Am J Cancer Res 2016;6(2):370-386
www.ajcr.us /ISSN:2156-6976/ajcr0022475

Review Article

Podoplanin - a small glycoprotein with many faces

Maciej Ugorski[1,2], Piotr Dziegiel[3,4], Jaroslaw Suchanski[2]

[1]Laboratory of Glycobiology and Cell Interactions, Ludwik Hirszfeld Institute of Immunology and Experimental Therapy, Polish Academy of Sciences, Wroclaw, Poland; [2]Department of Biochemistry, Pharmacology and Toxicology, Faculty of Veterinary Medicine, University of Environmental and Life Sciences, Wroclaw, Poland; [3]Department of Histology and Embryology, Medical University, Wroclaw, Poland; [4]Department of Physiotherapy, Wroclaw University of Physical Education, Wroclaw, Poland

Received December 12, 2015; Accepted January 6, 2016; Epub January 15, 2016; Published February 1, 2016

Abstract: Podoplanin is a small membrane glycoprotein with a large number of *O*-glycoside chains and therefore it belongs to mucin-type proteins. It can be found on the surface of many types of normal cells originating from various germ layers. It is present primarily on the endothelium of lymphatic vessels, type I pneumocytes and glomerular podocytes. Increased levels of podoplanin or its neo-expression have been found in numerous types of human carcinomas, but it is especially common in squamous cell carcinomas, such as cervical, larynx, oral cavity, skin and lung cancer. This small sialomucin is also seen on the surface of cancer-associated fibroblasts (CAFs) in lung adenocarcinomas, as well as in breast and pancreatic tumors. In most cancers, a high level of podoplanin expression, both in cancer cells, as well as in CAFs, is correlated with an increased incidence of metastasis to lymph nodes and shorter survival time of patients. Little is known about the biological role of podoplanin, however research carried out on mice with a knock-out gene of this glycoprotein shows that the presence of podoplanin determines normal development of lungs, the lymphatic system and heart. Podoplanin on cancer cells and CAFs seems to play an important role in the development and progression of various cancers. However, its role in these processes is both unclear and controversial. In this review, the role of podoplanin in both physiological processes and carcinogenesis is discussed.

Keywords: Podoplanin, physiological role, cancer marker, carcinogenesis, cancer-associated fibroblasts

Introduction

Podoplanin is a small membrane glycoprotein with a large number of *O*-glycoside chains and therefore it belongs to mucin-type proteins. Since it was discovered independently and almost simultaneously in human [1] and several mammal species in various cells and tissues, it has been given many names. In humans it is also known as gp36 [2] and T1α [3]. Its homologues in mice are known as OTS-8 [4], gp38 [5], aggrus [6], antigen PA2.26 [7] and RANDAM-2 (retinoic acid-induced neuronal differentiated-associated molecule-2) [8], in rats as T1α protein [9], E11 antigen [10] or podoplanin [11], in canine cells as gp40 [12] or aggrus [13], and in hamsters and cows also as aggrus [13].

Structure

Human podoplanin is an integral membrane protein composed of 162 amino acid residues [2, 14]. In its structure there is an extracellular domain rich in *O*-glycoside type carbohydrate chains, a transmembrane domain and a short intracellular domain having only 9 amino acids [15] (**Figure 1**). As a type-I membrane glycoprotein, its *N*-terminus is directed outside the cell. A comparison of podoplanin amino acid sequences from various species showed them to be conservative [2, 13, 15].

In the intracellular domain, there is a characteristic sequence of three basic amino acids (**Figure 1**) which also occur in other membrane proteins. It is known to be responsible for their binding to ezrin-radixin-moesin complex (ERM proteins, see section 4.2) [16]. On the other hand, within the extracellular domain three adjacent (tandem repeats) conservative amino acid sequences (EDxxVTPG) have been found. In humans, the first of those sequences interacts directly with podoplanin receptor CLEC-2 present on platelets (see section 4.2) [13]. This single sequence was named platelet aggrega-

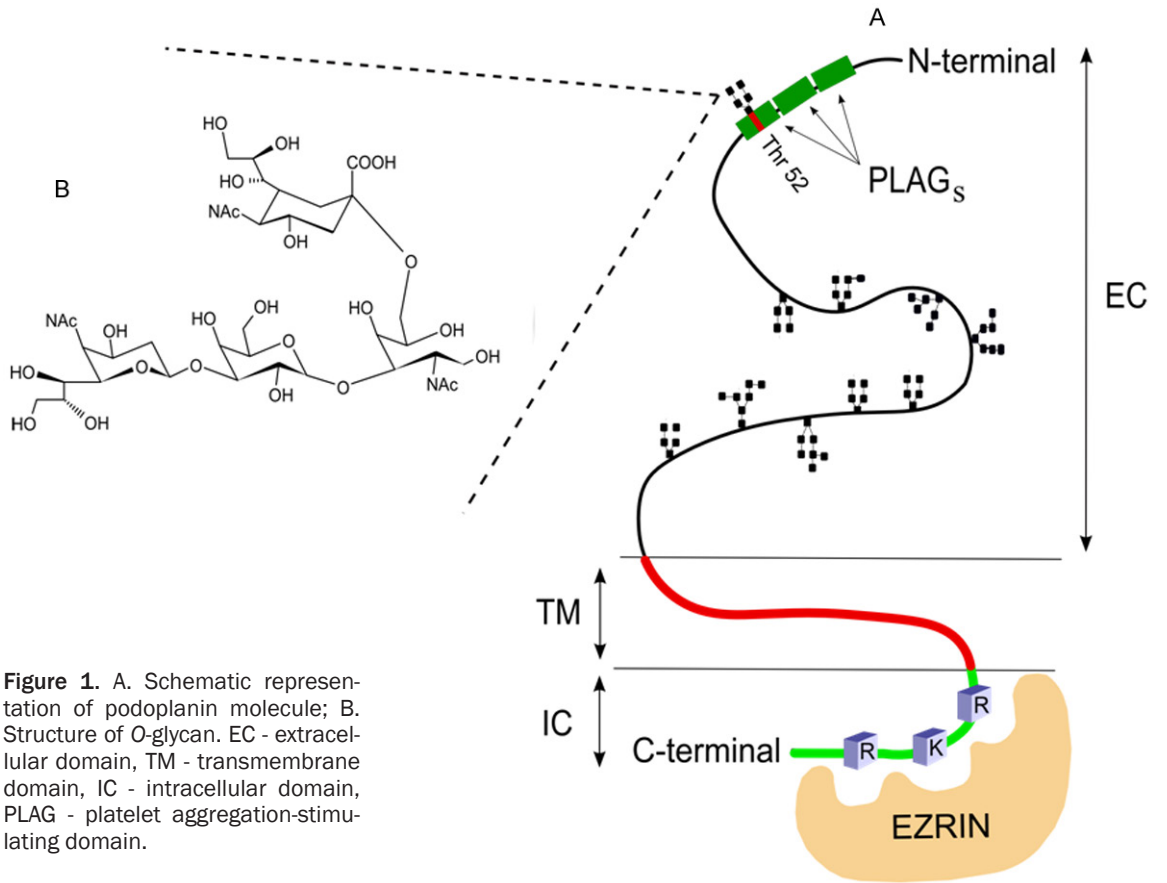

Figure 1. A. Schematic representation of podoplanin molecule; B. Structure of *O*-glycan. EC - extracellular domain, TM - transmembrane domain, IC - intracellular domain, PLAG - platelet aggregation-stimulating domain.

tion-stimulating domain (subdomain PLAG) [14]. A comparison of amino acid sequences of those subdomains from various species showed that they are conservative [2, 13, 15]. PLAG subdomain functions as a carrier for the *O*-glycosidic chain attached to threonine at position 52 in a polypeptide chain, which has the structure of a type-1 core with two sialic acid residues (**Figure 1**) [6, 17, 18], with which CLEC-2 interacts directly [19]. The participation of a second *O*-glycosidic chain attached to threonine at position 34 in those interactions was suggested by Kunita et al. [20].

Podoplanin in normal cells

Occurrence

Podoplanin was discovered for the first time in rat and mice lungs and on the surface of stromal cells in T-dependent zones of peripheral lymphatic tissues (e.g. lymph nodes) in mice [4, 5, 21]. Later it was seen on the surface of many types of normal cells originating from various germ layers. It is present primarily on the endothelium of lymphatic vessels [11, 22], type-I pneumocytes [2, 9, 10, 21, 23] and glomerular podocytes [1]. It was also found on the cells of the basal layer of human sweat glands and the external layer of hair follicles [24], peritoneal mesothelial cells [25, 26], ependymocytes (cells of the lining) of mouse and the human central nervous system [22, 26], rat and human osteocytes [10, 26], reticular cells, dendritic cells of lymphatic organs, granular cells of rat ovarian follicles [26] and mouse fibroblasts [7]. This glycoprotein was also seen on the epithelial cells of oral cavity mucous membrane and teeth during mouse embryonic development [7], as well as on glutaminergic neurones of mouse cerebrum [8].

Podoplanin expression changes during embryonic development. During initial lung morphogenesis, it is commonly present on the epithelium of pulmonary alveoli, whereas in the final stage, its expression is limited to type-I pneumocytes only and its level increases significantly together with the differentiation of those cells in the last stage of embryogenesis (see section 3.2) [9, 27]. During nervous system development, initially it is common and high

podoplanin expression decreases along with fetus growth, and in the brain of adults it is limited to choroid plexus [10, 23, 27].

