cytoskeleton. On the other hand, Hoshino and co-workers^[29] have found a synergistic interaction between exosomes and invadopodia in head and neck SCC cells by which invadopodia maturation and activity depends on the delivery of MMP14 via exosomes, being invadopodia critical docking sites for exosomes. Since we have found that knockdown of PDPN in oral SCC cells impairs both the stability of invadopodia (and degradation of extracellular matrix) and the secretion of exosomes^[19, 21], PDPN might well be a key element for the connection between invadopodia and exosomes in SCCs. Since CD44 is also a component of exosomes, we can speculate that PDPN-CD44 interaction plays also any role on this issue.

Interestingly, both PDPN-containing and non-containing exosomes promote *in vitro* angiogenesis; i.e., blood vessel formation by human umbilical vein endothelial cells on

Matrigel, but only PDPN-containing exosomes were able to promote the formation of lymphatic capillary structures by lymphatic endothelial cells (LECs). These results raise the possibility that PDPN-exosomes stimulate lymphangiogenesis during tumorigenesis *in vivo*. The molecular mechanism by which PDPN-exosomes modulate lymphangiogenesis remains to be investigated. However, our studies and those from other laboratories suggest a critical role for the extracellular domain since preincubation of PDPN-exosomes with a neutralizing monoclonal antibody directed against this region of PDPN block *in vitro* lymphangiogenesis^[19] as well as *in vivo* lymphangiogenesis (mediated by PDPN in lymphatic endothelial cells) associated with mouse corneal inflammation^[30]. Moreover, a soluble PDPN-Fc fusion protein comprising the extracellular domain of PDPN and the Fc region of IgG was shown to inhibit *in vitro* as well as *in vivo* lymphangiogenesis^[31].

Lymphangiogenesis is an important tread on metastasis, particularly in melanoma and breast cancer. Thus, the presence of tumor cells in regional or sentinel lymph nodes is

considered as a bad prognostic factor for cancer patients, and the presence of lymphangiogenesis in tumor-draining lymph nodes, which can be observed even before tumor cells arrive at the lymph nodes, facilitates dissemination of cancer cells to distant sites^[32, 33]. There is a close relationship between PDPN and lymphangiogenesis. PDPN is specifically expressed in lymphatic but not in blood vessels^[14, 34], and PDPN-deficient mice are born with a disrupted and dilated lymphatic vasculature and lymphedema^[35]. A correlation between high PDPN expression in tumor cells and increased metastasis to lymph nodes has been found in patients with oral SCCs^[31, 36]. Furthermore, ectopic PDPN expression in MCF7 breast cancer cells promoted tumor lymphangiogenesis and lymph node metastasis in an *in vivo* xenograft mouse model^[31]. On the contrary, expression of PDPN in EBC-1 lung squamoid cancer cells reduced tumor lymphangiogenesis and inhibited lymph node metastasis formation^[37], which is in agreement with PDPN being a favorable prognostic factor for patients with lung SCCs^[12]. A satisfactory explanation for these controversial results is still lacking. In addition, PDPN expression in stromal CAFs has been associated with enhanced intratumoral lymphangiogenesis, lymph node metastasis and lymphatic invasion in a wide variety of cancers, including prostate, ovary, bladder, pancreas, and invasive ductal breast carcinomas^[38, 39]. The molecular mechanism by which PDPN-positive CAFs stimulate lymphangiogenesis may be the secretion of lymphangiogenic growth factors and cytokines, such as VEGF-C, VEGF-D and CCL21^[33]. CCL21, which binds PDPN with high affinity, is secreted by LECs and tumor cells facilitating the entry of dendritic and cancer cells into lymphatic vessels^[2, 33]. Another possibility for PDPN-positive CAFs to promote lymphangiogenesis may be the release of PDPN-exosomes. In this respect, it has been shown that tumor cell-derived exosomes induce the differentiation of mesenchymal stem cells and fibroblasts into CAFs, and that CAFs secrete exosomes that promote tumor growth and metastasis^[40].

In summary, *in vitro* and *in vivo* studies have provided many experimental evidences suggesting that PDPN stimulates malignancy by a variety of strategies at different steps of the metastatic cascade (Figure 1): (1) PDPN induces invadopodia maturation and stability to promote tumor cell local invasion and infiltration into blood vessels. (2) PDPN-induced EMT stimulates individual tumor cell migration and invasion through the extracellular matrix. (3) PDPN-CD44s interaction assists directional migration of individual tumor cells. (4) PDPN induces collective tumor cell migration and invasion. (5) PDPN-CLEC-2 interaction promotes platelet aggregation facilitating survival of tumor cells into the bloodstream and metastasis. (6) PDPN promotes the biogenesis and/or release of exosomes and

microvesicles into the tumor microenvironment. (7) PDPN-containing exosomes stimulate lymphangiogenesis.

Conflicting interests

The authors have declared that no conflict of interests exist.

