

Lymphology. 2003 Jun;36(2):52-61.

Lymphatic vessels in the colonic mucosa in ulcerative colitis.

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In the normal colonic mucosa, lymphatics are found only in a narrow band associated with the muscularis mucosae and are absent from the rest of the mucosa. This study examined whether this arrangement of lymphatics is also valid in ulcerative colitis. Histological sections of colon from 15 long-standing cases were investigated with antibodies against CD 34 (negative for lymphatics; positive for blood vessel endothelium) and, in selected cases, **podoplanin** (positive for lymphatic endothelium; negative for blood vessel endothelium). Whereas inflammation of the mucosa was not associated with changes in lymphatics, an increase in intramucosal lymphatics was seen when the pathological changes included widening of the muscularis mucosae or penetration of the mucosa by muscle fibers, filiform changes in the mucosa, and hyperplasia of the mucosa-associated lymphoid tissue (MALT). In specimens with epithelial dysplasia, an association between the dysplastic epithelium and ectatic and quantitatively increased lymphatics was observed. With superimposed carcinoma, no relationship between the malignant tumor and lymphatics was identifiable. Nevertheless, pre-existing lymphatics in the muscularis mucosae were involved in lymphatic tumor spread. The immunohistochemical findings demonstrated that lymphatics occurred in all areas of the mucosa in ulcerative colitis (or, in effect, at sites which were not normally found under physiological conditions) and in regions that favored lymphatic tumor dissemination. Whether these lymphatics were actually involved in metastasis remains to be defined.

J Oral Pathol Med. 2003 Sep;32(8):455-460.

Expression of vascular endothelial growth factor-C correlates with the lymphatic microvessel density and the nodal status in oral squamous cell cancer.

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BACKGROUND: The cause of preferential metastatic spreading to cervical lymph nodes in oral squamous cell cancer (SCC) is not quite clear. As the density of microvessels may influence the metastatic behaviour, we were interested in how the density of blood/lymphatic microvessels are related to primary SCC and the clinical course of the disease. **METHODS:** Lymphatic and blood microvessels of 28 patients with oral SCC were identified immunohistochemically by antibodies against **podoplanin** and CD34, respectively. Lymphatic microvessel density (LVD) and blood microvessel density (MVD), and the expression of VEGF-C were determined. These findings were compared with the long-term clinicopathological data of the patients. **RESULTS:** LVD and MVD were significantly higher than in control tissues. The amount of lymphatic microvessels correlated positively with the expression of VEGF-C, the tumour grade, the nodal status and with later appearing metastasis. The latter three parameters, however, did not influence the clinical course of the disease. **CONCLUSIONS:** VEGF-C expression in oral SCC triggers lymphatic angiogenesis, which may result in a higher risk for cervical lymph node metastasis. The angiogenetic effect of VEGF-C may also favour the onset of late lymphatic and haematogenous metastases.

J Am Soc Nephrol. 2003 Aug;14(8):1981-9.

Lymphatic microvessels in the rat remnant kidney model of renal fibrosis: aminopeptidase p and podoplanin are discriminatory markers for endothelial cells of blood and lymphatic vessels.

Matsui K, Nagy-Bojarsky K, Laakkonen P, Krieger S, Mechtler K, Uchida S, Geleff S, Kang DH, Johnson RJ, Kerjaschki D.

ABSTRACT. Rat remnant kidney is an established model of renal tubulointerstitial fibrosis and progression to end-stage renal failure. The morphologic lesions comprise nephron loss and regenerative tubular hypertrophy, interstitial infiltration, predominately by macrophages, and progressive fibrosis. A critical role in this complex pathology was assigned to tubulointerstitial blood microvessels that regulate the supply of oxygen and nutrients of tubuli. Whereas some investigations reported a rarefaction of the vascular network in association with the degenerative cortical changes, others observed an increase in vascularization. Here these discrepant findings are addressed by reinvestigation of the vascularization of rat remnant kidneys by the use of two novel endothelial lineage specific, discriminatory markers, i.e., the membrane mucoprotein **podoplanin** with specificity for lymphatic endothelia, and the glycosyl-phosphatidylinositol (GPI)-anchored membrane enzyme aminopeptidase P that is recognized by a monoclonal antibody designated JG12 and that is specifically expressed by endothelial cells of blood vessels only. The results obtained confirm a regional rarefaction of aminopeptidase P-positive blood microvessels; they also establish major changes in the renal lymphatic vasculature. Massive proliferation of lymphatic vessels was observed in fibrotic tubulointerstitial regions, whereas in kidneys of sham-operated rats, only a few lymphatic vessels were found adjoined with arteries. The lymphatic vessels frequently contained mononuclear cells that were also encountered in the interstitial spaces and expressed relative large amounts of vascular endothelial growth factor-C mRNA by in situ hybridization. Collectively, these results indicate that a large proportion of the microvessels encountered in the cortex of remnant kidneys are of lymphatic origin and cannot be discriminated by common endothelial markers, such as CD34, that are expressed by both lymphatic and blood endothelia cells. As lymphatic endothelial cells secrete chemokines that attract dendritic cells, it is possible that the increase in lymphatic vascularization could enhance the immunologic surveillance of remnant kidneys.