Physiological role

The biological role of podoplanin is still poorly understood. However, observations made on knock-out mice show that the presence of podoplanin determines normal development and functioning of lungs, the lymphatic system and heart. Mice with *PDPN* gene knock-out die immediately after birth due to respiratory failure because their lungs can not be filled by air up to the necessary volume, and the structure of terminal respiratory units in the lungs is abnormal. Without podoplanin, differentiation of type-I pneumocytes is inhibited, causing underdevelopment of pulmonary alveoli [23]. It is possible that podoplanin regulates the proliferative potential of lung cells, which in turn determinates appropriate differentiation into type-I pneumocytes [23]. Moreover, it was shown that in type-I pneumocytes podoplanin is present together with caveolin-1 in lipid rafts found preferentially in protrusions localized on the apical side of the cell, which, as the authors believe, may be linked to unspecified biological activity of this glycoprotein [28]. In the lymphatic system of mice with *PDPN* gene knock-out, expanded malfunctioning lymphatic vessels were found, which causes lymphedema and may be linked with the fact that in *in vitro* assays podoplanin increased haptotactic migration and adhesion of endothelial cells to fibronectin and type-I collagen. It also accelerated the formation of capillary vessels [29]. The relation between podoplanin, migration and lymphatic vessel formation was also indicated in experiments showing that inhibition of podoplanin expression in the endothelial cells of lung lymphatic vessels reduced their mobility and prevented capillary tube formation [30]. Their adhesion, migration and formation of capillary tubes *in vitro* and capillary vessels *in vivo* were also reduced by a blocking function of podoplanin expressed by lymphatic vessel endothelium using fusion protein made of podoplanin extracellular domain and Fc fragment of IgG [31]. The important role of podoplanin in the formation of a normal lymphatic system during embryonic development is indicated by studies with knock-out mice model lacking expression of *C1galt1* gene only in endothelial and hematopoietic cells [32]. The lack of expression of this gene encoding core 1 β-1,3-galactosyltransferase (antigen T-synthase), responsible for synthesis of type-1 core *O*-glycans [33], was accompanied by a significant reduction of podoplanin expression in lymphatic endothelial cells. Interestingly, in embryos of such mice, blood-filled lymphatic vessels and partial lymphatic vessels separation from blood vessels was observed. The key role of podoplanin in this process was confirmed by further studies, where one more *PDPN* gene knock-out mouse model was used [34]. Based on that model, it was suggested that podoplanin occurring at the surface of cells of lymphatic endothelium induces formation of blood platelets aggregates (see section 4.2), which either mechanically close the aperture between the lymphatic sac and cardinal vein or are the source of factors causing vasoconstriction of blood vessels, which consequently lead to lymphatic - blood vessel separation. Podoplanin involvement in the formation of normal lymphatic vessels and their proper function to, among other things, activate and aggregate blood platelets was also revealed in the studies where the above mentioned fusion protein composed of podoplanin extracellular domain and Fc fragment of IgG was used to block podoplanin function [31].

In mice with podoplanin gene knock-out, increased fetus mortality was observed, which was linked to disorders in normal heart development [35]. Such embryos had underdeveloped structures which were differentiating into epicardium and changes in adhesion of epicardium cells together with impairment in their diffusion and migration. Embryos without podoplanin also showed hypoplasia and ventricular septal defect, as well as endocardial cushion hypoplasis leading to abnormalities in the structure of the atrio-ventricular valve. Abnormalities in the structure of coronary vessels were also found. The observed disorders are probably caused by the decreased ability of cells to the epithelial-mesenchymal transition (see section 4.2) due to an increased amount of cadherin E, which may be regulated by podoplanin. This mucin-type glycoprotein possibly takes part in the regulation of the shape of podocyte protrusions, which determinates proper blood filtration within glomeruli [1, 36].

Podoplanin is expressed by mouse keratinocytes during wound healing, which indicates its potential role in tissue regeneration [7]. Similarly, induction of podoplanin expression was observed on the cells of human epidermis damaged as a result of injury and on the epidermis from patients with psoriasis [24]. Since podoplanin expression increased in such keratinocytes under the influence of TGF-β1, IFN-γ, IL-6, the authors suggest that it may be involved, as in mice, in wound healing and pathogenesis of psoriasis. However, there is very little information regarding the role of podoplanin in the above processes on the molecular level. In this context, it has been proposed that podoplanin plays a significant role in the process of adhesion, on the one hand as an anti-adhesion molecule, and on the other hand as an adhesion-promoting molecule e.g. by interaction with CLEC-2 protein (see section 4.2). Another protein interacting with podoplanin is chemokine CCL21. The biological role of such interactions is to facilitate recruitment of lymphocytes expressing CCR7 receptor for this chemokine by lymphatic vessels that produce and secrete protein complexes composed of podoplanin and CCL21 chemokine [37]. Podoplanin is also bound by animal lectin, galectin-8 [38]. Since galectin-8 facilitates adhesion and haptotactic migration of lymphatic endothelial cells, it was suggested that interactions between podoplanin and galectin-8 promote endothelial cells anchoring into the surrounding extracellular matrix. It should be noted that all podoplanin interactions with other proteins into which extracellular domain is engaged, depend on its correct glycosylation. Therefore, it should not be surprising that it also is the main receptor for influenza type C virus due to the presence of 9-*O*-acetylated sialic acid residues [12].

Besides extracellular domains, both intracellular and transmembrane domains are engaged in interactions with other proteins, and this is linked to the role of podoplanin in cell migration, where such interactions have been analyzed mainly for their role in carcinogenesis and cancer progression (see section 4.2).

Podoplanin in cancers

Occurrence

Induction of podoplanin expression was observed in animal tumors generated experimentally. For example, significant amounts of this glycoprotein were found both on the surface of transformed mouse keratinocytes and on the stromal cells of tumors developed as a result of treating animals with 7,12-dimethylbenz[*a*]anthracene (DMBA) or 12-O-tetradecanoylphorbol-13-acetate (TPA) [7]. Increased levels of podoplanin or its neo-expression were shown in many types of human cancers. It occurs in several squamous cell carcinomas, such as oral cavity, tongue and pharynx [15, 39, 40], skin [26] and lung cancer [41, 42] (**Figure 2A** and **2B**). In oral cavity and tongue cancers, a high level of podoplanin expression was correlated with an increased incidence of metastasis to lymph nodes and shorter survival time of patients [39, 40], and in lung squamous cell carcinoma - with shorter survival time of patients [42]. Similarly, high podoplanin expression on the cells of squamous cell esophageal cancer was correlated with the severity of the disease, increased cancer cell invasion into lymphatic and blood vessels and a higher incidence of metastasis into regional lymph nodes, disease recurrences and shorter patients' survival [43, 44]. Wicki et al. [45] showed that generally in squamous cell carcinomas, including esophageal, skin, larynx, uterine cervix and lung cancers, as well as in some adenocarcinomas, such as lobular breast cancer, podoplanin expression was limited to cancer cells forming an external layer of invasion front. Shimada et al. [46] observed similar localization of the cells expressing podoplanin in squamous cell lung cancer. However according to these authors, as well as Ito et al. [47], and in contradiction to previous studies, the presence of podoplanin in primary tumors was correlated with the lack of invasion to lymphatic vessels and with better patients' survival. Low podoplanin expression was also an unfavorable prognostic factor in squamous cell uterine cervix cancer and it was associated with an increased infiltration of cancer cells into lymphatic vessels, an increased incidence of metastasis into regional lymph nodes and worse patients' survival [48]. Those results were confirmed by Longatto-Filho et al. [49], who linked the lack of podoplanin expression on cancer cells with an increased incidence of metastasis into regional lymph nodes and distant organs, as well as with shorter survival time of patients with mixed, glandular-epithelial form of uterine cervix cancer.

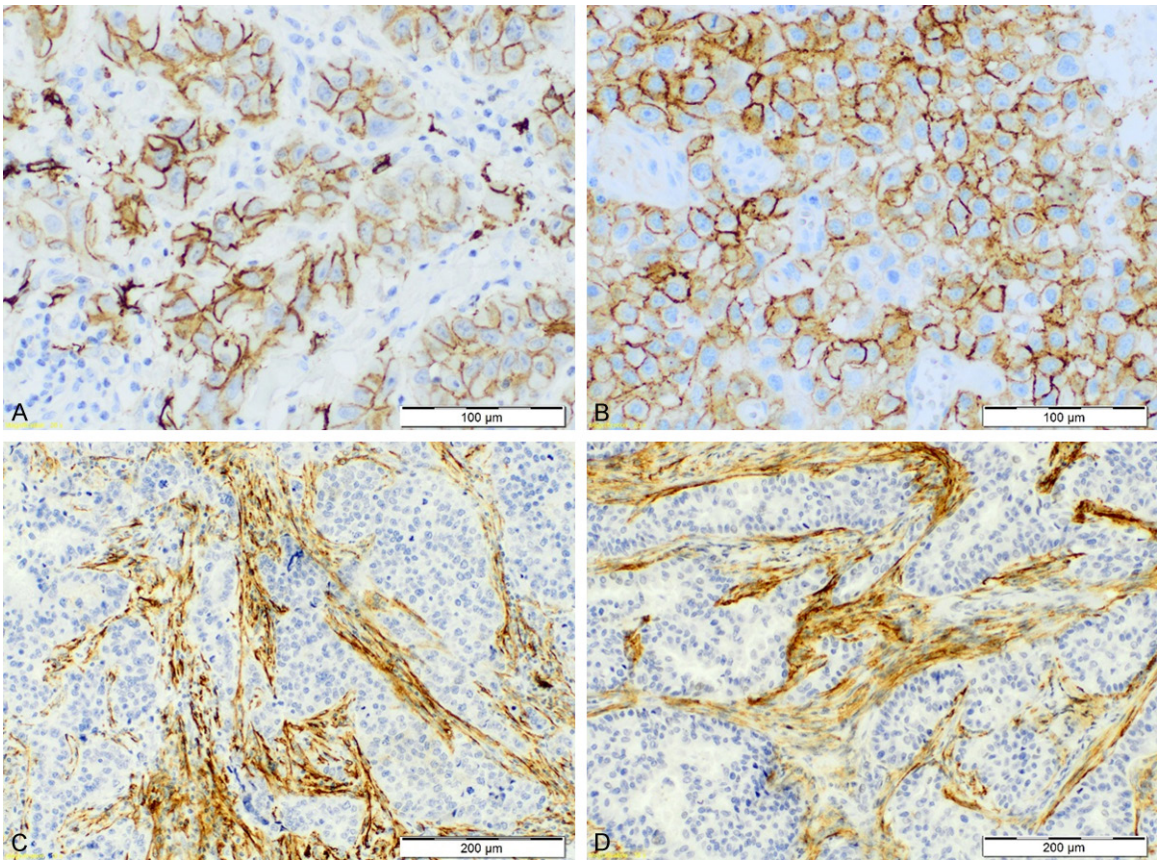

Figure 2. Immunohistochemistry method: podoplanin membrane staining of squamous cancer cells of skin (A) and lung (B) cancers; podoplanin expression in the cancer-associated fibroblasts (CAFs) of lung adenocarcinoma (C) and invasive ductal breast carcinoma (D) in tumor stroma.

