ANTICANCER RESEARCH *28*: 2997-3006 (2008)

Review

The Role of Podoplanin in Tumor Progression and Metastasis

MARIUS RAICA[1], ANCA MARIA CIMPEAN[1] and DOMENICO RIBATTI[2]

[1]*Department of Histology and Cytology, "Victor Babes" University of Medicine and Pharmacy, Timisoara, Romania;*
[2]*Department of Human Anatomy and Histology, Faculty of Medicine, University of Bari, Bari, Italy*

Abstract. *In the last decade, much data has been generated concerning the molecular mechanisms of lymphangiogenesis and its significance in pathological conditions. This was mainly due to the discovery of lymphatic endothelial cell (LEC)-specific markers, such as vascular endothelial growth factor receptor-3 (VEGFR-3), LYVE-1, Prox-1 and podoplanin. Podoplanin, originally detected on the surface of podocytes, belongs to the family of type-1 transmembrane sialomucin-like glycoproteins. Although specific for lymphatic vascular (LV) endothelium, podoplanin is expressed in a wide variety of normal and tumor cells. The expression of podoplanin is induced by the homeobox gene Prox-1 and a specific endogenous receptor was identified on platelets. Immunohistochemical detection of podoplanin/D2-40 in LECs was used in many studies to evaluate the LV microvascular density (LVMD) in peritumoral and tumoral areas, and to correlate LVMD with lymph node status and prognosis. Podoplanin significantly increases the detection of lymphovascular invasion in different types of malignant tumors. Podoplanin expression was found in tumor cells of various types of cancer, such as vascular tumors, malignant mesothelioma, tumors of the central nervous system (CNS), germ cell tumors and squamous cell carcinomas. This expression in tumor cells is useful for pathological diagnosis and podoplanin seems to be expressed by aggressive tumors, with higher invasive and metastatic potential. Based on these data, podoplanin might be considered as an attractive therapeutic target for both LVs and tumor cells. Further studies are necessary to investigate differences in the expression of podoplanin in normal and tumor-associated lymphatics, and between the expression of podoplanin in normal non-LECs and tumor cells.*

Correspondence to: Professor Domenico Ribatti, Department of Human Anatomy and Histology, University of Bari Medical School, Piazza G. Cesare, 11, Policlinico, 70124 Bari, Italy. Tel: + 39 0805478240, Fax: +39 0805478310, e-mail: ribatti@anatomia.uniba.it

Key Words: Lymphatic vessels, metastasis, podoplanin, tumor growth, review.

Cancer is presently the second most frequent disease with lethal potential in humans, and, despite all efforts in the field of early diagnosis and adjuvant therapy, morbidity and specific mortality continue to increase. One of the most important factors with direct impact on prognosis and therapeutic strategy in various types of cancer is that of lymph node status. Although the relevance of this factor is well documented, the mechanisms by which tumor cells enter the lymphatic vessels (LVs) and give rise to lymph node metastases are not completely understood (1). For decades, it was thought that lymphatic-borne metastases occured by a passive mechanism, based on the apparently simpler structure of lymphatic capillaries, as demonstrated by electron microscopy many years ago. The investigation of the lymphatic system at a molecular level started almost 10 years ago, because of the lack in specific markers of the lymphatic endothelium. With the introduction of the first three markers, LYVE-1 (2), Prox 1 (3) and podoplanin (4), it was shown that LVs are present in the tumor and peritumoral areas, and that their number correlates with prognosis in malignant melanoma (5), squamous cell carcinoma of the head and neck (6), breast cancer (7) and gastric adenocarcinoma (8). Thus, the idea was born that at the interface between LV and tumor cells, an active process takes place, which today is known as lymphangiogenesis.

Lymphangiogenesis is the process of new LV formation, but the origin of newly formed lymphatics in normal and pathological conditions is still a subject of debate (9, 10). There is accumulated evidence that supports the proliferative activity of lymphatic endothelial cells (LECs) in pre-/postnatal life and in physiological and pathological conditions (11, 12). Based on these observations, it was hypothesized that LV growth and/or growth factors that induce lymphangiogenesis, such as vascular endothelial growth factor-C and -D (VEGF-C and VEGF-D), platelet-derived growth factor-BB (PDGF-BB) and hepatocyte growth factor, may be inhibited by specific antibodies (13-15). In an effort to better characterize the molecular profile of LECs, other more specific markers were found, such as

VEGF receptor-3 (VEGFR-3), Prox-1, desmoplakin and podoplanin. Like many other markers used in molecular pathology, none of the LEC-associated molecules is entirely specific for lymphatic endothelium. Podoplanin was first used to identify LVs, but it was later shown that podoplanin is a useful marker of some malignant tumors.

LEC-specific markers have multiple functions in physiological and pathological conditions, are helpful to identify tumor tissue changes related to lymphangiogenesis, and to search for a rational therapeutic approach. Some questions regarding tumor lymphangiogenesis remain unanswered, including the mechanisms of migration and invasion of tumor cells into the LVs, which is the key factor for tumor metastasis, and the differences between preexisting and newly formed LVs. The immunohistochemical application of podoplanin has been used to investigate the relationship between LV density and lymph node metastasis, for tumor cell detection in LVs, and for the diagnosis of some vascular tumors (5, 16-19). In this review article, we summarize the literature data concerning podoplanin expression in LVs and tumor cells, and the identification of podoplanin as a potential therapeutic target.

Discovery of Podoplanin

Podoplanin mRNA was identified for the first time in the murine osteoblastic cell line MC3Y3-E1 (20). The first identification of podoplanin in LECs and some other normal cells was performed in 1996, by Wetterwald *et al*. (21). It was firstly designated as E11 antigen and was then was called podoplanin due its low level expression in podocytes of the renal corpuscle.

Podoplanin was originally detected in puromycin-induced nephrosis on the surface of rat podocytes, as a 38 kDa mucoprotein linked to the flattening of foot processes (22). Puromycin aminonucleoside nephrosis is a rat model of human minimal change nephropathy, characterized by extensive flattening and retraction of podocyte foot processes and severe proteinuria, associated with a significant decrease of podoplanin expression. Podoplanin probably plays a role in maintaining the unique shape of podocytes (23). Further investigations showed that podoplanin, the oncofetal antigen M2A recognized by the D2-40 antibody, and the type I alveolar cell marker hT1α-2 are identical proteins.

Gene Control and Synthesis of Podoplanin

The podoplanin gene is a functioning gene with 34.2 kb and 8 exons which controls the synthesis of podoplanin. The subcellular location of the encoded protein is the plasma membrane. Two species of podoplanin mRNA were identified by Northern blotting that probably originate from alternative splicing (24).

Gp38P, which showed strong homologies to rat podoplanin and gp38, expressed by the thymus and other tissues, was cloned from mouse glomerular cells and it was suggested that it plays a role in membrane transport (25).

Sp1/Sp3 members constitutively bind to three responsive elements of the podoplanin promoter in MG63 cells *in vivo* and the activity of this promoter depends on the integrity of two of these sites (26). Highly methylated chromatin leads to the auxiliary enhancement of transcriptional activity of the podoplanin gene. Other studies are ongoing to characterize regulation provided by this promoter in other podoplanin-expressing cell lines.