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EMBO J. 2003 Jul 15;22(14):3546-56.

T1alpha/podoplanin deficiency disrupts normal lymphatic vasculature formation and causes lymphedema.

Schacht V, Ramirez MI, Hong YK, Hirakawa S, Feng D, Harvey N, Williams M, Dvorak AM, Dvorak HF, Oliver G, Detmar M.

Within the vascular system, the mucin-type transmembrane glycoprotein T1alpha/**podoplanin** is predominantly expressed by lymphatic endothelium, and recent studies have shown that it is regulated by the lymphatic-specific homeobox gene Prox1. In this study, we examined the role of T1alpha/podoplanin in vascular development and the effects of gene disruption in mice. T1alpha/podoplanin is first expressed at around E11.0 in Prox1-positive lymphatic progenitor cells, with predominant localization in the luminal plasma membrane of lymphatic endothelial cells during later development. T1alpha/podoplanin(-/-) mice die at birth due to respiratory failure and have defects in lymphatic, but not blood vessel pattern formation. These defects are associated with diminished lymphatic transport, congenital lymphedema and dilation of lymphatic vessels. T1alpha/podoplanin is also expressed in the basal epidermis of newborn wild-type mice, but gene

disruption did not alter epidermal differentiation. Studies in cultured endothelial cells indicate that T1alpha/podoplanin promotes cell adhesion, migration and tube formation, whereas small interfering RNA-mediated inhibition of T1alpha/podoplanin expression decreased lymphatic endothelial cell adhesion. These data identify T1alpha/podoplanin as a novel critical player that regulates different key aspects of lymphatic vasculature formation.

Int J Oncol. 2003 Aug;23(2):533-9.

Quantitative analysis of lymphangiogenic markers in human colorectal cancer.

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Lymphatic spread of colorectal cancer cells to regional lymph nodes is one of the early events in metastatic cancer, and is often associated with distant metastatic spread and a poor prognosis. This study examined lymphangiogenic factors, and in particular a panel of newly discovered lymphangiogenic markers, in colorectal cancer tissues from a cohort of patients. Paired samples (background normal mucosa and cancer) of colon tissue were obtained from patients with colorectal cancer. The expression and levels of the VEGF-C and VEGF-D cytokines, the VEGF receptors VEGFR-2 and VEGFR-3, and newly described lymphatic endothelial markers, LYVE-1, Prox-1, **podoplanin** and 5'-nucleotidase were assessed. RNA was extracted from the frozen colon tissues. The level of expression for each factor/marker was determined using RT-PCR and quantified using a real-time quantitative PCR (RT-QPCR) technique, with respective cloned cDNA plasmids as internal standards. VEGF-D was expressed to a significantly higher degree in the colon tumour tissues. There was no significant difference between the expression levels for both VEGF-C and its receptor, VEGFR-2, in background and cancer tissues. However, levels of the VEGFR-3 receptor were found to be significantly higher in colon cancer than the normal background tissues. LYVE-1 levels were below detection in most cases. There was a significant increase in the degree of Prox-1 and 5'-nucleotidase expression in colon cancer tissue. Podoplanin expression was also increased in the cancer samples. These markers indicate an increase in lymphangiogenesis in colon cancer, and may therefore have prognostic value for colon cancer patients.

Cornea. 2003 Apr;22(3):273-81.

Corneal lymphangiogenesis: evidence, mechanisms, and implications for corneal transplant immunology.

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PURPOSE: The normal cornea is devoid of blood and lymphatic vessels but can become vascularized secondary to a variety of corneal diseases and surgical manipulations. Whereas corneal (hem)angiogenesis, i.e., the outgrowth of new blood vessels from preexisting limbal vessels, is obvious both clinically and histologically, proof of associated corneal lymphangiogenesis has long been hampered by invisibility and lack of specific markers. This has changed with the recent discovery of the lymphatic endothelial markers vascular endothelial growth factor receptor 3, LYVE-1 (a lymphatic endothelium-specific hyaluronan receptor), Prox 1, and **Podoplanin**. **METHODS:** We herein summarize the current evidence for lymphangiogenesis in the cornea and describe its molecular markers and mediators. Furthermore, the pathophysiologic implications of corneal lymphangiogenesis for corneal transplant immunology are discussed. **RESULTS:** Whereas corneal angiogenesis in vascularized high-risk beds provides a route of entry for immune effector

cells to the graft, lymphangiogenesis enables the exit of antigen-presenting cells and antigenic material from the graft to regional lymph nodes, thus inducing alloimmunization and subsequent graft rejection. **CONCLUSIONS:** Antilymphangiogenic strategies may improve transplant survival both in the high- and low-risk setting of corneal transplantation.

Microsc Res Tech. 2003 Feb 1;60(2):171-80.

Lymphangiogenesis in tumors: what do we know?

Reis-Filho JS, Schmitt FC.