In addition to squamous cell carcinomas, podoplanin expression was shown in germ cell tumors of the ovary [26], angiosarcomas [11], osteosarcomas, where its level was significantly higher in metastases in comparison to primary tumors [50], mesotheliomas [25, 51, 52], basal cell carcinomas of the skin [53], and follicular dendric cell tumors [54]. Podoplanin was also found in certain adenocarcinomas, such as colorectal [14] and stomach [55] carcinomas. In the latter case, it was related to highly invasive cancers with high metastatic potential. Increased amounts of podoplanin were also observed in some carcinomas of the central nervous system, such as germinoma-type tumors [56], as well as in glioblastoma multiforme in comparison to anaplastic astrocytomas [57].

The presence of podoplanin in hemangioblastomas facilitates and sometimes even enables its distinction from clear cell renal cell carcinoma [58]. Similarly, the presence of this glycoprotein on the cells of mesothelioma helps to distinguish it from lung adenocarcinoma [51], and in testicular germ cell tumors facilitates the distinction of seminomas from embryonal carcinoma [59, 60].

In human A431 cell line, podoplanin is a marker of cells with stem-cell-like properties, which are characterized by their high efficiency to form colonies *in vitro* and high tumorigenicity in nude mice model [61]. Based on these results, it is possible that podoplanin can be a marker of cancer stem cells in squamous cell skin carcinoma.

The role of podoplanin in carcinogenesis and cancer progression

In early studies on podoplanin it was shown that its presence on the surface of murine colon cancer cells and melanoma cells correlated with their ability to aggregate blood platelets and metastatic potential [6, 62, 63]. The

association between podoplanin expression and platelet aggregation, as well as the ability to metastasize, was confirmed by further studies which showed that antibodies directed against podoplanin inhibit both the formation of aggregates between murine colon cancer cells and platelets, as well as the formation of experimental lung metastases [64]. Ectopic podoplanin expression in Chinese hamster ovary cells (CHO) increased their ability to form experimental metastases in nude mice model, which was directly associated with the aggregation of platelets by those cells [19, 20]. It should be remembered that one of the necessary stages of metastasizing cascade is cancer cell infiltration into the blood vessels, where the majority of them is eliminated, due to either the activity of the immune system or mechanical damages caused by various factors associated with blood flow. One of the mechanisms of cancer cell protection, both against their recognition by the immune system, as well as haemodynamic factor influence, is an aggregation resulting from adhesion between cancer cells themselves and/or from adhesion between cancer cells and morphotic blood components, particularly platelets [65-67]. Aggregation is also supposed to increase the chance of metastasis by facilitated adhesion of cancer cell clusters to vascular endothelium. Moreover, platelet-derived factors increase the proliferation potential of cancer cells and facilitate their extravasation by retraction of endothelial cells. Podoplanin is bounded by blood platelets with the help of a "non-classical" C-type lectin receptor named CLEC-2 (C-type lectin-like receptor 2), which does not need Ca^{+2} ions for its activity [19, 68, 69]. The interaction between podoplanin and CLEC-2 causes blood platelets degranulation and their activation as the result of CLEC-2 oligomerisation [70], thus promoting formation of further platelet aggregates [68]. CLEC-2 is also present on the surface of leukocytes, including human dendric cells (DC) [71]. It was recently shown that interactions between CLEC-2 expressed by dendric cells and podoplanin expressed by lymphatic endothelial and fibroblastic reticular cells are necessary for DCs to spread and migrate along stromal surfaces and are sufficient to induce membrane protrusions [72]. Activation of dendric cells *via* podoplanin binding includes downregulation of RhoA activity, phosphorylation of myosin light-chain and formation of actin F rich protrusions.

In contrast to podoplanin binding by CLEC-2, which promotes metastases formation by cancer cells, its interaction with protein CD9 decreases their metastatic potential [73]. Protein CD9 belongs to the tetraspanin family of proteins, which regulate cell migration and are engaged in signaling pathways by forming in cell membranes multi-molecular complexes called tetraspanin-enriched microdomains or tetraspanin web [74]. Protein CD9 itself is known as a metastasis suppressor [75]. CD9 was shown to interact with podoplanin, with its transmembrane domains T1 and T2 being directly engaged in this process, and formation of such complexes on the surface of CHO cells results in a reduced number of experimental lung metastases in nude mice model [73]. The activity of CD9 as molecule inhibiting metastasis formation is most probably caused by lowered aggregation of blood platelets with the participation of podoplanin and CLEC-2.

For the first time the possible involvement of podoplanin in cancer formation and progression, other than the formation of aggregates by cancer cells and platelets, was taken into account during studies on carcinogenesis processes occurring in mouse skin. It was demonstrated that podoplanin appears on the surface of epidermis and dermis cells during processes associated with tissue remodeling e.g. wound healing, as well as carcinogenesis caused by chemical agents (see section 3.1) [7]. Expression of podoplanin as a result of treatment with 12-O-tetradecanoylphorbol-13-acetate (TPA) was also observed in murine osteoblastic cells transformed with Ras oncogene [4]. This glycoprotein accumulated in cellular protrusions, suggesting its association with the ability of those cells to migrate [7]. Therefore, its engagement in migration properties was evaluated in further studies with the use of immortalized mouse keratinocytes showing ectopic podoplanin expression [22]. It was shown that the occurrence of this glycoprotein in keratinocytes is associated with changes in their morphology i.e. formation of actin-enriched phyllopodia and lamellipodia, in addition to previously found actin-rich protrusions [7]. The accumulation of podoplanin molecules was observed on the surface of those protrusions where ezrin, radixin and moesin are also found. These so-called ERM proteins are believed to be universal linkers binding actin cytoskeleton with integral membrane proteins,

and therefore they are engaged in signaling pathways associated with cell adhesion and migration [76]. Furthermore, these studies showed that the intracellular domain of podoplanin directly interacts with ezrin and moesin. The occurrence of cellular protrusions was associated with profound changes in cytoskeleton organization and alterations in ezrin localization, which accumulated in those protrusions. Most importantly, morphological changes caused by podoplanin were associated with increased migration properties of keratinocytes. Motility is one of the key properties acquired by tumor cells during cancer progression. Therefore, in the next research stage the role of podoplanin in skin cancer development was analyzed [77]. Neo-expression of this glycoprotein in mouse MCA3D keratinocytes led to epithelial-mesenchymal transition (EMT) characterized by a loss of polarity and adhesion by epithelial cells, with a simultaneous acquirement of the migratory properties, which is a characteristic feature of fibroblasts [78, 79]. In embryonic development, EMT accompanies tissue formation and remodeling, as well as organogenesis. In pathology, EMT is observed during wound healing, inflammation and cancer progression, when cancer cells acquire the ability to infiltrate surrounding tissues and form metastasis. Various factors, from cytokines to transcription factors, are engaged in acquiring properties of mesenchymal cells by epithelial cells. In keratinocytes, podoplanin neo-expression led to reduced expression of characteristic cytokeratins, with a simultaneous increase in the expression of vimentin and cytokeratin K8. Additionally, the disappearance of adherens junctions was observed, which was associated with a decrease in the amount of E- and P-cadherins. In such cells the occurrence of actin-enriched phyllopodia and lamellipodia was observed, while the loss of actin filaments in cortical cytoplasm was also visible. These cells, in contrast to native and control cells (transfected with vector only), after being grafted to nude mice grew to form a tumor and metastasized into regional lymph nodes. These experimental data were supported by immunohistochemical studies on tissue sections from oral squamous cell carcinomas, which showed that reduced expression of E-cadherin correlated with podoplanin occurrence at those sites, where ezrin was also present [15]. A similar phenomenon was observed for HeLa cells and human immortalized keratinocytes transfected with podoplanin cDNA. Ectopic expression of podoplanin was accompanied by an increased number of longer cell protrusions associated with the transfer of ezrin into sites next to the plasma membrane, where podoplanin was localized (as described above). It was associated with lowered adhesive properties of cells and the phenomenon called “cell scattering” - diffused growth of epithelial cells in culture. Therefore it was suggested that changes in intercellular adhesion triggered by podoplanin are caused directly by the reorganization of actin cytoskeleton as a result of changes in ezrin localization. Direct interaction of podoplanin with ezrin and moesin was suggested based on the results of studies on canine MDCK cells, where ectopic expression of this sialomucin was observed [80]. Interestingly, although MDCK cells are non-cancerous, expression of human podoplanin caused a change in phenotype typical for epithelial-mesenchymal transition, which was associated with acquiring the ability to migrate and invade. It is quite surprising, because human immortalized non-cancerous keratinocytes transfected with podoplanin cDNA changed their phenotype to a limited degree only, in contrast to malignantly transformed mouse keratinocytes, which, similarly to canine MDCK cells, were fully subjected to EMT [15]. By using various mutated forms of podoplanin it was shown that for the change of phenotype of MDCK cells its cytoplasmic domain is responsible, and specifically a group of three basic amino acids responsible for binding ERM proteins (see section 2). Since GTP-binding proteins from the Rho family (RhoA, Rac1, Cdc42) are engaged in cells acquiring the ability to migrate and invade [81], their expression was analyzed in native and control MDCK cells, as well as in MDCK cells with ectopic podoplanin expression. It was found that the activity of RhoA protein is significantly increased in the latter ones, suggesting that podoplanin is engaged in the activation of this GTP-ase. In order to explain, how podoplanin activates RhoA protein, a mechanism similar to the one described for CD44 antigen and RhoA was suggested [82, 83]. According to this hypothesis, podoplanin-ERM protein complex binds activating GDP/GTP exchange protein (GEP) and this complex interacts in turn with the Rho-GDP dissociation inhibitor associated with RhA pro-