Acknowledgments

Our research is supported by grants SAF2013-46183-R from the *Ministerio de Economía y Competitividad*, and S2010/BMD-2359 (SkinModel) from the *Comunidad Autónoma de Madrid*. PC-R and LM-M were funded by the Spanish FPI (*Formación de Personal Investigador*) program. ILdS was funded by the brasilian PDSE-CAPES program (*Programa de Doutorado Sanduiche no Exterior*).

Author contribution

M.Q., J.R. and E.M-V. designed the study, P.C-R., L.M-M., A.R-L., I.L.S. and E.M-V conducted the experiments, M.Q. wrote the manuscript.

Abbreviations

PDPN: podoplanin; SCC: squamous cell carcinoma; EMT: epithelial-mesenchymal transition; CAF: cancer-associated fibroblast; MDCK: Madin-Darby canine kidney; FRET: fluorescence resonance energy transfer; FLIM: fluorescence lifetime imaging microscopy; LEC: lymphatic endothelial cell.

References

- Renart J, Carrasco-Ramirez P, Fernandez-Munoz B, Martin-Villar E, Montero L, Yurrita MM, *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.
- Peters A, Burkett PR, Sobel RA, Buckley CD, Watson SP, Bettelli E, *et al*. Podoplanin negatively regulates CD4+ effector T cell responses. *J Clin Invest* 2015; 125:129-140.
- Acton SE, Farrugia AJ, Astarita JL, Mourao-Sa D, Jenkins RP, Nye E, *et al*. Dendritic cells control fibroblastic reticular network tension and lymph node expansion. *Nature* 2014, 514:498-502.
- Astarita JL, Cremasco V, Fu J, Darnell MC, Peck JR, Nieves-Bonilla JM, *et al*. The CLEC-2-podoplanin axis controls the contractility of fibroblastic reticular cells and lymph node microarchitecture. *Nat Immunol* 2015; 16:75-84.
- Martin-Villar E, Scholl FG, Gamallo C, Yurrita MM, Munoz-Guerra M, Cruces J, *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.
7. Wicki A, Lehembre F, Wick N, Hantusch B, Kerjaschki D, Christofori G. Tumor invasion in the absence of epithelial-mesenchymal transition: podoplanin-mediated remodeling of the actin cytoskeleton. *Cancer Cell* 2006; 9:261-272.
 8. 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.
 9. Kolenda J, Jensen SS, Aaberg-Jessen C, Christensen K, Andersen C, Brunner N, et al. Effects of hypoxia on expression of a panel of stem cell and chemoresistance markers in glioblastoma-derived spheroids. *J Neurooncol* 2011; 103:43-58.
 10. Bortolomai I, Canevari S, Facetti I, De Cecco L, Castellano G, Zacchetti A, et al. Tumor initiating cells: development and critical characterization of a model derived from the A431 carcinoma cell line forming spheres in suspension. *Cell Cycle* 2010; 9:1194-1206.
 11. 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.
 12. Ugorski M, Dziegiel P, Suchanski J. Podoplanin - a small glycoprotein with many faces. *Am J Cancer Res* 2016; 6:370-386.
 13. Pula B, Witkiewicz W, Dziegiel P, Podhorska-Okolow M. Significance of podoplanin expression in cancer-associated fibroblasts: a comprehensive review. *Int J Oncol* 2013; 42:1849-1857.
 14. 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.
 15. 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.
 16. Fernandez-Munoz B, Yurrita MM, Martin-Villar E, Carrasco-Ramirez P, Megias D, Renart J, et al. The transmembrane domain of podoplanin is required for its association with lipid rafts and the induction of epithelial-mesenchymal transition. *Int J Biochem Cell Biol* 2011; 43:886-896.
 17. Scholl FG, Gamallo C, Quintanilla M. Ectopic expression of PA2.26 antigen in epidermal keratinocytes leads to destabilization of adherens junctions and malignant progression. *Lab Invest* 2000; 80:1749-1759.
 18. Mei Y, Zhang P, Zuo H, Clark D, Xia R, Li J, et al. Ebp1 activates podoplanin expression and contributes to oral tumorigenesis. *Oncogene* 2014; 33:3839-3850.
 19. Carrasco-Ramirez P, Greening DW, Andres G, Gopal SK, Martin-Villar E, Renart J, et al. Podoplanin is a component of extracellular vesicles that reprograms cell-derived exosomal proteins and modulates lymphatic vessel formation. *Oncotarget* 2016; 7:16070-16089.
 20. Hwang YS, Xianglan Z, Park KK, Chung WY. Functional invadopodia formation through stabilization of the PDPN transcript by IMP-3 and cancer-stromal crosstalk for PDPN expression. *Carcinogenesis* 2012; 33:2135-2146.
 21. Martin-Villar E, Borda-d'Agua B, Carrasco-Ramirez P, Renart J, Parsons M, Quintanilla M, et al. Podoplanin mediates ECM degradation by squamous carcinoma cells through control of invadopodia stability. *Oncogene* 2015; 34:4531-4544.
 22. Martin-Villar E, Fernandez-Munoz B, Parsons M, Yurrita MM, Megias D, Perez-Gomez E, et al. Podoplanin associates with CD44 to promote directional cell migration. *Mol Biol Cell* 2010; 21:4387-4399.
 23. Zhao P, Xu Y, Wei Y, Qiu Q, Chew TL, Kang Y, et al. The CD44s splice isoform is a central mediator for invadopodia activity. *J Cell Sci* 2016; 129:1355-1365.
 24. Lowe KL, Navarro-Nunez L, Watson SP. Platelet CLEC-2 and podoplanin in cancer metastasis. *Thromb Res* 201; 129 Suppl 1:S30-37.
 25. Suzuki-Inoue K, Hughes CE, Inoue O, Kaneko M, Cuyun-Lira O, Takafuta T, et al. Involvement of Src kinases and PLCgamma2 in clot retraction. *Thromb Res* 2007; 120:251-258.
 26. Nakazawa Y, Sato S, Naito M, Kato Y, Mishima K, Arai H, et al. Tetraspanin family member CD9 inhibits Aggrus/podoplanin-induced platelet aggregation and suppresses pulmonary metastasis. *Blood* 2008; 112:1730-1739.
 27. Raposo G, Stoorvogel W. Extracellular vesicles: exosomes, microvesicles, and friends. *J Cell Biol* 2013; 200:373-383.
 28. Colombo M, Raposo G, Thery C. Biogenesis, secretion, and intercellular interactions of exosomes and other extracellular vesicles. *Annu Rev Cell Dev Biol* 2014; 30:255-289.
 29. Hoshino D, Kirkbride KC, Costello K, Clark ES, Sinha S, Grega-Larson N, et al. Exosome secretion is enhanced by invadopodia and drives invasive behavior. *Cell Rep* 2013; 5:1159-1168.
 30. Maruyama Y, Maruyama K, Kato Y, Kajiya K, Moritoh S, Yamamoto K, et al. The effect of podoplanin inhibition on lymphangiogenesis under pathological conditions. *Invest Ophthalmol Vis Sci* 2014; 55:4813-4822.
 31. Cueni LN, Chen L, Zhang H, Marino D, Huggerberger R, Alitalo A, et al. Podoplanin-Fc reduces lymphatic vessel formation in vitro and in vivo and causes disseminated intravascular coagulation when transgenically expressed in the skin. *Blood* 2010; 116:4376-4384.
 32. Alitalo A, Detmar M. Interaction of tumor cells and lymphatic vessels in cancer progression. *Oncogene* 2012; 31:4499-4508.
 33. Stacker SA, Williams SP, Karnezis T, Shayan R, Fox SB, Achen MG. Lymphangiogenesis and lymphatic vessel remodelling in cancer. *Nat Rev Cancer* 2014; 14:159-172.
 34. Breiteneder-Geleff S, Soleiman A, Kowalski H, Horvat R, Amann G, Kriehuber E, et al. Angiosarcomas express mixed endothelial phenotypes of blood and lymphatic capillaries: podoplanin as a specific marker for lymphatic endothelium. *Am J Pathol* 1999; 154:385-394.
 35. Schacht V, Ramirez MI, Hong YK, Hirakawa S, Feng D, Harvey N, et al. T1alpha/podoplanin deficiency disrupts normal lymphatic vasculature formation and causes lymphedema. *EMBO J* 2003; 22:3546-3556.