Lymphangiogenesis, the growth of new lymphatic vessels, has long been regarded as a putative efficient pathway to neoplastic metastization. However, until recently consistent data regarding reliable lymphatic endothelial cell markers were lacking. Moreover, the presence of new formed lymphatic vessels was considered a largely disputable concept. Now, this scenario has changed significantly, owing to consistent reports describing novel lymphatic endothelial cell (LEC) markers, the demonstration of new formed lymphatic vessels within the bulk of the tumor in animal models and human neoplasms, and the characterization of the VEGF-C/VEGFR-3 pathway. We herein review the major breakthroughs in the field of lymphangiogenesis, with special emphasis on novel and reliable LEC markers, such as prox-1, LYVE-1, and **podoplanin**, as well as on the pathological assessment of lymphangiogenesis as a putative prognostic factor for human neoplasms.

Clin Cancer Res. 2003 Jan;9(1):250-6.

Independent prognostic impact of lymphatic vessel density and presence of low-grade lymphangiogenesis in cutaneous melanoma.

Straume O, Jackson DG, Akslen LA.

The aim of this study was to determine lymphatic vessel density (LVD) in a series of nodular melanoma and correlate the findings with the expression of several angiogenic factors, including vascular endothelial growth factor-C, basic fibroblast growth factor (bFGF), patient survival, and clinico-pathologic data. Patients with nodular melanoma and complete follow-up information were included. Lymphatic vessels were immunostained with the LYVE-1 and **Podoplanin** antibodies, and LVD was evaluated in both intra- and peri-tumoral (LVDpt) areas. Median LVD was 6.3 and 12.5 vessels/mm² in intra- and peri-tumoral areas, and coexpression of LYVE-1 and Ki-67/MIB-1 in lymphatic endothelial cells within the tumor was demonstrated, indicating active but low-grade lymphangiogenesis. Increased LVDpt was significantly associated with localization on the extremities (P = 0.005), decreased tumor thickness (P = 0.036), absence of vascular invasion (P = 0.004), brisk lymphocytic infiltration (P = 0.018), low proliferative rate by Ki-67 (P = 0.011), increased bFGF expression in tumor cells (P = 0.01) as well as in endothelial cells (P = 0.008), and decreased tumor cell expression of Ephrin-A1 (P = 0.009). Decreased LVD in intra-tumoral areas and LVDpt both predicted improved survival rates in multivariate analyses (for LVDpt, Hazard ratio: 2.1, P = 0.009). We found that decreased LVD was present in thicker and more proliferative tumors (Ki-67) and that increased LVD was significantly associated with improved patient survival in multivariate analysis. In addition, our data suggest the presence of low-grade intra-tumoral lymphangiogenesis in melanoma and a stimulating role of bFGF in lymphangiogenesis.

Dev Dyn. 2002 Nov;225(3):351-7.

Prox1 is a master control gene in the program specifying lymphatic endothelial cell fate.

Hong YK, Harvey N, Noh YH, Schacht V, Hirakawa S, Detmar M, Oliver G.

Early during development, one of the first indication that lymphangiogenesis has begun is the polarized expression of the homeobox gene *Prox1* in a subpopulation of venous endothelial cells. It has been shown previously that *Prox1* expression in the cardinal vein promotes and maintains the budding of endothelial cells that will form the lymphatic vascular system. *Prox1*-deficient mice are devoid of lymphatic vasculature, and in these animals endothelial cells fail to acquire the lymphatic phenotype; instead, they remain as blood vascular endothelium. To investigate whether *Prox1* is sufficient to induce a lymphatic fate in blood vascular endothelium, *Prox1* cDNA was ectopically expressed by adenoviral gene transfer in primary human blood vascular endothelial cells and by transient plasmid cDNA transfection in immortalized microvascular endothelial cells. Transcriptional profiling combined with quantitative real-time reverse transcription-polymerase chain reaction and Western blotting analyses revealed that *Prox1* expression up-regulated the lymphatic endothelial cell markers **podoplanin** and vascular endothelial growth factor receptor-3. Conversely, genes such as laminin, vascular endothelial growth factor-C, neuropilin-1, and intercellular adhesion molecule-1, whose expression has been associated with the blood vascular endothelial cell phenotype, were down-regulated. These results were confirmed by the use of specific antibodies against some of these markers in sections of embryonic and adult tissues. These findings validate our previous proposal that *Prox1* is a key player in the molecular pathway leading to the formation of lymphatic vasculature and identify *Prox1* as a master switch in the program specifying lymphatic endothelial cell fate. That a single gene product was sufficient to re-program the blood vascular endothelium toward a lymphatic phenotype corroborates the close relationship between these two vascular systems and also suggests that during evolution, the lymphatic vasculature originated from the blood vasculature by the additional expression of only a few gene products such as *Prox1*.

Blood. 2003 Jan 1;101(1):168-72. Epub 2002 Aug 15.

VEGFR-3 and CD133 identify a population of CD34+ lymphatic/vascular endothelial precursor cells.