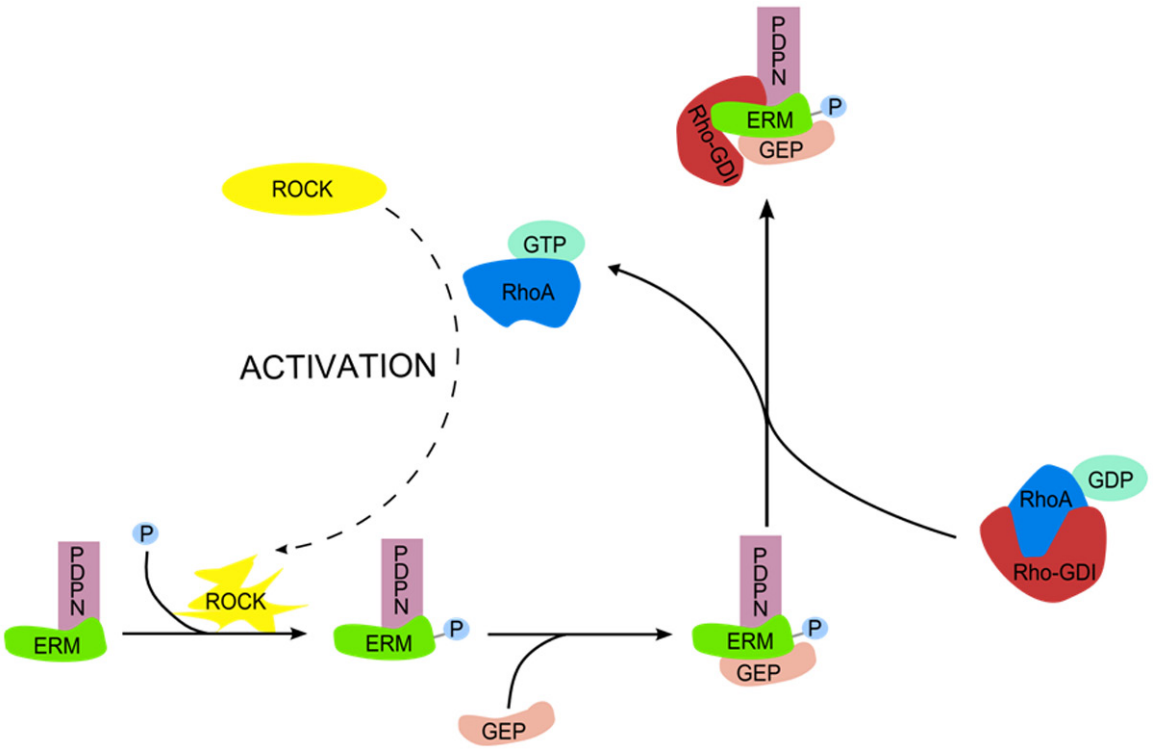

Figure 3. A model for the activation of RhoA protein by podoplanin and the role of activated RhoA protein in PDPN-ERM protein interaction. PDPN - podoplanin, ERM - ERM proteins, GEP - GDP/GTP exchange protein, Rho-GDI - Rho-GDP dissociation inhibitor, ROCK - RhoA-associated kinase.

tein with GDP molecule bounded (RhoA-GDP) (**Figure 3**). It causes the release of RhoA-GDP, which simultaneously allows for its activation by the GDP exchange for GTP with the engagement of GDP-GTP exchange factor. An increase in the activity of RhoA protein leads to the activation of RhoA-associated kinase (ROCK), which phosphorylates ERM proteins. This then stabilizes their active conformation [84] and enhances interactions between podoplanin and a cell's cytoskeleton. Therefore, podoplanin interactions with ERM proteins are engaged in EMT by not only binding it physically to a cell's cytoskeleton, but also by the activation of RhoA protein. Recent studies have shown that podoplanin on the surface of MDCK cells is localized within lipid rafts and both transmembrane and cytoplasmic domains are responsible for this [85]. This localization is necessary for podoplanin to actively participate in epithelial-mesenchymal transition and cell migration.

By its extracellular domain, podoplanin present on the surface of cancer cells interacts not only with CLEC-2 protein localized on platelets, but also it specifically binds to CD44 protein expressed on the same cancer cell. As shown in the studies of Martin-Villar et al. [86], the so-called standard form of CD44 antigen (CD44s) [87] and podoplanin are subjected to an increased expression on the same cells during the progression of mouse squamous cell skin cancer. In cancer cells, both glycoproteins are present on the cellular protrusions, where they directly interact with each other, and their carbohydrate chains are most probably engaged in this process. The interactions with CD44 are required for podoplanin to facilitate directed movement of cancer cells (directional migration).

Podoplanin engagement in cancer progression, mainly by increasing migration and invasiveness of cancer cells, was also suggested by Wicki and Chrstofori [88]. However, their hypothesis on podoplanin's role in this process differs significantly from the one proposed above. While analyzing podoplanin expression in tissue sections from squamous cell carcinomas of lung, uterine cervix, larynx, skin and esophagus, they showed expression of podo-

planin only on cancer cells forming the tumor invasion front and only those which were in direct contact with stroma and normal tissues [45]. Interestingly, in addition to podoplanin, these cells expressed E-cadherin on their surface, so despite their ability to migrate and infiltrate surrounding tissues, they were not subjected to EMT. In cancer progression, EMT is crucial for invasion of single-cells or small groups of cells and early cancer cell spreading [78], while it seems to play a less significant role in the case of massive invasion with collective cell invasion [89]. In the latter form of invasion observed in the analyzed clinical samples, despite maintenance of epithelial-specific adhesion molecules, cancer cells invade surrounding tissues. Thus, in further studies, the function of podoplanin in this form of invasive infiltration was addressed using transgenic Rip1Podo mice expressing podoplanin under the control of the rat insulin promoter in the β cells of islets of Langerhans, which were crossed into the Rip1Tag2 mice representing mouse model of pancreatic β cell cancer [90]. The transition from benign adenoma to malignant cancer in Rip1Tag2 mice is associated with the loss of E-cadherin expression by β cells of islets of Langerhans and the occurrence of N-cadherin. However, podoplanin expression in those cells caused the development of malignant cancer without changes in cadherin expression and EMT. It was associated with changes in cancer invasiveness and instead of invasion with single cells of modified morphology, invasion of large groups of cells was observed in the absence of EMT [45, 88]. Localization of actin fibres was significantly affected by the presence of podoplanin and they were displaced from the subcortical layer of the cell into phyllopodia-type protrusions. The results obtained from animal models were confirmed in *in vitro* studies, where human breast cancer MCF7 cells with ectopic expression of podoplanin were used [45]. As in the case of other cells with ectopic expression of podoplanin (see above), its presence caused significant changes in their appearance and migration abilities, which were associated with the formation of multiple protrusions (phyllopodia) and ezrin re-localization. However, in contrast to previous studies, no loss of E-cadherin expression was observed in such cells, despite the occurrence of N-cadherin and vimentin, which indicates that they were subjected to EMT. MCF7 cells with podoplanin over-expression were characterized by significantly higher migration and invasion potential in comparison to parental cells and control cells transfected with vector alone. Besides ERM proteins, podoplanin also affected the activity of RhoA protein, which can be connected with higher migration capabilities of those cells too. Contradicting previous studies [80], expression of this sialomucin decreased the activity of RhoA [40, 45].

In certain carcinomas, such as breast cancer, tumor cell spreading leading to metastasis occurs mainly in the lymphatic system, however, the molecular basis of these processes are poorly understood. Podoplanin engagement in this phenomenon is indicated by the studies of Suzuki et al. [91]. They showed that ectopic podoplanin expression in squamoid cancer EBC-1 cells inhibits lymphangiogenesis and attenuates formation of metastases to regional lymph nodes in nude mice model. The inhibitory effects of podoplanin expression was associated with activation of the JNK signaling pathway, which in turn inhibits the production of vascular endothelial growth factor-C (VEGF-C) playing a key role in lymphangiogenesis stimulated by cancer cells [92]. These results are supported by clinical data showing that the presence of podoplanin in lung squamous cell carcinoma is a favorable prognostic factor, and correlates with a lack of invasion into lymphatic vessels and better survival of patients [46, 47]. In contrast to the above studies, Cueni et al. [40] showed that podoplanin present on breast cancer cells induces lymphangiogenesis and promotes metastases to regional lymph nodes, which was shown in nude mice model following orthotopic engraftment of MCF7 cells expressing this glycoprotein. A comparison of gene expression profiles in podoplanin-expressing MCF7 cells and control MCF7 cells showed that lymphangiogenic activity of podoplanin is associated with an increased expression of endothelin-1 (ET-1), which binds to lymphatic endothelial cells through specific B receptor (ENDRB). It should be stressed that podoplanin is only rarely expressed by breast cancer cells, and in breast cancer it is mainly found on stromal cells [93]. Therefore it raises doubts about the appropriateness of such a cellular model, especially in *in vivo* studies.