Molecular Characterization of Podoplanin

Podoplanin, also known as aggrus, was generated from studies on platelet aggregation. Aggrus was able to induce platelet aggregation with no requirement for plasma components (27).

It was demonstrated that 8F11 monoclonal antibody, which inhibited platelet aggregation *in vitro* and mouse lung metastasis from colon carcinoma *in vivo* (28), recognized a cell-surface sialoglycoprotein and podoplanin belongs to the family of type-1 transmembrane sialomucin-like glycoproteins. It consists of 162 amino acids, with an extracellular domain rich in serine and threonine residues, a single transmembrane portion, and a short cytoplasmic tail, with sites for protein kinase C and cAMP phosphorylation (27).

Kato *et al*. (28) showed that the EDxxVTPG segment of the extracellular domain (platelet aggregation-stimulating domain, PLAG) is important for the activity of podoplanin and, in particular, threonine residues of this domain are important in platelet aggregation. Mutation of threonine residue in the PLAG domain abolished the platelet aggregation-inducing abilities of human and mouse aggrus protein (28).

Podoplanin possesses a disialyl-core1 structure in the PLAG domain, which is necessary for the binding of podoplanin to its specific receptor (29). A C-type lectin-like receptor-2 (CLEC-2) was identified as an endogenous receptor of podoplanin on platelets (30). By CLEC-2-Fc deletion mutants, inhibition of podoplanin-induced platelet aggregation was induced, and this indicates that CLEC-2 is a physiological ligand of podoplanin (29). Association between CLEC-2 and podoplanin was confirmed by flow cytometry and was found to be dependent on sialic acid present on *O*-glycans of podoplanin. Recombinant CLEC-2 inhibited platelet aggregation induced by podoplanin-expressing tumor cells and LECs (30). These findings suggest that CLEC-2 is a physiological target protein of podoplanin and the interaction between podoplanin and CLEC-2 may regulate tumor invasion and metastasis and these might be potential targets for therapy of metastasis. Podoplanin expressed on the surface of tumor cells induces platelet aggregation by interacting with CLEC-2. Kato *et al*. (29) showed that CLEC-2-Fc inhibits podoplanin-induced platelet aggregation.

Functions of Podoplanin under Normal Conditions

Podoplanin plays an important role in preventing cellular adhesion and is involved in the regulation of the shape of podocyte foot processes and in the maintenance of glomerular permeability (22, 31, 32).

Moreover, podoplanin is involved in LV formation and does not influence formation of blood vessels (33). Podoplanin knockout mice have lymphatic defects associated with diminished lymphatic transport, congenital lymphoedema and dilation of lymphatic vessels (4).

Markers Useful for Identification of Podoplanin

The demonstration of podoplanin expression is largely based on immunohistochemistry. The most frequently used antibodies are directed against podoplanin and D2-40 (which recognizes the formalin-resistant epitope of podoplanin) (34).

Double immunostaining based on anti-podoplanin or anti-D2-40 and CD34 demonstrated that the final product of reaction for podoplanin is restricted to the lymphatic endothelium in both normal and neoplastic tissues (35). On the other hand, it was shown that the sialomucin CD34, a recognized vascular endothelial marker, is expressed at a low level by podoplanin-positive tumor-associated LECs in more than 60% of colon, breast, lung and skin tumors (36). Podoplanin-positive vessels are also stained with an anti-VEGFR-3 antibody in double labeling experiments, and the final product of reaction is predominantly found at the luminal surface (36).

LECs can be defined as podoplanin-expressing cells that co-express CD34 at low levels (24). Coculture of podoplanin-positive LECs with podoplanin-negative ECs produces islands of LECs surrounded by vascular ECs, demonstrating that lymphatic and vascular ECs are involved in homotypic associations and vascular tube formation. This finding supports the hypothesis that LECs and blood vessel ECs belong to two different EC lineages.

Induction of Podoplanin Expression

By using embryonic stem cells, it was shown that vascular structures expressing podoplanin are also positive for Prox1 and CD31, and embryoid bodies treated with VEGF-C or VEGF-C and VEGF-A gave rise to vascular structures composed of cells expressing classical LEC markers (including podoplanin) (37).

The expression of podoplanin is regulated by the lymphatic-specific homeobox gene *Prox 1*, a master gene that controls the development of lymphatic progenitors from embryonic veins (38). *Prox-1* 'reprograms' vascular endothelial cells in culture to become podoplanin-expressing LECs (39).

Schacht *et al*. (40) showed that myoblasts transfected with a human podoplanin overexpression vector when stained cytoplasmic labeling. Podoplanin expression was induced by epidermal growth factor, basic fibroblast growth factor and tumor necrosis factor alpha in the MCF7 breast cancer cell line, and by bradykinin in 3T3 fibroblasts (41, 42), by interleukin-3 in dermal LECs (43) and by transforming growth factor beta in human fibrosarcoma cells (44).

Podoplanin as Marker of Lymphatic Endothelial Cells

Normal lymphatic vessels. Podoplanin is a specific marker of the lymphatic endothelium and is not expressed in blood vessels (Figure 1 A). It is expressed by both developing and mature LECs and seems to be a more specific marker of LECs, as LYVE-1 was detected in only a subset of cultured podoplanin-positive ECs (45). Podoplanin staining is detected in small lymphatic vessels co-expressing VEGFR-3, lymphatic collecting vessels and hepatic sinusoids, but not large lymphatic vessels with perivascular cells, nor high endothelial venules of the lymph nodes (4). By electron microscopy and immunoelectron microscopy, it was demonstrated that podoplanin is mainly expressed on the luminal surface of LECs and only rarely on the abluminal surface or lateral domain and in cytoplasmic organelles of ECs (46).

Podoplanin is not an exclusive marker of the lymphatic endothelium. In normal human tissue, podoplanin was demonstrated in podocytes, osteoblastic cells, osteocytes, basal keratinocytes, choroid plexus epithelial cells, type I epithelial cells of the thymus, myoepithelial cells (Figure 1 B), reserve cells of sebaceous gland (unpublished data) (Figure 1 C), myofibroblasts of the prostate, granulosa cells of the ovary, follicular dendritic cells (Figure 1 D) and alveolar type I cells (21, 40, 47).

Peritumoral lymphatic vessels. These are larger and more irregular than the intratumoral lymphatics, with a significantly lower density (46). Numerous podoplanin-expressing lymphatics were found in the intralobular pancreatic parenchyma, close to blood vessels and ducts. It seems that peritumoral lymphatics are more important in tumor cell spreading, through a sprouting process under the influence of interstitial fluid hypertension and VEGF-C secreted by tumor cells (15, 48, 49). An increased lymphatic microvascular density (LMVD) was found in the peritumoral area in early stages of squamous cell carcinoma of the uterine cervix (50), in non-small lung carcinoma, and in lung adenocarcinoma, where peritumoral LMVD strongly correlated with lymph node metastasis (51, 52). Several studies provided evidence for a direct correlation between LMVD and lymph node status in colorectal carcinoma (53),

oral squamous cell carcinoma (Figure 2 A) (54) and breast cancer (55-57), but not in cutaneous melanoma (58).