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Human CD133 (AC133)(+)CD34(+) stem and progenitor cells derived from fetal liver and from bone marrow and blood incorporate a functional population of circulating endothelial precursor cells. Vascular endothelial growth factor receptor 3 (VEGFR-3) regulates cardiovascular development and physiological and pathological lymphangiogenesis and angiogenesis. However, the origin of VEGFR-3(+) endothelial cells (ECs) and the mechanisms by which these cells contribute to postnatal physiological processes are not known, and the possible existence of VEGFR-3(+) lymphatic or vascular EC progenitors has not been studied. Using monoclonal antibodies to the extracellular domain of VEGFR-3, we show that 11% +/- 1% of CD34(+) cells isolated from human fetal liver, 1.9% +/- 0.8% CD34(+) cells from human cord blood, and 0.2% +/- 0.1% of CD34(+) cells from healthy adult blood donors are positive for VEGFR-3. CD34(+)VEGFR-3(+) cells from fetal liver coexpress the stem/precursor cell marker CD133 (AC133). Because mature ECs do not express CD133, coexpression of VEGFR-3 and CD133 on CD34(+) cells identifies a unique population of stem and progenitor cells. Incubation of isolated CD34(+)VEGFR-3(+) cells in EC growth medium resulted in a strong proliferation (40-fold in 2

weeks) of nonadherent VEGFR-3(+) cells. Plating of these cells resulted in the formation of adherent VEGFR-3(+)Ac-LDL(+) (Ac-LDL = acetylated low-density lipoprotein) EC monolayers expressing various vascular and lymphatic endothelial-specific surface markers, including CD34, VE-cadherin, CD51/61, CD105, LYVE-1, and podoplanin. These data demonstrate that human CD34(+)CD133(+) cells expressing VEGFR-3 constitute a phenotypically and functionally distinct population of endothelial stem and precursor cells that may play a role in postnatal lymphangiogenesis and/or angiogenesis.

Histol Histopathol. 2002;17(3):863-70.

Lymphangiogenesis and breast cancer metastasis.

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Breast cancer is one of the commonest malignancies in women in the western world. It spreads predominantly via the lymphatic system. However, the understanding of the formation of lymphatics, lymphangiogenesis, has been limited. This has been largely due to the previous lack of lymphatic specific markers. The most specific marker used in humans has been the vascular endothelial growth factor receptor 3 (VEGFR-3). However, this is also found on blood vessel endothelium. The other vascular endothelial factor receptors (VEGFR-1 and -2) are primarily blood vessel receptors. More recently, novel, specific markers for lymphatics have been discovered, such as LYVE-1, prox I and **podoplanin**, enabling further research into this new field. Each of these new markers is described in detail. The article also outlines the current understanding in breast cancer metastasis, with an emphasis on the more recent research into lymphangiogenesis. Since these specific markers are now available, quantitation of lymphangiogenesis is now possible by using either immunohistochemistry or quantitative PCR approaches. In addition, to breast cancer, research into many other cancers is now possible using these methods and new markers. With this in mind, possible therapeutic strategies for the future are discussed.

Invest Ophthalmol Vis Sci. 2002 Jul;43(7):2127-35.

Lymphatic vessels in vascularized human corneas: immunohistochemical investigation using LYVE-1 and podoplanin.

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PURPOSE: To determine whether lymphatic vessels exist in vascularized human corneas, by using immunohistochemistry with novel markers for lymphatic endothelium. **METHODS:** Human corneas exhibiting neovascularization secondary to keratitis, transplant rejection, trauma, and limbal insufficiency (n = 21) were assessed for lymphatic vessel content by conventional transmission electron microscopy and by immunostaining and immunoelectron microscopy with antibodies specific for the lymphatic endothelial markers, lymphatic vessel endothelial hyaluronan receptor (LYVE-1) and the 38-kDa integral membrane glycoprotein **podoplanin**. In addition, corneas were stained for the lymphangiogenic growth factor VEGF-C, and its receptor VEGFR3 by immunohistochemistry and in situ RNA hybridization, respectively. **RESULTS:** Thin-walled, erythrocyte-free vessels staining with lymphatic markers (LYVE-1 and podoplanin) were found to constitute 8% of all vessels, to be more common in the early course of neovascularization, to be always associated with blood vessels and stromal inflammatory cells, and to correlate significantly

with the degree of corneal hemangiogenesis ($r = 0.6$; $P = 0.005$). VEGF-C, VEGFR3, podoplanin, and LYVE-1 colocalized on the endothelial lining of lymphatic vessels. With immunogold labeling, LYVE-1 and podoplanin antigen were found on endothelial cells lining vessels with ultrastructural features of lymph vessels. **CONCLUSIONS:** Immunohistochemistry with novel lymph-endothelium markers and ultrastructural analyses indicate the existence of lymphatic vessels in vascularized human corneas. Human corneal lymphangiogenesis appears to be correlated with the degree of corneal hemangiogenesis and may at least partially be mediated by VEGF-C and its receptor VEGFR3.

Nephrol Dial Transplant. 2002 Jun;17(6):978-84.

Cloning and expression of the mouse glomerular podoplanin homologue gp38P.