As already mentioned, expression of podoplanin by cancer cells increases their migration and invasion capabilities, which raises questions about the regulation of its expression in those cells. Src oncogen, whose role in carcinogenesis is well-documented [94], was shown to be responsible for podoplanin expression in mouse cells [95]. Src kinase induces podoplanin expression by the phosphorylation of Cas adaptor protein (Crk-associated substrate), which promotes cancer cell migration [96]. It may also happen through stimulatory activity of Src oncogen on AP-1 transcription factor, whose component is Fos protein increasing podoplanin expression in mouse keratinocytes and fibroblasts during carcinogenesis [97].

Podoplanin on cancer-associated fibroblasts (CAF) is involved in tumor progression

In early studies regarding the role of podoplanin in carcinogenesis, the presence of podoplanin was confirmed not only on the surface of malignantly transformed mouse keratinocytes, but also in mouse fibroblasts [7]. Podoplanin expression was also observed in fibroblasts from chronically inflamed tissues and organs [98]. Furthermore, this small sialomucin was found on the surface of cancer-associated fibroblasts (CAF) in lung adenocarcinomas, where it was also detected on cancer cells, but in a significantly smaller number of cases (30.5% v. 5.1%) [99]. The presence of podoplanin-expressing fibroblasts was positively correlated (statistically significant) with tumor size, metastases to regional lymph nodes, clinical stage, the degree of differentiation, cancer cell invasion into vessels and pleura, as well as patients' survival [99-101]. The above studies have been expanded by additional cases and additional types of cancers, such as breast, kidney, bile ducts, thyroid, liver, colon, stomach, prostate, pancreas, urinary bladder and uterus [102]. Generally podoplanin expression in tumor stromal myofibroblasts (cells belonging to cancer-associated fibroblasts) (**Figure 2C** and **2D**) significantly correlated with the size of the tumor, lymph node metastases and lymphatic and blood vessel invasion by cancer cells. However, in the case of lung cancers, the correlation between podoplanin expression and patients' survival was observed only in adenocarcinomas, but not in squamous cell carcinomas. Based on these results it was suggested that podoplanin produced by myofibroblasts induces lymphangiogenesis, and therefore promotes metastases formation by lymphatic means, and its presence is an unfavorable prognostic factor correlating with shorter patients' survival. However, there is no direct experimental evidence to confirm it. These authors own research confirmed the significance of podoplanin on the surface of CAF as an unfavorable prognostic marker in invasive forms of ductal breast cancer [93]. Fibroblasts expressing podoplanin were positively correlated (statistically significant) with tumor size, degree of malignancy, lymph node metastases, invasion into lymphatic and blood vessels, expression of Ki67 antigen, shorter patients' survival, and VEGF-C expression [93, 103]. Schoppmann et al. [104] confirmed these results and also showed that in invasive breast cancer, the presence of podoplanin-expressing CAF correlates with poor patients' prognosis. Similar results were recently obtained in invasive forms of ductal pancreatic cancer [105]. Expression of podoplanin found only on the surface of cancer-associated fibroblasts, just as in breast cancer, was positively correlated with tumor size, the degree of malignancy, invasion of lymphatic and blood vessels, as well as shorter patients' survival. Podoplanin was also present on the surface of myofibroblasts found in tissue sections of liver cancer originating from bile ductules [106]. The percentage of podoplanin-expressing myofibroblasts was correlated with metastases into regional lymph nodes.

Contradicting the above studies on the role of podoplanin present on CAF in cancer progression, which see podoplanin as an unfavourable prognostic factor in a few types of cancer, its expression on the CAFs in colon cancer is supposed to indicate (statistically significant) longer survival of patients with this cancer [107]. Podoplanin was limited to cancer-associated fibroblasts and co-cultures of CAF with suppressed expression of podoplanin with colon cancer cells increased the invasive potential of the latter. Based on these results it was suggested that podoplanin-expressing fibroblasts limit invasiveness of cancer cells in this type of cancer. Studies by Carvalho et al. [108] also indicated that podoplanin expressed by stromal cells in squamous cell uterine cervix cancer was a favorable prognostic marker. They showed that podoplanin expression by the cells

with fibroblast phenotype was negatively correlated with metastasis into lymph nodes. However, this conclusion should be treated with caution, because fibroblasts expressing podoplanin were found only in a small number of patients (n=27) relative to the total number of cases (n=143). Moreover, they are incompatible with the results of studies done by Kitano et al. [102], which, interestingly, are not cited in this publication.

CAFs in the tumor stroma may promote cancer growth by secretion of chemokines, growth factors, induction of angiogenesis and recruitment of progenitor endothelial cells from bone marrow, or extracellular matrix remodeling [109, 110]. Thereby, they affect proliferation, survival and invasive properties of cancer cells [111, 112]. Thus the question arises regarding the role of podoplanin present on the surface of CAFs in these processes. In order to answer that question, Hoshino et al. [100] used human vascular adventitial fibroblasts (hVAFs), which come in contact with cancer cells during their migration into the lumen of blood vessels (intravasation), as well as human lung fibroblasts (hLFs), and human lung adenocarcinoma cells. With the use of nude mice model it was shown that hVAFs increase the ability of cancer cells to develop tumors to a greater degree in comparison to hLF. These differences in biological properties of fibroblasts originating from the same organ, but from different tissues, were correlated with the level of podoplanin expression in those cells, which was significantly higher in hVAF than in hLF. Direct involvement of podoplanin in increasing tumorigenicity, as well as metastatic potential of lung cancer cells was confirmed by experiments, where hVAF with inhibited podoplanin expression or its over-expression were used. Based on experiments, as well as the results of *in vitro* studies regarding the ability of cancer cells to form colonies in the presence and absence of hVAF, it was suggested that podoplanin-enriched fibroblasts affect the development of cancer cells in early stages of tumor growth, before the stage of cancer vascularisation. No direct interaction between those fibroblasts and cancer cells affected the proliferative potential and apoptotic properties of the latter. Therefore, based on the above studies, it was not possible to explain how podoplanin present on the surface of CAFs stimulates cancer growth on the molecular level. Taking into account the fact that A549 cells used in these studies do not have CLEC-2 protein on their surface (see section 4.2), the same group of researchers focused on the role of podoplanin's intracellular domain in cancer progression, using a mutated form of podoplanin lacking this domain [113]. Based on these studies, it was found, firstly, that human fibroblasts expressing wild type podoplanin promote subcutaneous tumor formation by A549 cells, which is associated with increased activity of RhoA protein in comparison to control podoplanin-negative fibroblasts. Secondly, the absence of cytoplasmic domain in podoplanin molecules decreases the effect of podoplanin overexpressing fibroblasts on cancer cells. Since RhoA protein is engaged in cancer migration processes and extracellular matrix remodeling [114, 115], it was proposed that enhanced RhoA activity facilitates podoplanin-positive-fibroblasts to create a microenvironment promoting cancer growth. Recently, Shindo et al. [105] analyzed the role of podoplanin-expressing CAFs in their interactions with pancreatic cancer cells. They showed that fibroblasts with high podoplanin expression increase migration and invasiveness of cancer cells, but on the other hand, inhibition of expression of this glycoprotein with siRNA did not affect their migration or invasiveness. This suggests that the presence of podoplanin on the surface of CAFs does not have a direct, functional link with changes in biological properties of pancreatic cancer cells. Rather it is a marker of a specific CAF subpopulation, which interacts with cancer cells. The above studies are contrary to the results obtained for colon cancer, where it was shown that inhibition of podoplanin expression in fibroblasts co-cultured with colon cancer cells increases their invasiveness [107]. This suggests that CAFs with expression of podoplanin may not so much promote, as inhibit cancer progression.

Summary

In spite of more than 30 years of research, questions regarding the role of podoplanin, both in physiological processes, as well as in cancer development and progression, are still far from being answered. The lack of consistency and often contrary results obtained by different research teams are troublesome. For example, based on the available literature, it is

impossible to clearly answer the question as to the role of podoplanin in such phenomenon as EMT (discussed in section 4.2) or its role in CAFs (discussed in section 5). In contrast to others, in these authors own research it was impossible to show that ectopic expression of podoplanin changes the phenotype and activity of breast cancer cells or that its presence on the surface of fibroblasts has any impact on biological properties of cancer cells (manuscript in preparation). Therefore, further studies regarding this protein, which is undoubtedly important for the proper functioning of many cells and tissues, are still necessary.

Acknowledgements

Grant support for this work was provided by the National Science Center (Poland); grant no. N N401 570340.

Disclosure of conflict of interest

None.

Address to correspondence to: Maciej Ugorski, Laboratory of Glycobiology and Cell Interactions, Ludwik Hirszfeld Institute of Immunology and Experimental Therapy, Polish Academy of Sciences, Wrocław, Poland. E-mail: maciej.ugorski@up.wroc.pl

References

[1] Breiteneder-Geleff S, Matsui K, Soleiman A, Meraner P, Poczewski H, Kalt R, Schaffner G and Kerjaschki D. Podoplanin, novel 43-kd membrane protein of glomerular epithelial cells, is down-regulated in puromycin nephrosis. Am J Pathol 1997; 151: 1141-1152.

[2] Zimmer G, Oeffner F, Von Messling V, Tschernig T, Groness HJ, Klenk HD and Herrler G. Cloning and characterization of gp36, a human mucin-type glycoprotein preferentially expressed in vascular endothelium. Biochem J 1999; 341: 277-284.