Intratumoral lymphatic vessels. These are found in a large variety of tumors and are usually small, flattened and irregular (Figure 2 B) and occasionally, contain tumor cells. Several authors found podoplanin-positive vessels within the stroma in ovarian (59), cervical (17), pancreatic endocrine (60, 61) and breast (7) malignant tumors. The proliferative activity of LECs in the tumor stroma was demonstrated by podoplanin and/or D2-40/Ki67 (62) in squamous cell carcinoma of the head and neck (54, 63), melanoma (64), colorectal carcinoma (65) and non-small lung carcinoma by double labeling podoplanin or D2-40/Ki67 (51). In inflammatory breast cancer, it was shown that proliferating endothelial cells are found in both tumor and peritumor areas, with a significantly higher rate in peritumoral podoplanin/D2-40-positive LVs (66). The clinical significance of intratumoral lymphatics is still controversial and many authors consider that they are less efficient in tumor cell trafficking (67). In a recent experimental study based on hybridoma-induced tumors, it was shown that both intra- and peritumoral lymphatics identified with anti-podoplanin and LYVE-1 antibodies participated in tumor cell adhesion, invasion and migration (46).

Podoplanin/D2-40 staining is useful not only to evaluate LMVD, but also to demonstrate lymphovascular invasion (Figure 3), which has an important prognostic value and expresses a high risk for lymph node metastasis. It was shown that lymphovascular invasion was detected in 13.8 to 16% of cases of invasive breast cancer on slides marked with conventional staining, whereas by using the staining for podoplanin, detection increased to 28.5% in the same cases (68, 69). Moreover, lymphovascular invasion identified by using D2-40 antibody correlated with lymph node metastasis and extramammary Paget disease (70).

Expression of Podoplanin in Human Tumors

Specific markers of LECs, namely VEGFR-3, LYVE-1, Prox1 and podoplanin, have given new insights into the biology of malignant tumors. Coexpression of podoplanin, VEGFR-3 and CD31 by dual-staining using confocal microscopy was found in Kaposi's sarcoma (71). Podoplanin is expressed in tumor cells of various neoplasms, such as squamous cell carcinoma (42, 72, 73), mesothelioma (74), germ cell tumors (75, 76), tumors (77) and some subtypes of vascular tumors (78-80).

Vascular tumors. Benign vascular tumors (capillary, cavernous and venous hemangioma) do not express podoplanin, with the exception of lymphangioma, Dabska's tumor, and, to a lesser extent, mixed lymphatic/hemangioma (4, 78). Strong and uniform expression of podoplanin was found in epithelioid hemangioendothelioma, a low-grade vascular neoplasm (79) and a single study reported a positive reaction for podoplanin in 10 cases of lymphangioleiomyomatosis (80).

In angiosarcoma, the presence of podoplanin-expressing tumor cells, arranged in clusters or as single cells, with intensely stained membrane was demonstrated (4, 81). In Kaposi's sarcoma, tumor cells expressed podoplanin in all cases in one study (4) and in 9/10 cases in another (81). The observation that in the early stages all tumor cells expressed podoplanin provides evidence that spindle cells of Kaposi's sarcoma originate in the lymphatic endothelium. This hypothesis is supported by gene expression analysis of tumor cells (82) and by the infection of dedifferentiated endothelial cells with human herpes virus 8 that leads to their lymphatic differentiation and induction of 70% of the main lymphatic lineage-specific genes, including *Prox-1* (83).

Mesothelioma. In all mesothelioma, tumor cells are intensely stained with podoplanin, with a continuous and strong staining pattern, especially on the luminal surfaces (74). The reaction is strong in all better differentiated and papillary tumors, and less consistent in less differentiated mesotheliomas. Results obtained for podoplanin overlapped with those for D2-40, and the majority of epithelioid mesotheliomas were positively stained (84, 85). Initially, it was thought that sarcomatoid mesothelioma lacked podoplanin expression (86), but more recently, it was shown that podoplanin expression was found in 13/18 cases of sarcomatoid mesothelioma (87).

Germ cell tumors. Podoplanin immunoreactivity was detected in 98% pure germinoma and germinomatous components in mixed germ cell tumors of the central nervous system (CNS) (76). The immunostaining showed a diffuse cell surface pattern in germinoma cells. The same authors reported weak staining in 12 /17 cases of immature teratoma of the CNS, restricted to the basal cell layer of the immature epithelium, whereas no positive reaction was found in choriocarcinoma, yolk sac tumor or normal brain tissue.

Ovarian tumors. A role for podoplanin was suggested in early differentiation of the granulose cell layer of the ovarian follicle (40), where primary and secondary ovarian follicles show strong podoplanin expression, while the reaction was absent in the luteal and albicans bodies. In the same study, podoplanin expression in ovarian tumor cells was found in 4/4 cases of dysgerminoma and in 1/3 cases of granulosa cell tumors, whereas the other ovarian tumors were negative.

Tumors of the testis. Podoplanin expression was demonstrated in immature fetal germ cells, developing Sertoli cells, intratubular germ cell neoplasia and seminoma (88, 89). Tumor cells of intratubular germ cells strongly express podoplanin (88, 89). In primary seminomas and their

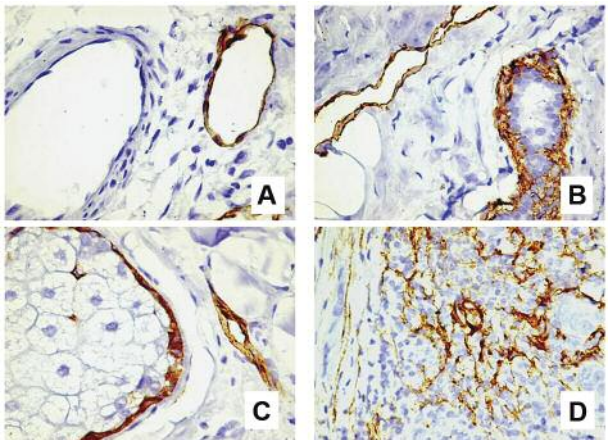

Figure 1. *(A), D2-40 expression in lymphatic vessel endothelium. Immunostaining of podoplanin in a small lymphatic vessel of a sebaceous gland (B), in myoepithelial cells of a normal breast duct (C) and in follicular dendritic cells in a reactive lymph node (D). Original magnification, A-D, ×400.*

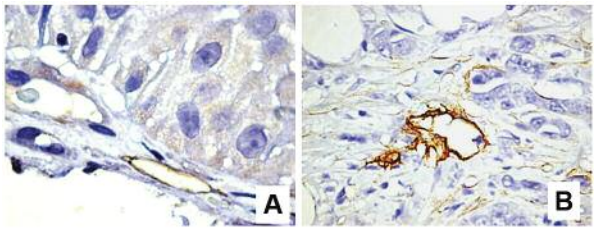

Figure 2. *Immunostaining with podoplanin of peritumoral lymphatic vessel in squamous cell carcinoma (A). Immunostaining with D2-40 of intratumoral lymphatic vessels in breast carcinoma (B). Original magnification, A-B, ×400.*