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BACKGROUND: Puromycin aminonucleoside nephrosis (PAN) is a rat model for human minimal change nephropathy. During PAN, severe proteinuria is induced that is paralleled by a reduced expression of a rat podocyte protein, named **podoplanin**. The protein probably plays a role in maintaining the unique shape of podocytes. Recently, attenuated amino acid transport has been observed in cultured mouse glomerular epithelial cells treated with puromycin aminonucleoside (PA). In the present study, gp38P, a protein homologous to rat podoplanin was cloned from mouse glomerular epithelial cells and was found to be down-regulated by PA. A role for gp38P in membrane transport in mouse podocytes has been suggested. **METHODS:** Based on homology to rat podoplanin, the protein gp38P was cloned from mouse glomerular epithelial cells by RT-PCR. Mouse glomerular epithelial cells, mouse cortical collecting duct cells, and *Xenopus* oocytes were treated with PA and the expression of gp38P was examined by RT-PCR and western blot analysis. Expression of gp38P in other mouse tissues was demonstrated by RT-PCR. The possible impact of gp38P on amino acid transport and folic acid uptake was examined in *Xenopus* oocytes. **RESULTS:** gp38P cloned from mouse glomerular epithelial cells showed strong homologies to rat podoplanin and gp38, a protein expressed in the thymus and other tissues. RT-PCR analysis demonstrated ubiquitous expression of gp38P in epithelial and non-epithelial tissues. Quantitative RT-PCR and western blot analysis indicated down-regulation of gp38P in PA-treated glomerular epithelial cells along with loss of cell shape and cell lysis, which was not observed in other cell types. When expressed in *Xenopus* oocytes, gp38P had no impact on folic acid uptake or transport activity of the amino acid co-transporters CAT1, EAAC1, and rBAT. **CONCLUSION:** Cultured mouse glomerular epithelial cells express the podoplanin homologue gp38P, which is down-regulated by PAs. gp38P is ubiquitously expressed and is likely to control specifically the unique shape of podocytes.

Semin Cell Dev Biol. 2002 Feb;13(1):9-18.

Lymphatic endothelial regulation, lymphoedema, and lymph node metastasis.

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Vascular endothelial growth factor receptor-3 (VEGFR-3) mediates lymphatic endothelial cell (LEC) growth, migration, and survival by binding VEGF-C and VEGF-D. Recent studies have revealed new regulators of the lymphatic endothelium, such as the transcription factor Prox1, and the cell surface proteins **podoplanin** and lymphatic vessel endothelial hyaluronan receptor-1 (LYVE-1). Furthermore, the isolation of LECs now allows detailed molecular studies of the factors

regulating the lymphatic vasculature. These studies are aimed at targeting the lymphatic vasculature in the treatment of various diseases, such as tumour metastasis and lymphoedema.

Anticancer Res. 2001 Sep-Oct;21(5):3419-23.

Inflammatory stromal reaction correlates with lymphatic microvessel density in early-stage cervical cancer.

Schoppmann SF, Schindl M, Breiteneder-Geleff S, Soleima A, Breitenecker G, Karner B, Birner P.

BACKGROUND: In early-stage cervical cancer, high lymphatic microvessel density (LMVD) indicates favorable prognosis. This unexpected finding was thought to be an effect of local immunological response, although no data supported this thesis. **MATERIALS AND METHODS:** LMVD and lymphovascular invasion (LVI) were assessed in 85 specimens of cervical cancer stage pT1b by immunostaining for **podoplanin**, a marker for lymphatic endothelia. Local immunological response, evident by inflammatory stromal reaction (ISR), was determined in H&E-stained slides and rated from grade 1 (absent or weak) to 3 (strong) **RESULTS:** A good correlation of LMVD and ISR was found ($p=0.002$). While a strong correlation between LMVD and the presence of LVI was found ($p<0.001$), no association between LMVD and pelvic lymph node involvement ($p=0.732$) was observed. ISR indicated favourable prognosis of patients ($p=0.0247$, log-rank test). **CONCLUSION:** Our findings suggest that ISR might play a role in the induction of lymphangiogenesis in early stage cervical cancer.

Anticancer Res. 2001 Jul-Aug;21(4A):2351-5.

Lymphatic microvessel density and lymphovascular invasion assessed by anti-podoplanin immunostaining in human breast cancer.

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BACKGROUND: The development of an antibody against **podoplanin** has enabled us to selectively stain lymphatic vessels in breast cancer samples for the first time. **MATERIALS AND METHODS:** We investigated lymphatic vessels in 45 specimens of invasive breast cancer by immunostaining for podoplanin. Lymphatic microvessel density (LMVD) was correlated with various clinical and histopathological parameters. LMVD was also compared to blood microvessel density (BMVD), assessed by CD34 -immunostaining. **RESULTS:** LMVD as well as lymphovascular invasion (LVI) correlated significantly with the lymph node status ($p=0.001/p=0.035$). Logistic regression revealed that LVI was the more important factor for development of lymph node metastasis ($p=0.043$). There was no significant association between various clinical and histopathological parameters and LMVD or LVI, nor was a correlation found between LMVD and BMVD ($p=0.121$). **CONCLUSION:** High LMVD and the presence of LVI are strongly associated with lymph node metastasis in breast cancer.