[3] Ma T, Yang B, Matthay MA and Verkman AS. Evidence against a role of mouse, rat, and two cloned human t1alpha isoforms as a water channel or a regulator of aquaporin-type water channels. Am J Respir Cell Mol Biol 1998; 19: 143-149.

[4] Nose K, Saito H and 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.

[5] Farr AG, Berry ML, Kim A, Nelson AJ, Welch MP and Aruffo A. Characterization and cloning of a novel glycoprotein expressed by stromal cells in T-dependent areas of peripheral lymphoid tissues. J Exp Med 1992; 176: 1477-1482.

[6] Toyoshima M, Nakajima M, Yamori T and Tsuruo T. Purification and characterization of the platelet-aggregating sialoglycoprotein gp44 expressed by highly metastatic variant cells of mouse colon adenocarcinoma 26. Cancer Res 1995; 55: 767-773.

[7] Gandarillas A, Scholl FG, Benito N, Gamallo C and 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.

[8] Kotani M, Tajima Y, Osanai T, Irie A, Iwatsuki K, Kanai-Azuma M, Imada M, Kato H, Shitara H, Kubo H and Sakuraba H. Complementary DNA cloning and characterization of RANDAM-2, a type I membrane molecule specifically expressed on glutamatergic neuronal cells in the mouse cerebrum. J Neurosci Res 2003; 73: 603-613.

[9] Rishi AK, Joyce-Brady M, Fisher J, Dobbs LG, Floros J, VanderSpek J, Brody JS and Williams MC. Cloning, characterization, and development expression of a rat lung alveolar type I cell gene in embryonic endodermal and neural derivatives. Dev Biol 1995; 167: 294-306.

[10] Wetterwald A, Hoffstetter W, Cecchini MG, Lanske B, Wagner C, Fleisch H and Atkinson M. Characterization and cloning of the E11 antigen, a marker expressed by rat osteoblasts and osteocytes. Bone 1996; 18: 125-132.

[11] Breiteneder-Geleff S, Soleiman A, Kowalski H, Horvat R, Amann G, Kriehuber E, Diem K, Weninger W, Tschachler E, Alitalo K and Kerjaschki D. 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.

[12] Zimmer G, Klenk HD and Herrler G. Identification of a 40-kDa cell surface sialoglycoprotein with the characteristics of a major influenza C virus receptor in a Madin-Darby canine kidney cell line. J Biol Chem 1995; 270: 17815-17822.

[13] Kaneko MK, Kato Y, Kitano T and Osawa M. Conservation of a platelet activating domain of Aggrus/podoplanin as a platelet aggregation-inducing factor. Gene 2006; 378: 52-57.

[14] Kato Y, Fujita N, Kunita A, Sato S, Kaneko M, Osawa M and Tsuruo T. Molecular identification of Aggrus/T1alpha as a platelet aggregation-inducing factor expressed in colorectal tumors. J Biol Chem 2003; 278: 51599-51605.

[15] Martin-Villar E, Scholl FG, Gamallo C, Yurrita MM, Munoz-Guerra M, Cruces J and Quintanilla M. 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.

[16] Yonemura S, Hirao M, Doi Y, Takahashi N, Kondo T, Tsukita S and Tsukita S. Ezrin/radixin/moesin (ERM) proteins bind to a positively charged amino acid cluster in the juxta-membrane cytoplasmic domain of CD44, CD43, and ICAM-2. J Cell Biol 1998; 140: 885-895.

[17] Kaneko M, Kato Y, Kunita A, Fujita N, Tsuruo T and Osawa M. Functional sialylated O-glycan to platelet aggregation on Aggrus (T1alpha/Podoplanin) molecules expressed in Chinese hamster ovary cells. J Biol Chem 2004; 279: 38838-38843.

[18] Kaneko MK, Kato Y, Kameyama A, Ito H, Kuno A, Hirabayashi J, Kubota T, Amano K, Chiba Y, Hasegawa Y, Sasagawa I, Mishima K and Narimatsu H. Functional glycosylation of human podoplanin: glycan structure of platelet aggregation-inducing factor. FEBS Lett 2007; 581: 331-336.

[19] Kato Y, Kaneko MK, Kunita A, Ito H, Kameyama A, Ogasawara S, Matsuura N, Hasegawa Y, Suzuki-Inoue K, Inoue O, Ozaki Y and Narimatsu H. Molecular analysis of the pathophysiological binding of the platelet aggregation-inducing factor podoplanin to the C-type lectin-like receptor CLEC-2. Cancer Sci 2008; 99: 54-61.

[20] Kunita A, Kashima TG, Morishita Y, Fukayama M, Kato Y, Tsuruo T and Fujita N. The platelet aggregation-inducing factor aggrus/podoplanin promotes pulmonary metastasis. Am J Pathol 2007; 170: 1337-1347.

[21] Dobbs LG, Williams MC and Gonzalez R. Monoclonal antibodies specific to apical surfaces of rat alveolar type I cells bind to surfaces of cultured, but not freshly isolated, type II cells. Biochim Biophys Acta 1988; 970: 146-156.

[22] Scholl FG, Gamallo C, Vilaro S and 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.

[23] Ramirez MI, Millien G, Hinds A, Cao YX, Seldin DC and Williams MC. T1 alpha, a lung type I cell differentiation gene, is required for normal lung cell proliferation and alveolus formation at birth. Developmental Biology 2003; 256: 61-72.

[24] Honma M, Minami-Hori M, Takahashi H and Iizuka H. Podoplanin expression in wound and hyperproliferative psoriatic epidermis: Regulation by TGF-beta and STAT-3 activating cytokines, IFN-gamma, IL-6, and IL-22. J Dermatol Sci 2012; 65: 134-140.

[25] Ordonez NG. D2-40 and podoplanin are highly specific and sensitive immunohistochemical markers of epithelioid malignant mesothelioma. Hum Pathol 2005; 36: 372-380.

[26] Schacht V, Dadras SS, Johnson LA, Jackson DG, Hong YK and Detmar M. Up-regulation of the lymphatic marker podoplanin, a mucin-type transmembrane glycoprotein, in human squamous cell carcinomas and germ cell tumors. Am J Pathol 2005; 166: 913-921.

[27] Williams MC, Cao Y, Hinds A, Rishi AK and Wetterwald A. T1 alpha protein is developmentally regulated and expressed by alveolar type I cells, choroid plexus, and ciliary epithelia of adult rats. Am J Respir Cell Mol Biol 1996; 14: 577-585.

[28] Barth K, Blasche R and Kasper M. T1alpha/podoplanin shows raft-associated distribution in mouse lung alveolar epithelial E10 cells. Cell Physiol Biochem 2010; 25: 103-112.

[29] Schacht V, Ramirez MI, Hong YK, Hirakawa S, Feng D, Harvey N, Williams M, Dvorak AM, Dvorak HF, Oliver G and Detmar M. T1alpha/podoplanin deficiency disrupts normal lymphatic vasculature formation and causes lymphedema. EMBO J 2003; 22: 3546-3556.

[30] Navarro A, Perez RE, Rezaiekhaligh M, Mabry SM and Ekekezie II. T1alpha/podoplanin is essential for capillary morphogenesis in lymphatic endothelial cells. Am J Physiol Lung Cell Mol Physiol 2008; 295: L543-551.

[31] Cueni LN, Chen L, Zhang H, Marino D, Huggenberger R, Alitalo A, Bianchi R and Detmar M. 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] Fu J, Gerhardt H, McDaniel JM, Xia B, Liu X, Ivanciu L, Ny A, Hermans K, Silasi-Mansat R, McGee S, Nye E, Ju T, Ramirez MI, Carmeliet P, Cummings RD, Lupu F and Xia L. Endothelial cell O-glycan deficiency causes blood/lymphatic misconnections and consequent fatty liver disease in mice. J Clin Invest 2008; 118: 3725-3737.

[33] Argueso P, Guzman-Aranguez A, Mantelli F, Cao Z, Ricciuto J and Panjwani N. Association of cell surface mucins with galectin-3 contributes to the ocular surface epithelial barrier. J Biol Chem 2009; 284: 23037-23045.

[34] Uhrin P, Zaujec J, Breuss JM, Olcaydu D, Chrenek P, Stockinger H, Fuertbauer E, Moser M, Haiko P, Fassler R, Alitalo K, Binder BR and Kerjaschki D. Novel function for blood platelets and podoplanin in developmental separation of blood and lymphatic circulation. Blood 2010; 115: 3997-4005.

[35] Mahtab EA, Wijffels MC, Van Den Akker NM, Hahurij ND, Lie-Venema H, Wisse LJ, Deruiter MC, Uhrin P, Zaujec J, Binder BR, Schalij MJ, Poelmann RE and Gittenberger-De Groot AC. Cardiac malformations and myocardial abnormalities in podoplanin knockout mouse embryos: Correlation with abnormal epicardial development. Dev Dyn 2008; 237: 847-857.

[36] Matsui K, Breiteneder-Geleff S and Kerjaschki D. Epitope-specific antibodies to the 43-kD glomerular membrane protein podoplanin cause proteinuria and rapid flattening of podocytes. J Am Soc Nephrol 1998; 9: 2013-2026.

[37] Kerjaschki D, Regele HM, Moosberger I, Nagy-Bojarski K, Watschinger B, Soleiman A, Birner P, Krieger S, Hovorka A, Silberhumer G, Laakkonen P, Petrova T, Langer B and Raab I. Lymphatic neoangiogenesis in human kidney transplants is associated with immunologically active lymphocytic infiltrates. J Am Soc Nephrol 2004; 15: 603-612.

[38] Cueni LN and Detmar M. Galectin-8 interacts with podoplanin and modulates lymphatic endothelial cell functions. Exp Cell Res 2009; 315: 1715-1723.