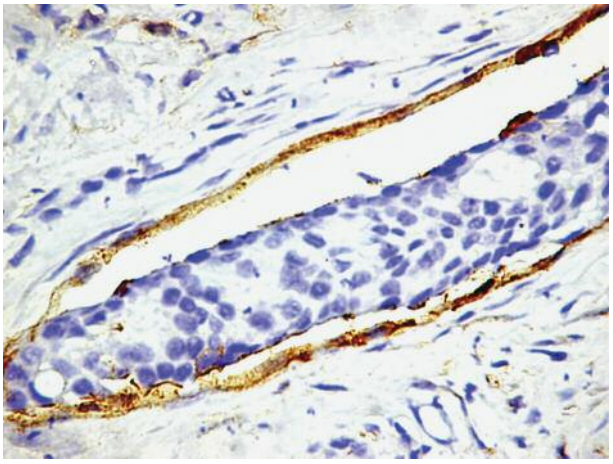

Figure 3. *Immunostaining with D2-40 in lymphovascular invasion in breast carcinoma. Original magnification, ×400.*

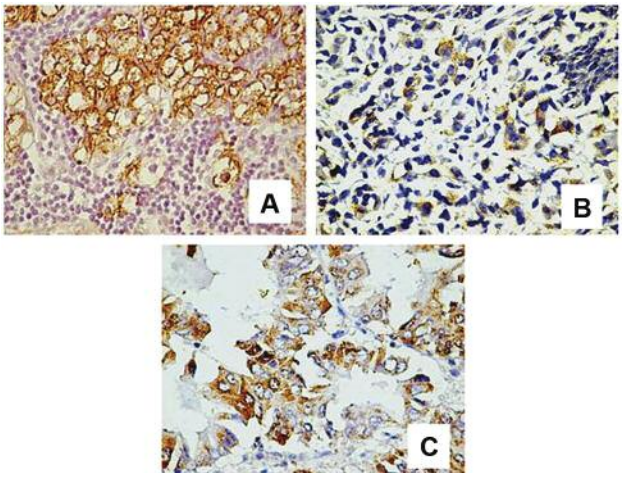

Figure 4. *Podoplanin expression in tumor cells of primary seminoma of the thymus (A), of mastocytoma (B) and colon adenocarcinoma (C). Original magnification, A-C, ×400.*

corresponding metastases, immunoreaction for podoplanin was found in 97% of the cases. Moreover, extragonadal seminomas showed strong expression for podoplanin (Figure 4 A) (90). Podoplanin expression in tumor cells of seminoma allows their differentiation from embryonal carcinoma (75).

Tumors of the CNS. Although glial cells do not express podoplanin, immunoreactivity to podoplanin was reported in 52.9 to 82.9% of glioblastomas and in 35.7 to 47.1% of anaplastic astrocytomas, but not in diffuse astrocytoma (77, 91). The expression of podoplanin mRNA and protein correlated with malignant progression from anaplastic astrocytoma to glioblastoma (77). Podoplanin expression was found in 96.6% of ependymal tumors, in 27.3% of cases with medulloblastoma and in 28.5% oligodendrogliomas. Meningiomas showed reactivity to podoplanin, irrespective of their histological subtype (91). Podoplanin-positive cells with membrane pattern of expression were found mainly around microvascular proliferations and necrotic tissue.

Chondroid tumors. Strong and diffuse expression of podoplanin was found in all cases of enchordoma and in 17/20 cases of chondrosarcoma, while no expression was found in chordoma. The expression of podoplanin in tumor cells of chondrosarcoma allows the use of podoplanin as the first marker to discriminate between low-grade chondrosarcoma and chordoma (92).

Squamous cell carcinoma. Podoplanin expression was demonstrated in 22/28 cases of squamous cell carcinoma, whereas well-differentiated carcinoma did not express podoplanin (40).

Mastocytoma. In high-grade invasive mastocytoma, podoplanin was found in tumor cells, with a cytoplasmic pattern of expression and membrane enhancement (unpublished data)

(Figure 4 B), with the immunoreactivity being stronger at the interface between tumor cells and stroma.

Podoplanin in Tumor Diagnosis

Podoplanin seems to be a useful marker in tumor diagnosis. Podoplanin may be useful to discriminate between mesothelioma (tumor cells are frequently podoplanin-positive) and lung adenocarcinoma (few positive cases have been reported) (93).

Podoplanin is expressed in 93% of cases of mesothelioma, as a continuous, strong membranous pattern (85, 94). The reaction is strong and diffuse in the large majority of cases, more evident along the apical cell membranes in all well-differentiated and papillary tumors, whereas the reaction is discontinuous in solid and less differentiated tumors. Only 13% of serous carcinomas expressed podoplanin, restricted to the apical surface of tumor cells. Podoplanin is not absolutely specific for epitheloid mesothelioma, but is more so than calretinin is. Despite its sensitivity being lower than that of other markers, podoplanin is considered a good marker to discriminate between these two tumor types.

Potential Role of Podoplanin in Tumor Invasion and Metastasis

A role for podoplanin in invasion and metastasis has been suggested (77, 95). This hypothesis is mainly based on the observation that high expression of podoplanin is consistently correlated with the presence of metastases. It was reported that podoplanin-expressing cells were found at the invasion front in more than 80% human squamous cell carcinomas (42).

Podoplanin might favor tumor invasion through its ability to remodel actin in the cytoskeleton of tumor cells, contributing to their increased motility (96). The association between podoplanin and the actin cytoskeleton seems to be mediated by ezrin, which is markedly phosphorylated in the presence of podoplanin overexpression (41, 42, 97). In addition, podoplanin increases the activities of Rho GTPases, mainly RhoA, and this might reflect a different organization of the cytoskeleton in different cell types. Inhibition of RhoA leads to reduced motility of tumor cells (97).

Podoplanin increases cell migration of MCF7 cells and HaCaT keratinocytes in a down-regulation of E-cadherin expression. These findings suggest that podoplanin does not suppress the cadherin switch and can mediate tumor invasion by an alternative pathway. Wicki and Christofori (98) showed that invasion of podoplanin-expressing tumor cells was correlated with an overexpression of matrix metalloproteinases, and that it could be inhibited by specific inhibitors of matrix metalloproteinases.

Anti-human Podoplanin Antibody as an Antimetastatic Strategy

An anti-human podoplanin antibody (NZ-1) completely inhibited podoplanin-induced platelet aggregation and inhibited experimental metastasis (29, 38). The effects of NZ-1 antibody is based on cell-mediated toxicity and neutralization of the interaction between podoplanin and CLEC-2. In animals injected with podoplanin-transfected cells of the Chinese hamster ovary and NZ-1, the number of lung metastases was significantly lower than in the controls and no metastases were found in the liver, kidney, spleen, colon or ovary.