Histopathology. 2001 Oct;39(4):373-81.

Ordered array of dendritic cells and CD8+ lymphocytes in portal infiltrates in chronic hepatitis C.

Galle MB, DeFranco RM, Kerjaschki D, Romanelli RG, Montalto P, Gentilini P, Pinzani M, Romagnoli P.

AIMS: Despite the importance of dendritic cells in stimulating primary and secondary immune responses by presenting antigens to T-lymphocytes in draining lymph nodes and peripheral tissues, respectively, very limited information is available on the presence and localization of these cells in hepatitis C virus (HCV)-related chronic active hepatitis. Therefore, we addressed the ultrastructure, immunophenotype, distribution and relationships to lymphatics of dendritic cells in portal infiltrates of this disease. METHODS AND RESULTS: Part of percutaneous diagnostic liver biopsies (Knodell's histological assessment index: 9-13) was processed for electron microscopy and for immunohistochemical detection of immune system cell membrane antigens and of the lymphatic endothelium marker **podoplanin**. In portal infiltrates, cells with electron microscopical and cell marker features of dendritic cells and expressing the activation markers CD54, CD80, CD83 and CD86 were organized in a discontinuous network, that embedded CD8+ lymphocytes in close contact with dendritic cells and came in contact with hepatocytes, sometimes infiltrating beyond the limiting plate. Also, dendritic cells were found within newly formed lymphatic capillaries in thin, infiltrated septa among hepatocytes. CONCLUSIONS: This evidence strongly suggests a critical role of dendritic cells and newly formed lymphatics in the pathogenesis and organization of the immune infiltrate that characterizes HCV-related chronic active hepatitis.

Microsc Res Tech. 2001 Oct 15;55(2):108-21.

Lymphatic versus blood vascular endothelial growth factors and receptors in humans.

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Three different growth factor systems have been described acting via endothelial cell-specific receptor tyrosine kinases (RTKs). These are vascular endothelial growth factors (VEGFs), angiopoietins, and ephrins. Recent studies on gene targeting suggest that they play critical roles in embryonic development and contribute to the integrity and responses to environmental factors in the adult vasculature. Coagulation, inflammation, immune response regulation, vascular tone, stromal component synthesis, and angiogenesis are all dependent on the physiological and pathological events that affect endothelial cells in the heart, arteries, veins, and lymphatic vessels. Angiogenesis, the formation of new blood vessels from preexisting ones, takes place in adults only during hormonal control of female reproduction. All other activation of angiogenesis in adulthood occurs in response to injury or pathological processes such as tumorigenesis, diabetes, or inflammatory conditions. Insufficient growth of collateral vessels is a major problem in atherosclerotic cardiovascular disease. Controlled stimulation of angiogenesis would be of therapeutic value. Lymphangiogenesis, the mechanisms involved in the development of lymphatic vessels, was studied intensively nearly a century ago, although since then it has been neglected, perhaps because, unlike the disorders of blood vessels, those of the lymphatic vessels are seldom life-threatening. Interrupting this one-way system can cause severe disorders, including liver dysfunction, genetic disease (e.g., Milroy's disease), and degenerative disease (e.g., primary lymphangiosclerosis). Recently, novel growth factors, receptors, cell surface proteins, and transcription factors have been found which play a role in the lymphatic endothelium. These are VEGF-C, VEGF-D, VEGFR-3, LYVE-1, **podoplanin**, and Prox-1. Until recently lymphatic vessels have been difficult to study due to a lack of appropriate tools. Monoclonal antibodies raised against VEGFR-3 and against its ligands, VEGF-C and VEGF-D, have offered an insight into expression studies in tissues. In this review, we summarize the recent data on VEGFs in the human vasculature.

Microsc Res Tech. 2001 Oct 15;55(2):61-9.

Markers for the lymphatic endothelium: in search of the holy grail?

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The ability to discriminate reliably at the histological level between blood and lymphatic microcapillaries would greatly assist the study of a number of biological and pathological questions and may also be of clinical utility. A structure-function comparison of these types of microcapillary suggests that differences which could function as markers to allow discrimination between blood and lymphatic endothelium should exist. Indeed, to date a variety of such markers have been proposed, including basement membrane components, constituents of junctional complexes such as desmoplakin and enzymes such as 5'-nucleotidase. Additionally, a variety of cell surface molecules are thought to be differentially expressed, including PAL-E, VEGFR-3, **podoplanin**, and LYVE-1. Several of the lymphatic markers proposed in the literature require further characterization to demonstrate fully their lymphatic specificity and some have proven not to be reliable. The relative merits and drawbacks of each of the proposed markers is discussed.

Graefes Arch Clin Exp Ophthalmol. 2001 Aug;239(8):628-32.