[39] Yuan P, Temam S, El-Naggar A, Zhou X, Liu DD, Lee JJ and Mao L. Overexpression of podoplanin in oral cancer and its association with poor clinical outcome. Cancer 2006; 107: 563-569.

[40] Cueni LN, Hegyi I, Shin JW, Albinger-Hegyi A, Gruber S, Kunstfeld R, Moch H and Detmar M. Tumor lymphangiogenesis and metastasis to lymph nodes induced by cancer cell expression of podoplanin. Am J Pathol 2010; 177: 1004-1016.

[41] Kato Y, Kaneko M, Sata M, Fujita N, Tsuruo T and Osawa M. Enhanced expression of Aggrus (T1alpha/podoplanin), a platelet-aggregation-inducing factor in lung squamous cell carcinoma. Tumour Biol 2005; 26: 195-200.

[42] Kadota K, Huang CL, Liu D, Nakashima N, Yokomise H, Ueno M and Haba R. The clinical significance of the tumor cell D2-40 immunoreactivity in non-small cell lung cancer. Lung Cancer 2010; 70: 88-93.

[43] Chuang WY, Yeh CJ, Wu YC, Chao YK, Liu YH, Tseng CK, Chang HK, Liu HP and Hsueh C. Tumor cell expression of podoplanin correlates with nodal metastasis in esophageal squamous cell carcinoma. Histol Histopathol 2009; 24: 1021-1027.

[44] Rahadiani N, Ikeda J, Makino T, Tian T, Qiu Y, Mamat S, Wang Y, Doki Y, Aozasa K and Morii E. Tumorigenic role of podoplanin in esophageal squamous-cell carcinoma. Ann Surg Oncol 2010; 17: 1311-1323.

[45] Wicki A, Lehembre F, Wick N, Hantusch B, Kerjaschki D and Christofori G. Tumor invasion in the absence of epithelial-mesenchymal transition: podoplanin-mediated remodeling of the actin cytoskeleton. Cancer Cell 2006; 9: 261-272.

[46] Shimada Y, Ishii G, Nagai K, Atsumi N, Fujii S, Yamada A, Yamane Y, Hishida T, Nishimura M, Yoshida J, Ikeda N and Ochiai A. Expression of podoplanin, CD44, and p63 in squamous cell carcinoma of the lung. Cancer Sci 2009; 100: 2054-2059.

[47] Ito T, Ishii G, Nagai K, Nagano T, Kojika M, Murata Y, Atsumi N, Nishiwaki Y, Miyazaki E, Kumamoto T and Ochiai A. Low podoplanin expression of tumor cells predicts poor prognosis in pathological stage IB squamous cell carcinoma of the lung, tissue microarray analysis of 136 patients using 24 antibodies. Lung Cancer 2009; 63: 418-424.

[48] Dumoff KL, Chu CS, Harris EE, Holtz D, Xu X, Zhang PJ and Acs G. Low podoplanin expression in pretreatment biopsy material predicts poor prognosis in advanced-stage squamous cell carcinoma of the uterine cervix treated by primary radiation. Mod Pathol 2006; 19: 708-716.

[49] Longatto-Filho A, Pinheiro C, Pereira SM, Etlinger D, Moreira MA, Jube LF, Queiroz GS, Baltazar F and Schmitt FC. Lymphatic vessel density and epithelial D2-40 immunoreactivity in pre-invasive and invasive lesions of the uterine cervix. Gynecol Oncol 2007; 107: 45-51.

[50] Kunita A, Kashima TG, Ohazama A, Grigoriadis AE and Fukayama M. Podoplanin is regulated by AP-1 and promotes platelet aggregation and cell migration in osteosarcoma. Am J Pathol 2011; 179: 1041-1049.

[51] Chu AY, Litzky LA, Pasha TL, Acs G and Zhang PJ. Utility of D2-40, a novel mesothelial marker, in the diagnosis of malignant mesothelioma. Mod Pathol 2005; 18: 105-110.

[52] Kimura N and Kimura I. Podoplanin as a marker for mesothelioma. Pathol Int 2005; 55: 83-86.

[53] Ishida M, Kushima R and Okabe H. Immunohistochemical demonstration of D2-40 in basal cell carcinomas of the skin. J Cutan Pathol 2008; 35: 926-930.

[54] Yu H, Gibson JA, Pinkus GS and Hornick JL. Podoplanin (D2-40) is a novel marker for follicular dendritic cell tumors. Am J Clin Pathol 2007; 128: 776-782.

[55] Raica M, Ribatti D, Mogoanta L, Cimpean AM and Ioanovici S. Podoplanin expression in advanced-stage gastric carcinoma and prognostic value of lymphatic microvessel density. Neoplasma 2008; 55: 455-460.

[56] Mishima K, Kato Y, Kaneko MK, Nakazawa Y, Kunita A, Fujita N, Tsuruo T, Nishikawa R, Hirose T and Matsutani M. Podoplanin expres-

sion in primary central nervous system germ cell tumors: a useful histological marker for the diagnosis of germinoma. Acta Neuropathol 2006; 111: 563-568.
[57] Mishima K, Kato Y, Kaneko MK, Nishikawa R, Hirose T and Matsutani M. Increased expression of podoplanin in malignant astrocytic tumors as a novel molecular marker of malignant progression. Acta Neuropathol 2006; 111: 483-488.
[58] Roy S, Chu A, Trojanowski JQ and Zhang PJ. D2-40, a novel monoclonal antibody against the M2A antigen as a marker to distinguish hemangioblastomas from renal cell carcinomas. Acta Neuropathol 2005; 109: 497-502.
[59] Kato Y, Sasagawa I, Kaneko M, Osawa M, Fujita N and Tsuruo T. Aggrus: a diagnostic marker that distinguishes seminoma from embryonal carcinoma in testicular germ cell tumors. Oncogene 2004; 23: 8552-8556.
[60] Iczkowski KA, Butler SL, Shanks JH, Hossain D, Schall A, Meiers I, Zhou M, Torkko KC, Kim SJ and MacLennan GT. Trials of new germ cell immunohistochemical stains in 93 extragonadal and metastatic germ cell tumors. Hum Pathol 2008; 39: 275-281.
[61] Atsumi N, Ishii G, Kojima M, Sanada M, Fujii S and Ochiai A. Podoplanin, a novel marker of tumor-initiating cells in human squamous cell carcinoma A431. Biochem Biophys Res Commun 2008; 373: 36-41.
[62] Watanabe M, Okochi E, Sugimoto Y and Tsuruo T. Identification of a platelet-aggregating factor of murine colon adenocarcinoma 26: Mr 44,000 membrane protein as determined by monoclonal antibodies. Cancer Res 1988; 48: 6411-6416.
[63] Watanabe M, Sugimoto Y and Tsuruo T. Expression of a Mr 41,000 Glycoprotein Associated with Thrombin-Independent Platelet-Aggregation in High Metastatic Variants of Murine-B16 Melanoma. Cancer Research 1990; 50: 6657-6662.
[64] Sugimoto Y, Watanabe M, Ohhara T, Sato S, Isoe T and Tsuruo T. Suppression of Experimental Lung Colonization of a Metastatic Variant of Murine Colon Adenocarcinoma-26 by a Monoclonal-Antibody 8f11 Inhibiting Tumor Cell-Induced Platelet-Aggregation. Cancer Research 1991; 51: 921-925.
[65] Mehta P. Potential Role of Platelets in the Pathogenesis of Tumor-Metastasis. Blood 1984; 63: 55-63.
[66] Honn KV, Tang DG and Crissman JD. Platelets and Cancer Metastasis - a Causal Relationship. Cancer Metastasis Rev 1992; 11: 325-351.
[67] Erpenbeck L and Schon MP. Deadly allies: the fatal interplay between platelets and metastasizing cancer cells. Blood 2010; 115: 3427-3436.
[68] Suzuki-Inoue K, Kato Y, Inoue O, Kaneko MK, Mishima K, Yatomi Y, Yamazaki Y, Narimatsu H and Ozaki Y. Involvement of the snake toxin receptor CLEC-2, in podoplanin-mediated platelet activation, by cancer cells. J Biol Chem 2007; 282: 25993-26001.
[69] Christou CM, Pearce AC, Watson AA, Mistry AR, Pollitt AY, Fenton-May AE, Johnson LA, Jackson DG, Watson SP and O'Callaghan CA. Renal cells activate the platelet receptor CLEC-2 through podoplanin. Biochem J 2008; 411: 133-140.
[70] Hughes CE, Pollitt AY, Mori J, Eble JA, Tomlinson MG, Hartwig JH, O'Callaghan CA, Futterer K and Watson SP. CLEC-2 activates Syk through dimerization. Blood 2010; 115: 2947-2955.
[71] Colonna M, Samaridis J and Angman L. Molecular characterization of two novel C-type lectin-like receptors, one of which is selectively expressed in human dendritic cells. Eur J Immunol 2000; 30: 697-704.
[72] Acton SE, Astarita JL, Malhotra D, Lukacs-Kornek V, Franz B, Hess PR, Jakus Z, Kuligowski M, Fletcher AL, Elpek KG, Bellemare-Pelletier A, Sceats L, Reynoso ED, Gonzalez SF, Graham DB, Chang J, Peters A, Woodruff M, Kim YA, Swat W, Morita T, Kuchroo V, Carroll MC, Kahn ML, Wucherpfennig KW and Turley SJ. Podoplanin-rich stromal networks induce dendritic cell motility via activation of the C-type lectin receptor CLEC-2. Immunity 2012; 37: 276-289.
[73] Nakazawa Y, Sato S, Naito M, Kato Y, Mishima K, Arai H, Tsuruo T and Fujita N. Tetraspanin family member CD9 inhibits Aggrus/podoplanin-induced platelet aggregation and suppresses pulmonary metastasis. Blood 2008; 112: 1730-1739.
[74] Hemler ME. Tetraspanin proteins mediate cellular penetration, invasion, and fusion events and define a novel type of membrane microdomain. Annu Rev Cell Dev Biol 2003; 19: 397-422.
[75] Takeda T, Hattori N, Tokuhara T, Nishimura Y, Yokoyama M and Miyake M. Adenoviral transduction of MRP-1/CD9 and KAI1/CD82 inhibits lymph node metastasis in orthotopic lung cancer model. Cancer Res 2007; 67: 1744-1749.
[76] Bretscher A, Edwards K and Fehon RG. ERM proteins and merlin: integrators at the cell cortex. Nat Rev Mol Cell Biol 2002; 3: 586-599.
[77] Scholl FG, Gamallo C and 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.