Transgenic expression of podoplanin in pancreatic β-cell tumors of a Rip1Tag2 mouse model of tumor progression led to the formation of carcinoma in the absence of cadherin switch or epithelial-mesenchymal transition (98).

Podoplanin as a Potential Target of Lymphatic Vessels

Targeting lymphatic vessels and/or lymphangiogenic growth factors is an attractive therapeutic strategy in the treatment of tumor progression and metastasis. In preclinical studies, molecules which have been shown to be effective in inhibiting tumor lymphangiogenesis and lymph node metastasis include neutralizing anti-VEGF-D antibodies and VEGFR-3-Ig fusion protein (99, 100).

In an experimental model with VEGF-D-expressing tumors, administration of VEGF-D monoclonal antibody reduced the growth rate of the primary tumors and development of lymph node metastases (101, 102). Similar results were obtained with anti-VEGFR-3, but no effect was noticed on the development of lung metastasis (99). Endostatin inhibited tumor lymphangiogenesis by down-regulation of VEGF-C (103). Rapamycin, a specific inhibitor of mTOR, had an antilymphangiogenic effect and inhibited lymphatic metastasis and reduced VEGF-C levels and the rate of metastasis, without a complete response (104).

These data show that these therapeutic strategies may be useful, but are not efficient enough for a complete inhibition of lymphangiogenic metastasis. In a study on prostate tumors, it was shown that 92% ablation of intratumoral lymphatics did not inhibit lymph node metastasis and preexisting peritumoral lymphatics may be sufficient for tumor cell spreading (105).

The use of an anti-podoplanin-based therapeutic strategy could be suggested in the treatment of lymphatic metastases based on four considerations: (i) podoplanin is a well-known marker of LECs; (ii) it is expressed in tumor cells of various types of human cancer; (iii) its expression seems to be associated with bad prognosis and high risk for lymph node metastases; (iv) it is involved in tumor invasion.

A major drawback is that, to date, no differences in the expression of podoplanin in normal lymphatics and tumor-associated lymphatics have been reported, nor between the expression of podoplanin in normal non-LECs and tumor cells. As a consequence, a humanized anti-podoplanin antibody administered to cancer patients might inhibit not only tumor-associated lymphatics, but also normal cells that express podoplanin.

In conclusion, podoplanin is a sensitive marker of LECs and is very useful in evaluating lymphatic microvascular density. Immunohistochemical detection of podoplanin is helpful in the diagnosis of lymphovascular invasion. Finally, its expression in tumor cells of various neoplasms is useful for both differential diagnosis and prediction of tumor progression and metastasis.

Acknowledgements

This work was supported by Grant PNII/41-054/2007 of the Romanian National Agency for Scientific Research and by Fondazione Carime, Bari, Italy. The Authors are grateful to Diana Tatucu for her excellent technical assistance.

References

1 Reis-Filho JS and Schmitt FC: Lymphangiogenesis: what do we know? Microsc Res Tech *60*: 171-180, 2003.

2 Banerji S, Ni J, Wang SX, Clasper S, Su J, Tammi R, Jones M and Jackson DG: LYVE-1, a new homologue of the CD44 glycoprotein, is a lymph-specific receptor for hyaluronan. J Cell Biol *144*: 789-801, 1999.

3 Wigle JT and Oliver G: Prox 1 function is required for the development of the murine lymphatic system. Cell *98*: 769-778, 1999.

4 Breiteneder-Geleff S, Soleiman A, Kowalski H, Horvat R, Amann G, Kriehuber E, Diem K, Weninger W, Tschachler E, Alitalo K and Kerjanschki D: Angiosarcomas express mixed endothelial phenotypes of blood and lymphatic capillaries. Am J Pathol *154*: 385-394, 1999.

5 Straume O, Jackson DG and Akslen LA: Independent prognostic impact of lymphatic vessel density and presence of low-grade lymphangiogenesis in cutaneous melanoma. Clin Cancer Res *9*: 250-256, 2003.

6 Beasley NJ, Prevo R, Banerji S, Leek DR, Moore J, van Trappen Ph, Cox G, Harris AL and Jackson DG: Intratumoral lymphangiogenesis and lymph node metastasis in head and neck cancer. Cancer Res *62*: 1315-1320, 2002.

7 Schoppmann SF, Birner P, Studer P and Breiteneder-Geleff S: Lymphatic microvessel density and lymphovascular invasion assessed by anti-podoplanin immunostaining in human breast cancer. Anticancer Res *21*: 2351-2355, 2001.

8 Yonemura Y, Fushida S, Bando E, Kinoshita K, Miwa K, Endo Y, Sugiyama K, Partanen T, Yamamoto H and Sasaki T: Lymphangiogenesis and the vascular endothelial growth factor receptor (VEGFR)-3 in gastric cancer. Eur J Cancer *37*: 918-923, 2001.

9 Shayan R, Achen MG and Stacker SA: Lymphatic vessels in cancer metastasis: bridging the gap. Carcinogenesis *27*: 1729-1738, 2006.

10 He Y, Rajantie I, Ilmonen M, Makinen T, Karkkainen MJ, Haiko P, Salven P and Alitalo K: Preexisting lymphatic endothelium but not endothelial progenitor cells are essential for tumor lymphangiogenesis and lymphatic metastasis. Cancer Res *64*: 3737-3740, 2004.

11 Wilting J, Tomarev SI, Christ B and Schweigerer L: Lymphangioblasts in embryonic lymphangiogenesis. Lymphat Res Biol *1*: 33-40, 2003.

12 Jeltsch M, Tammela T, Alitalo K and Wilting J: Genesis and pathogenesis of lymphatic vessels. Cell Tissue Res *314*: 69-84, 2003.

13 Saintigny P, Morere JF, Breau JL, Bernaudin JF and Kraemer M: Lymph node metastasis as a new target for cancer treatment. Targ Oncol *2*: 49-57, 2007.

14 Cao Y: Emerging mechanisms of tumour lymphangiogenesis and lymphatic metastasis. Nat Rev Cancer *5*: 735-743, 2005.

15 Ji RC: Lymphatic endothelial cells, tumor lymphangiogenesis and metastasis: new insights into intratumoral and peritumoral lymphatics. Cancer Metastasis Rev *25*: 677-694, 2006.

16 Sinzelle E, Van Huyen JP, Breiteneder Geleff S, Braunberger E, Deloche A, Kerjanschki D and Bruneval P: Intrapericardial lymphangioma with podoplanin immunohistochemical characterization of lymphatic endothelial cells. Histopathology *37*: 93-94, 2000.

17 Birner P, Obermair A, Schindl M, Kowalski H, Breitenecker G and Oberhuber G: Selective immunohistochemical staining of blood and lymphatic vessels reveals independent prognostic influence of blood and lymphatic vessel invasion in early-stage cervical cancer. Clin Cancer Res *7*: 93-97, 2001.

18 Cunnick GH, Jiang WG, Gomez KF and Mansel RE: Lymphangiogenesis and breast cancer metastasis. Histol Histopathol *17*: 863-870, 2002.