Orbital lymphangioma with positive immunohistochemistry of lymphatic endothelial markers (vascular endothelial growth factor receptor 3 and podoplanin).

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BACKGROUND: Existence of true orbital lymphangiomas has been questioned in recent years. Therefore an orbital lymphangioma was analyzed with two new specific markers of lymphatic endothelium. **METHODS:** Case-report with clinicopathological, immunohistochemical, and ultrastructural findings. A 25-year-old man presented with recurrent lower lid "hematomas" and a pea-sized tumor palpable in the left lower lid. Magnetic resonance imaging showed an inferonasally located orbital tumor which extended to the posterior pole of the eye. The highly vascularized tumor was excised by medial orbitotomy. **RESULTS:** Histopathologically, the mass consisted of large, erythrocyte-filled cavernous vessels without evidence of smooth muscle cells or pericytes surrounding them. Numerous lymph follicles and small arterioles were scattered between them. Immunohistochemically, endothelial cells lining the lumina of the cavernous vessels were partly positive for **podoplanin** and vascular endothelial growth factor receptor 3 (flt-4), two markers of lymphatic endothelium. These markers did not react with endothelial cells lining the arterioles. Ultrastructurally, cavernous vessels displayed features characteristic of lymphatic vessels, and the smaller vessels demonstrated signs of arterioles. **CONCLUSION:** Ultrastructural analysis and immunohistochemistry using two new markers of lymphatic endothelium suggest a lymphatic nature of large vessels in an orbital lymphangioma. A greater series of vascular orbital tumors must be studied with these new lymph endothelial markers to confirm the existence of true orbital lymphangiomas and to analyze different profiles of lymph endothelial marker expression.

J Exp Med. 2001 Sep 17;194(6):797-808.

Isolation and characterization of dermal lymphatic and blood endothelial cells reveal stable and functionally specialized cell lineages.

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A plexus of lymphatic vessels guides interstitial fluid, passenger leukocytes, and tumor cells toward regional lymph nodes. Microvascular endothelial cells (ECs) of lymph channels (LECs) are difficult to distinguish from those of blood vessels (BECs) because both express a similar set of markers, such as CD31, CD34, podocalyxin, von Willebrand factor (vWF), etc. Analysis of the specific properties of LECs was hampered so far by lack of tools to isolate LECs. Recently, the 38-kD mucoprotein **podoplanin** was found to be expressed by microvascular LECs but not BECs in vivo. Here we isolated for the first time podoplanin(+) LECs and podoplanin(-) BECs from dermal cell suspensions by multicolor flow cytometry. Both EC types were propagated and stably expressed VE-cadherin, CD31, and vWF. Molecules selectively displayed by LECs in vivo, i.e., podoplanin, the hyaluronate receptor LYVE-1, and the vascular endothelial cell growth factor (VEGF)-C receptor, fms-like tyrosine kinase 4 (Flt-4)/VEGFR-3, were strongly expressed by expanded LECs, but not BECs. Conversely, BECs but not LECs expressed VEGF-C. LECs as well as BECs formed junctional contacts with similar molecular composition and ultrastructural features. Nevertheless, the two EC types assembled in vitro in vascular tubes in a strictly homotypic fashion. This EC specialization extends to the secretion of biologically relevant chemotactic factors: LECs, but not BECs, constitutively secrete the CC chemokine receptor (CCR)7 ligand secondary lymphoid tissue chemokine (SLC)/CCL21 at their basal side, while both subsets, upon activation, release macrophage inflammatory protein (MIP)-3 α /CCL20 apically. These results demonstrate that LECs and BECs constitute stable and specialized EC lineages equipped with the potential to navigate leukocytes and, perhaps also, tumor cells into and out of the tissues.

Int J Cancer. 2001 Jan 20;95(1):29-33.

Lymphatic microvessel density as a novel prognostic factor in early-stage invasive cervical cancer.

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Few data on the influence of lymphatic microvessel density (MVD) on survival in cancer are available since until recently there was no reliable immunohistological marker for lymphatic endothelium. Using an antibody staining **podoplanin**, a novel marker for lymphatic endothelium, lymphatic MVD in tissue samples of 85 patients with cervical cancer classification pT1b treated by radical hysterectomy was investigated. Survival was determined using univariate and multivariate analyses. Lymphatic MVD was also compared to MVD assessed by immunostaining against factor VIII-related antigen, which is considered a marker for blood vessels. Patients with >5 lymphatic microvessels/0.25 mm² field had significantly better overall survival (mean 91.8 months) than those with \leq 5 lymphatic microvessels/field in univariate analysis (mean 113 months) ($p = 0.0105$, log-rank test). In multivariate analysis, lymphatic node involvement ($p = 0.0183$), vessel infiltration ($p = 0.0158$) and lymphatic MVD ($p = 0.0269$) remained independent prognostic factors. No correlation between lymphatic MVD and various clinical and histopathological parameters was observed. Correlation between lymphatic MVD and MVD assessed by immunostaining against factor VIII was only weak ($p = 0.004$, $r = 0.312$, Spearman's coefficient of correlation). Our results suggest that increased lymphatic MVD is associated with favorable prognosis in early-stage cervical cancer.