[78] Lee JM, Dedhar S, Kalluri R and Thompson EW. The epithelial-mesenchymal transition: new insights in signaling, development, and disease. J Cell Biol 2006; 172: 973-981.
[79] Polyak K and Weinberg RA. Transitions between epithelial and mesenchymal states: acquisition of malignant and stem cell traits. Nat Rev Cancer 2009; 9: 265-273.
[80] Martin-Villar E, Megias D, Castel S, Yurrita MM, Vilaro S and Quintanilla M. Podoplanin binds ERM proteins to activate RhoA and promote epithelial-mesenchymal transition. J Cell Sci 2006; 119: 4541-4553.
[81] Klopocka W and Baranska J. The role of Rho family proteins in controlling the migration of crawling cells. Postepy Biochem 2005; 51: 36-43.
[82] Takahashi K, Sasaki T, Mammoto A, Takaishi K, Kameyama T, Tsukita S and Takai Y. Direct interaction of the Rho GDP dissociation inhibitor with ezrin/radixin/moesin initiates the activation of the Rho small G protein. J Biol Chem 1997; 272: 23371-23375.
[83] Takahashi K, Sasaki T, Mammoto A, Hotta I, Takaishi K, Imamura H, Nakano K, Kodama A and Takai Y. Interaction of radixin with Rho small G protein GDP/GTP exchange protein Dbl. Oncogene 1998; 16: 3279-3284.
[84] Matsui T, Maeda M, Doi Y, Yonemura S, Amano M, Kaibuchi K, Tsukita S and Tsukita S. Rho-kinase phosphorylates COOH-terminal threonines of ezrin/radixin/moesin (ERM) proteins and regulates their head-to-tail association. J Cell Biol 1998; 140: 647-657.
[85] Fernandez-Munoz B, Yurrita MM, Martin-Villar E, Carrasco-Ramirez P, Megias D, Renart J and Quintanilla M. 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.
[86] Martin-Villar E, Fernandez-Munoz B, Parsons M, Yurrita MM, Megias D, Perez-Gomez E, Jones GE and Quintanilla M. Podoplanin associates with CD44 to promote directional cell migration. Mol Biol Cell 2010; 21: 4387-4399.
[87] Screaton GR, Bell MV, Jackson DG, Cornelis FB, Gerth U and Bell JI. Genomic structure of DNA encoding the lymphocyte homing receptor CD44 reveals at least 12 alternatively spliced exons. Proc Natl Acad Sci U S A 1992; 89: 12160-12164.
[88] Wicki A and Christofori G. The potential role of podoplanin in tumour invasion. Br J Cancer 2007; 96: 1-5.
[89] Friedl P and Wolf K. Tumour-cell invasion and migration: diversity and escape mechanisms. Nat Rev Cancer 2003; 3: 362-374.
[90] Hanahan D. Heritable formation of pancreatic beta-cell tumours in transgenic mice expressing recombinant insulin/simian virus 40 oncogenes. Nature 1985; 315: 115-122.
[91] Suzuki H, Onimaru M, Yonemitsu Y, Maehara Y, Nakamura S and 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.
[92] Alitalo K, Tammela T and Petrova TV. Lymphangiogenesis in development and human disease. Nature 2005; 438: 946-953.
[93] Pula B, Jethon A, Piotrowska A, Gomulkiewicz A, Owczarek T, Calik J, Wojnar A, Witkiewicz W, Rys J, Ugorski M, Dziegiel P and Podhorska-Okolow M. Podoplanin expression by cancer-associated fibroblasts predicts poor outcome in invasive ductal breast carcinoma. Histopathology 2011; 59: 1249-1260.
[94] Yeatman TJ. A renaissance for SRC. Nat Rev Cancer 2004; 4: 470-480.
[95] Shen Y, Chen CS, Ichikawa H and Goldberg GS. SRC induces podoplanin expression to promote cell migration. J Biol Chem 2010; 285: 9649-9656.
[96] Chodniewicz D and Klemke RL. Regulation of integrin-mediated cellular responses through assembly of a CAS/Crk scaffold. Biochim Biophys Acta 2004; 1692: 63-76.
[97] Durchdewald M, Guinea-Viniegra J, Haag D, Riehl A, Lichter P, Hahn M, Wagner EF, Angel P and Hess J. Podoplanin is a novel fos target gene in skin carcinogenesis. Cancer Res 2008; 68: 6877-6883.
[98] Muller AM, Franke FE and Muller KM. D2-40: a reliable marker in the diagnosis of pleural mesothelioma. Pathobiology 2006; 73: 50-54.
[99] Kawase A, Ishii G, Nagai K, Ito T, Nagano T, Murata Y, Hishida T, Nishimura M, Yoshida J, Suzuki K and Ochiai A. Podoplanin expression by cancer associated fibroblasts predicts poor prognosis of lung adenocarcinoma. Int J Cancer 2008; 123: 1053-1059.
[100] Hoshino A, Ishii G, Ito T, Aoyagi K, Ohtaki Y, Nagai K, Sasaki H and Ochiai A. Podoplanin-positive fibroblasts enhance lung adenocarcinoma tumor formation: podoplanin in fibroblast functions for tumor progression. Cancer Res 2011; 71: 4769-4779.
[101] Ito M, Ishii G, Nagai K, Maeda R, Nakano Y and Ochiai A. Prognostic impact of cancer-associated stromal cells in patients with stage I lung adenocarcinoma. Chest 2012; 142: 151-158.
[102] Kitano H, Kageyama S, Hewitt SM, Hayashi R, Doki Y, Ozaki Y, Fujino S, Takikita M, Kubo H and Fukuoka J. Podoplanin expression in cancerous stroma induces lymphangiogenesis and predicts lymphatic spread and patient survival. Arch Pathol Lab Med 2010; 134: 1520-1527.

[103] Pula B, Wojnar A, Witkiewicz W, Dziegiel P and 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.
[104] Schoppmann SF, Berghoff A, Dinhof C, Jakesz R, Gnant M, Dubsky P, Jesch B, Heinzl H and Birner P. Podoplanin-expressing cancer-associated fibroblasts are associated with poor prognosis in invasive breast cancer. Breast Cancer Res Treat 2012; 134: 237-244.
[105] Shindo K, Aishima S, Ohuchida K, Fujiwara K, Fujino M, Mizuuchi Y, Hattori M, Mizumoto K, Tanaka M and Oda Y. Podoplanin expression in cancer-associated fibroblasts enhances tumor progression of invasive ductal carcinoma of the pancreas. Mol Cancer 2013; 12: 168.
[106] Aishima S, Nishihara Y, Iguchi T, Taguchi K, Taketomi A, Maehara Y and Tsuneyoshi M. Lymphatic spread is related to VEGF-C expression and D2-40-positive myofibroblasts in intrahepatic cholangiocarcinoma. Mod Pathol 2008; 21: 256-264.
[107] Yamanashi T, Nakanishi Y, Fujii G, Akishima-Fukasawa Y, Moriya Y, Kanai Y, Watanabe M and Hirohashi S. Podoplanin expression identified in stromal fibroblasts as a favorable prognostic marker in patients with colorectal carcinoma. Oncology 2009; 77: 53-62.
[108] Carvalho FM, Zaganelli FL, Almeida BG, Goes JC, Baracat EC and Carvalho JP. Prognostic value of podoplanin expression in intratumoral stroma and neoplastic cells of uterine cervical carcinomas. Clinics (Sao Paulo) 2010; 65: 1279-1283.
[109] Orimo A, Gupta PB, Sgroi DC, Arenzana-Seisdedos F, Delaunay T, Naeem R, Carey VJ, Richardson AL and Weinberg RA. Stromal fibroblasts present in invasive human breast carcinomas promote tumor growth and angiogenesis through elevated SDF-1/CXCL12 secretion. Cell 2005; 121: 335-348.
[110] Pietras K, Pahler J, Bergers G and Hanahan D. Functions of paracrine PDGF signaling in the proangiogenic tumor stroma revealed by pharmacological targeting. PLoS Med 2008; 5: e19.
[111] Kalluri R and Zeisberg M. Fibroblasts in cancer. Nat Rev Cancer 2006; 6: 392-401.
[112] Tlsty TD and Coussens LM. Tumor stroma and regulation of cancer development. Annu Rev Pathol 2006; 1: 119-150.
[113] Ito S, Ishii G, Hoshino A, Hashimoto H, Neri S, Kuwata T, Higashi M, Nagai K and Ochiai A. Tumor promoting effect of podoplanin-positive fibroblasts is mediated by enhanced RhoA activity. Biochem Biophys Res Commun 2012; 422: 194-199.
[114] Takai Y, Sasaki T and Matozaki T. Small GTP-binding proteins. Physiol Rev 2001; 81: 153-208.
[115] Provenzano PP, Inman DR, Eliceiri KW, Trier SM and Keely PJ. Contact guidance mediated three-dimensional cell migration is regulated by Rho/ROCK-dependent matrix reorganization. Biophys J 2008; 95: 5374-5384.