19 Sedivy R, Beck-Mannagetta J, Haverkampf C, Battistutti W and Honigschnable S: Expression of vascular endothelial growth factor-C correlates with the lymphatic microvessel density and the nodal status in oral squamous cell cancer. J Oral Pathol Med *32*: 455-460, 2003.

20 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 *1*: 511-518, 1990.

21 Wetterwald A, Hofstetter 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 *18*: 125-132, 1996.

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

23 Matsui K, Breiteneder Geleff S and Kerjaschki D: Epitope specific antibodies to the 43 kDa glomerular membrane protein podoplanin cause proteinuria and rapid flattening of podocytes. J Am Soc Nephrol *9*: 2013-1026, 1998.

24 Kriehuber E, Breitender-Gelleff S, Groeger M, Soleiman A, Schoppmann SF, Stingl G, Kerjaschki D and Maurer D: Isolation and characterization of dermal lymphatic and blood endothelial cells reveal stable and functionally specialized cell lineages. J Exp Med *194*: 797-808, 2001.

25 Boucherot A, Schreiber R, Pavenstadt H and Kunzelmann K: Cloning and expression of the mouse glomerular podoplanin homologue gp38P. Nephrol Dial Transplant *17*: 978-984, 2002.

26 Hantusch B, Kalt R, Krieger S, Puri C and Kerjanschki D: Sp1/Sp3 and DNA-methylation contribute to basal transcriptional activation of human podoplanin in MG63 *versus* Saos-2 osteoblastic cells. BMC Mol Biol *8*: 20, 2007.

27 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 *378*: 52-57, 2006.

28 Kato Y, Fujita N, Kunita A, Sato S, Kaneko M, Osawa M and Tsuruo T: Molecular identification of Aggrus/T1α as a platelet aggregation-inducing factor expressed in colorectal tumors. J Biol Chem *278*: 51599-51605, 2003.

29 Kato Y, Kaneko MK, Kunita A, Ito H, Kameyama A, Ogasawara S, Matsuura N, Hasegawa Y, 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 *3*: 1-8, 2007.

30 Suzuki-Inoue K, Kato Y, Inoue O, Kaneko MK, Mishima K, Yatomi 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 *282*: 25993-26001, 2007.

31 Schmid H, Henger A, Cohen CD, Frach K, Gröne HJ, Schlöndorff D and Kretzler M: Gene expression profiles of podocyte-associated molecules as diagnostic markers in acquired proteinuric diseases. J Am Soc Nephrol *14*: 2958-2966, 2003.

32 Levidiotis V and Power DA: New insights into the molecular biology of the glomerular filtration barrier and associated disease. Nephrology *10*: 157-166, 2005.

33 Schacht V, Ramirez MI, Hong YK, Hirakawa S, Feng D, Harvey N, Williams M, Dvorak AM, Oliver G and Detmar M: T1α/podoplanin deficiency disrupts normal lymphatic vasculature formation and causes lymphedema. EMBO J *22*: 3546-3556, 2003.

34 Marks A, Sutherland DR, Bailey D, Iglesias J, Law J, Lei M, Yeger H, Banerjee D and Baumal R: Characterization and distribution of an oncofetal antigen (M2A antigen) expressed on testicular germ cell tumours. Br J Cancer *80*: 569-578, 2000.

35 Xuan M, Fang YR, Wato M, Hata S and Tanaka A: Immunohistochemical co-localization of lymphatics and blood vessels in oral squamous cell carcinomas. J Oral Pathol Med *34*: 334-339, 2005.

36 Fiedler U, Christian S, Koidl S, Kerjanschki D, Emmett MS, Bates DO, Christofori G and Augustin HG: The sialomucin CD34 is a marker of lymphatic endothelial cells in human tumors. Am J Pathol *168*: 1045-1053, 2006.

37 Kreuger J, Nilsson I, Kerjaschki D, Petrova T, Alitalo K and Claesson-Welsh L: Early lymph vessel development from embryonic stem cells. Arterioscler Thromb Vasc Biol *26*: 1073-1078, 2006.

38 Kato Y, Kaneko MK, Kuno A, Uchiyama N, Amano K, Chiba Y, Hasegawa Y, Hirabayashi M, Narimatsu H, Mishima K and Osawa ML: Inhibition of tumor cell-induced platelet aggregation using a novel anti-podoplanin antibody reacting with its platelet-aggregation-stimulating domain. Biochem Biophys Res Commun *349*: 1301-1307, 2006.

39 Petrova TV, Makinen T, Makela TP, Saarela J, Virtanen I, Ferrell RE, Finegold DN, Kerjaschki D, Yla-Herttuala S and Alitalo K: Lymphatic endothelial reprogramming of vascular endothelial cells by the Prox-1 homeobox transcription factor. EMBO J *21*: 4593-4599, 2002.

40 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 *166*: 913-921, 2005.

41 Scholl FG, Gamallo C, Vilar 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 *112*: 4601-4613, 1999.

42 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 *9*: 261-272, 2006.

43 Gröger M, Loewe R, Holnthoner W, Embacher R, Pillinger M, Herron SG, Wolff K and Petzelbauer P: Il-3 induces expression of lymphatic markers Prox-1 and podoplanin in human endothelial cells. J Immunol *173*: 7161-7169, 2004.

44 Suzuki H, Kato Y, Kaneko MK, Okita Y, Narimatsu H and Kato M: Induction of podoplanin by transforming growth factor-beta in human fibrosarcoma. FEBS Lett *582*: 341-345, 2008.

45 Makinen T, Jussila L, Veikkola T, Karpanen T, Kettunen MI, Pulkkanen KJ, Kauppinen R, Jackson DG, Kubo H, Nishikawa S, Yla-Herttuala S and Alitalo K: Inhibition of lymphangiogenesis with resulting lymphedema in transgenic mice expressing soluble VEGF receptor-3. Nat Med *7*: 199-205, 2001.

46 Ji RC, Eshita Y and Kato S: Investigation of intratumoural and peritumoral lymphatics expressed by podoplanin and LYVE-1 in the hybridoma-induced tumours. Int J Exp Path *88*: 257-270, 2007.

47 Vanderbilt JN and Dobbs LG: Characterization of the gene and promoter for RTI40, a differentiation marker of type I alveolar cells. Am J Respir Cell Mol Biol *19*: 662-671, 1998.

48 Alitalo K, Tammela T and Petrova TV: Lymphangiogenesis in development and human disease. Nature Insight *438*: 946-953, 2005.

49 Schoppmann SF, Fenzl A and Laengle F: Surgical oncology: impact of lymphangiogenesis for metastasis formation. Eur Surg *37*: 153-158, 2005.

50 Gombos Z, Xu X, Chu CS, Zhang PJ and Acs G: Peritumoral lymphatic vessel density and vascular endothelial growth factor C expression in early stage squamous cell carcinoma of the uterine cervix. Clin Cancer Res *11*: 8367-8371, 2005.

51 Renyi-Vamos F, Tovari J, Fillinger J, Timar J, Paku S, Kenessey I, Ostoros G, Agocs L, Soltesz I and Dome B: Lymphangiogenesis correlates with lymph node metastasis, prognosis, and angiogenic phenotype in human non-small cell lung cancer. Clin Cancer Res *11*: 7344-7353, 2005.