36. Yuan P, Temam S, El-Naggar A, Zhou X, Liu DD, Lee JJ, *et al.* Overexpression of podoplanin in oral cancer and its association with poor clinical outcome. *Cancer* 2006; 107:563-569.
37. Suzuki H, Onimaru M, Yonemitsu Y, Maehara Y, Nakamura S, Sueishi K. Podoplanin in cancer cells is experimentally able to attenuate prolymphangiogenic and lymphogenous metastatic potentials of lung squamoid cancer cells. *Mol Cancer* 2010; 9:287.
38. Pula B, Wojnar A, Witkiewicz W, Dziegiel P, Podhorska-Okolow M. Podoplanin expression in cancer-associated fibroblasts correlates with VEGF-C expression in cancer cells of invasive ductal breast carcinoma. *Neoplasma* 2013; 60:516-524.
39. Kitano H, Kageyama S, Hewitt SM, Hayashi R, Doki Y, Ozaki Y, *et al.* Podoplanin expression in cancerous stroma induces lymphangiogenesis and predicts lymphatic spread and patient survival. *Arch Pathol Lab Med* 2010; 134:1520-1527.
40. Fu H, Yang H, Zhang X, Xu W. The emerging roles of exosomes in tumor-stroma interaction. *J Cancer Res Clin Oncol* 2016; doi: 10.1007/s00432-016-2145-0.