52 Adachi Y, Nakamura H, Kitamura Y, Taniguchi Y, Araki K, Shomori K, Horie Y, Kurozawa Y, Ito H and Hayashi K: Lymphatic vessel density in pulmonary adenocarcinoma immunohistochemically evaluated with anti-podoplanin or anti-D2-40 antibody is correlated with lymphatic invasion or lymph node metastases. Pathol Intern *57*: 171-177, 2007.

53 Fenzl A, Schopmann SF, Geleff S, Birner P, Wrba F, Herberger B, Teleky B, Herbst F, Kührer I, Jakesz R and Längle F: Vascular endothelial growth factor-C expression and lymphangiogenesis in colorectal cancer. Eur Surg *38*: 149-154, 2006.

54 Ohno F, Nakanishi H, Abe A, Seki Y, Kinoshita A, Hasegawa Y, Masae T and Kurita K: Regional differences in intratumoral lymphangiogenesis of oral squamous cell carcinomas evaluated by immunohistochemistry using D2-40 and podoplanin antibody: an analysis in comparison with angiogenesis. J Oral Pathol *36*: 281-289, 2007.

55 Nakamura Y, Yasouka H, Tsujimoto M, Imabun S, Nakahara M, Nakao K, Nakamura M, Mori I and Kakudo K: Lymph vessel density correlates with nodal status, VEGF-C expression, and prognosis in breast cancer. Breast Cancer Res Treat *91*: 125-132, 2005.

56 Choi WWL, Lewis MM, Lawson D, Yin-Goen Q, Birdsong GG, Cotsonis GA, Cohen C and Young AN: Angiogenic and lymphangiogenic microvessel density in breast carcinoma: correlation with clinicopathologic parameters and VEGF-family gene expression. Mod Pathol *18*: 143-152, 2005.

57 Schoppmann SF, Fenzl A, Schindl M, Bachleitner-Hoffman T, Nagy K, Gnant M, Horvat R, Jakesz R and Birner P: Hypoxia inducible factor-1α correlates with VEGF-C expression and lymphangiogenesis in breast cancer. Breast Cancer Res Treat *99*: 135-141, 2006.

58 Wobster M, Siedel C, Schrama D, Bröcker EB, Becker JC and Vetter-Kauczok CS: Expression pattern of the lymphatic and vascular markers VEGFR-3 and CD31 does not predict regional lymph node metastasis in cutaneous melanoma. Arch Dermatol Res *297*: 352-357, 2006.

59 Birner P, Schindl M, Obermair A, Plank C, Breitenecker G, Kowalski H and Oberhuber G: Lymphatic microvessel density in epithelial ovarian cancer: its impact on prognosis. Anticancer Res *20*: 2981-2985, 2000.

60 Sipos B, Klapper W, Kruse ML, Kalthoff H, Kerjanschki D and Klöppel G: Expression of lymphangiogenic factors and evidence of intratumoral lymphangiogenesis in pancreatic endocrine tumors. Am J Pathol *165*: 1187-1197, 2004.

61 Rubbia-Brandt L, Terris B, Giostra E, Dousset B, Morel Ph and Pepper MS: Lymphatic vessel density and vascular endothelial growth factor-C expression correlate with malignant behavior in human pancreatic endocrine tumors. Clin Cancer Res *10*: 6919-6928, 2004.

62 Van der Auwera I, Cao Y, Pepper MS, Jackson DG, Fox SB, Harris AL, Dirix LY and Vermeulen PB: First international consensus on the methodology of lymphangiogenesis quantification in solid human tumours. Br J Cancer *95*: 1611-1625, 2006.

63 Maula SM, Luukkaa M, Grenman R, Jackson D, Jalkanen S and Ristamaki R: Intratumoral lymphatics are essential for the metastatic spread and progression in squamous cell carcinomas of the head and neck region. Cancer Res *63*: 1920-1926, 2003.

64 Dadras SS, Paul T, Bertoncini J, Brown LF, Muzikansky A, Jackson DG, Ellwanger U, Garbe C, Mihm MC and Detmar M: Tumor lymphangiogenesis: a novel prognostic indicator for cutaneous melanoma metastasis and survival. Am J Pathol *162*: 1951-1960, 2003.

65 Omachi T, Kawai Y, Mizuno R, Nomiyama T, Miyagawa S, Ohhashi T and Nakayama J: Immunohistochemical demonstration of proliferating lymphatic vessels in colorectal carcinoma and its clinicopathological significance. Cancer Lett *246*: 167-172, 2007.

66 Van der Auwera I, van der Eynden GG, Colpaert CG, van Laere SJ, van Dam P, Van Marck EA, Dirix LY and Vermeulen PB: Tumor lymphangiogenesis in inflammatory breast carcinoma: a histomorphometric study. Clin Cancer Res *11*: 7637-7642, 2005.

67 Jain RK and Fenton BT: Intratumoral lymphatic vessels: a case of mistaken identity or malfunction? J Natl Cancer Inst *94*: 417-421, 2002.

68 Marinho VFZ, Metze K, Sanches FSF, Rocha GFS and Gobbi H: Lymph vascular invasion in invasive mammary carcinomas identified by the endothelial lymphatic marker D2-40 is associated with other indicators of poor prognosis. BMC Cancer *8*: 64, 2008.

69 Braun M, Flucke U, Debald M, Walgenbach-Bruenagel G, Walgenbach KJ, Hőller T, Pőlcher M, Wolfgarten M, Sauerwald A, Keyver-Paik M, Kühr M, Büttner R and Kuhn W: Detection of lymphovascular invasion in early breast cancer by D2-40 (podoplanin): a clinically useful predictor for axillary lymph node metastases. Breast Cancer Res Treat, 2007.

70 Yamada Y, Matsumoto T, Arakawa A, Ikeda S, Fujime M, Komuro Y and Takeda S: Evaluation using a combination of lymphatic invasion on D2-40 immunostain and depth of dermal invasion is a strong predictor for nodal metastasis in extramammary Paget's disease. Pathol Intern *58*: 114-117, 2008.

71 Weninger W, Partanen T, Breitender-Gelleff S, Mayer C, Kowalski H, Mildner M, Pammer J, Stürzl M, Kerjanschki D, Alitalo K and Tschachler E: Expression of vascular endothelial growth factor receptor-3 and podoplanin suggests a lymphatic endothelial cell origin of Kaposi's sarcoma tumor cells. Lab Invest *78*: 243-251, 1999.

72 Kato Y, Kaneko M, Sata M, Fujita N, Tsuruo T and Osawa M: Enhanced expression of Aggrus (T1α/podoplanin), a platelet aggregation-inducing factor in lung squamous cell carcinoma. Tumor Biol *26*: 195-200, 2005.

73 Martin-Villar E, Scholl FG, Gamallo C, Yurrita MM, Munoz-Guerra M, Cruces J and Quintanilla M: Characterization of human PA2.26 antigen (T1α-2, podoplanin) a small membrane mucin induced in oral squamous cell carcinomas. Int J Cancer *113*: 899-910, 2005.

74 Kimura N and Kimura I: Podoplanin as a marker for mesothelioma. Pathol Int *55*: 83-86, 2005.

75 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 *23*: 8552-8556, 2004.

76 Mishima K, Kato Y, Kaneko MK, Nakazawa Y, Kunita A, Fujita N, Tsuruo T, Nishikawa R, Hirose T and Matsutani M: Podoplanin expression in primary central nervous system germ cell tumors: a useful histological marker for the diagnosis of germinoma. Acta Neuropathol *111*: 563-568, 2006.

77 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 *111*: 483-488, 2006.

78 Suurmeijer AJH and Fletcher CDM: Papillary haemangioma. A distinctive cutaneous haemangioma of the head and neck area containing eosinophilic hyaline globules. Histopathol *51*: 638-648, 2007.

79 Naqvi J, Ordonez NG, Luna MA, Williams MD, Weber RS and El-Naggar AK: Epitheloid hemangioendothelioma of the head and neck: role of podoplanin in the differential diagnosis. Head Neck Pathol *2*: 25-30, 2007.

80 Hansen T, Katenkamp K, Bittinger F, Kirkpatrick CJ and Katenkamp D: D2-40 labeling in lymphangiomyoma/lymphangiomyomatosis of the soft tissue: further evidence of lymphangiogenic tumor histogenesis. Virchows Arch *450*: 449-453, 2007.

81 Fukunaga M: Expression of D2-40 in lymphatic endothelium of normal tissues and in vascular tumours. Histopathol *46*: 396-402, 2005.

82 Wang HW, Trotter MW, Lagos D, Bourboulia D, Henderson S, Makinen T, Elliman S, Flanagan AM, Alitalo K and Boshoff C: Kaposi sarcoma herpesvirus-induced cellular reprogramming contributes to the lymphatic endothelial gene expression in Kaposi sarcoma. Nat Genet *36*: 687-693, 2004.

83 Hong YK, Foreman K, Shin JW, Hirakawa S, Curry CL, Sage DR, Libermann T, Dezube BJ, Fingeroth JD and Detmar M: Lymphatic reprogramming of blood vascular endothelium by Kaposi sarcoma-associated herpes virus. Nat Genet *36*: 683-685, 2004.

84 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 *18*: 105-110, 2005.

85 Ordonez NG: The diagnostic utility of immunohistochemistry and electron microscopy in distinguishing between peritoneal mesotheliomas and serous carcinomas: a comparative study. Mod Pathol *19*: 34-48, 2006.

86 Ordonez NG: What are the current best immunohistochemical markers for the diagnosis of epitheloid mesothelioma? A review and update. Human Pathol *38*: 1-16, 2007.

87 Padgett DM, Cathro HP, Wick MR and Mills SE: Podoplanin is a better immunohistochemical marker for sarcomatoid mesothelioma than calretinin. Am J Surg Pathol *32*: 123-127, 2008.

88 Sonne SB, Herlihy AS, Hoei-Hansen CE, Nielsen JE, Almstrup K, Skakkebaek NE, Marks A, Leffers H and Rajpert-De Meyts E: Identity of M2A (D2-40) antigen and gp36 (Aggrus, T1A-2, podoplanin) in human developing testis, testicular carcinoma in situ and germ-cell tumours. Virchows Arch *449*: 200-206, 2006.

89 Zayed AE, Abd-Elnaeim MM, Abd-Elghaffar SKh, Hild A, Brehm R and Steger K: Prenatal development of murine gonads with special reference to germ cell differentiation: a morphological and immunohistochemical study. Andrologia *39*: 93-100, 2007.

90 Biermann K, Klingmuller D, Koch A, Pietsch T, Schorle H, Büttner R and Zhou H: Diagnostic value of markers M2A, OCT3/4, AP-2γ, PLAP and c-KIT in the detection of extragonadal seminomas. Histopathol *49*: 290-297, 2006.

91 Shibahara J, Kashima T, Kikuchi Y, Kunita A and Fukayama M: Podoplanin is expressed in subsets of tumors of the central nervous system. Virchows Arch *448*: 493-499, 2006.

92 Huse JT, Pasha TL and Zhang PJ: D2-40 functions as an effective chondroid marker distinguishing true chondroid tumors from chordoma. Acta neuropathol *113*: 87-94, 2007.

93 Kushitani K, Takeshima Y, Amatya VJ, Furonaka O, Sakatani A and Inai K: Immunohistochemical marker panels for distinguishing between epithelioid mesothelioma and lung adenocarcinoma. Pathol Intern *57*: 190-199, 2007.

94 Maeda M and Hino O: Molecular tumor markers for asbestos-related mesothelioma: serum diagnostic markers. Pathol Intern *56*: 649-654, 2006.

95 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 *170*: 1337-1347, 2007.

96 Moustakas A and Heldin CH: Signaling networks guiding epithelial-mesenchymal transitions during embryogenesis and cancer progression. Cancer Sci *98*: 1512-1520, 2007.

97 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 *119*: 4541-4553, 2006.

98 Wicki A and Christofori G: The potential role of podoplanin in tumour invasion. Br J Cancer *96*: 1-5, 2007.

99 He Y, Kokaki K, Karpanen T, Koshikawa K, Ila-Herttuala S, Takahashi T and Alitalo K: Suppression of tumor lymphangiogenesis and lymph node metastasis by blocking vascular endothelial growth factor receptor 3 signaling. JNCI *94*: 819-825, 2002.

100 Pepper MS, Tille JC, Nisato R and Skobe M: Lymphangiogenesis and tumor metastasis. Cell Tissue Res *314*: 167-177, 2003.

101 Stacker SA, Caesar C, Baldwin ME, Thornton GE, Williams RA, Prevo R, Jackson DG, Nishikawa S, Kubo H and Achen MG: VEGF-D promotes the metastatic spread of tumor cells *via* the lymphatics. Nat Med *7*: 186-191, 2001.

102 Jain RK and Padera TP: Prevention and treatment of lymphatic metastasis by antilymphangiogenic therapy. J Natl Cancer Inst *94*: 785-787, 2002.

103 Fukumoto S, Morifuji M, Katakura Y, Ohishi M and Nakamura S: Endostatin inhibits lymph node metastasis by a down-regulation of the vascular endothelial growth factor C expression in tumor cells. Clin Exp Metastasis *22*: 31-38, 2005.

104 Kobayashi S, Kishimoto T, Kamata S, Otsuka M, Miyazaki M and Ishikura H: Rapamycin, a specific inhibitor of the mammalian target rapamycin, suppresses lymphangiogenesis and lymphatic metastasis. Cancer Sci *98*: 726-733, 2007.

105 Wong SY, Haack H, Crowley D, Barry M, Bronson RT and Hynes RO: Tumor-secreted vascular endothelial growth factor-C is necessary for prostate cancer lymphangiogenesis, but lymphangiogenesis is unnecessary for lymph node metastasis. Cancer Res *65*: 9789-9798, 2005.

Received April 4, 2008
Revised June 11, 2008
Accepted June 18, 2008