Am J Pathol. 2001 Mar;158(3):867-77.

The beta-chemokine receptor D6 is expressed by lymphatic endothelium and a subset of vascular tumors.

Nibbs RJ, Kriehuber E, Ponath PD, Parent D, Qin S, Campbell JD, Henderson A, Kerjaschki D, Maurer D, Graham GJ, Rot A.

The lymphatic vessels (lymphatics) play an important role in channeling fluid and leukocytes from the tissues to the secondary lymphoid organs. In addition to driving leukocyte egress from blood, chemokines have been suggested to contribute to leukocyte recirculation via the lymphatics. Previously, we have demonstrated that binding sites for several pro-inflammatory beta-chemokines are found on the endothelial cells (ECs) of lymphatics in human dermis. Here, using the MIP-1alpha isoform MIP-1alphaP, we have extended these studies to further support the contention that the in situ chemokine binding to afferent lymphatics exhibits specificity akin to that observed in vitro with the promiscuous beta-chemokine receptor D6. We have generated monoclonal antibodies to human D6 and showed D6 immunoreactivity on the ECs lining afferent lymphatics, confirmed as such by staining serial skin sections with antibodies against **podoplanin**, a known lymphatic EC marker. In parallel, in situ hybridization on skin with antisense D6 probes demonstrated the expression of D6 mRNA by lymphatic ECs. D6-immunoreactive lymphatics were also abundant in mucosa and submucosa of small and large intestine and appendix, but not observed in several other organs tested. In lymph nodes, D6 immunoreactivity was present on the afferent lymphatics and also in subcapsular and medullary sinuses. Tonsillar lymphatic sinuses were also D6-positive. Peripheral blood cells and the ECs of blood vessels and high endothelial venules were consistently nonreactive with anti-D6 antibodies. Additionally, we have demonstrated that D6 immunoreactivity is detectable in some malignant vascular tumors suggesting they may be derived from, or phenotypically similar to, lymphatic ECs. This is the first demonstration of chemokine receptor expression by lymphatic ECs, and suggests that D6 may influence the chemokine-driven recirculation of leukocytes through the lymphatics and modify the putative chemokine effects on the development and growth of vascular tumors.

Clin Cancer Res. 2001 Jan;7(1):93-7.

Selective immunohistochemical staining of blood and lymphatic vessels reveals independent prognostic influence of blood and lymphatic vessel invasion in early-stage cervical cancer.

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Lymphovascular space invasion was shown to play a key role in the progression of cervical cancer. Because of the absence of a specific marker for lymphatic vessels, earlier studies could not reliably distinguish between blood and lymphatic vessel invasion. By immunostaining for **podoplanin**, a novel marker for lymphatic endothelium, and for factor VIII-related antigen, we determined lymphatic and blood vessel invasion in tissue samples of 98 patients with cervical cancer pT1b treated by radical hysterectomy. Eleven (11.2%) specimens showed invasion of blood vessels, 20 (20.4%) showed invasion of lymphatic vessels, and 15 (15.3%) showed invasion of blood and lymphatic vessels. There was a strong association of lymphatic vessel invasion and lymph node involvement ($P < 0.001$). In univariate analysis, both blood and lymphatic vessel invasion failed to reach a statistically significant influence on overall survival, but a significant influence on disease-free survival was found ($P = 0.0002$ and $P < 0.0001$, respectively). In multivariate analysis of disease-free survival, only blood vessel invasion remained statistically significant ($P = 0.0457$). Lymphatic vessel invasion reached significance when lymph node status was excluded from the

model ($P = 0.0025$). Both lymphatic vessel and blood vessel invasion occur frequently in early-stage cervical cancer. Determination of the vessel status may be of clinical importance because it signifies the risk of recurrent disease.

Anticancer Res. 2000 Sep-Oct;20(5A):2981-5.

Lymphatic microvessel density in epithelial ovarian cancer: its impact on prognosis.

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BACKGROUND: The influence of lymphatic microvessel density (MVD) on survival in epithelial ovarian cancer is still unknown, owing to the fact that until recently no reliable immunohistologic markers for lymphatic endothelium were available. **MATERIALS AND METHODS:** By using a polyclonal antibody staining **podoplanin**, a novel marker for lymphatic endothelium, lymphatic MVD in tissue samples of 90 patients with epithelial ovarian cancer treated by radical surgery and chemotherapy was investigated. Survival analysis was performed using univariate and multivariate analysis. Furthermore, lymphatic MVD was compared to MVD assessed by CD34 immunostaining. **RESULTS:** Lymphatic MVD was significantly lower than CD34 MVD ($p < 0.0001$). There was no significant association between lymphatic MVD and various histological and clinical parameters. Lymphatic MVD had no influence on overall survival and disease free survival ($p = 0.4627$ and $p = 0.4337$, respectively; log-rank test). **CONCLUSION:** The formation of lymphatic vessels has no influence on the progression of epithelial ovarian cancer.

Verh Dtsch Ges Pathol. 1999;83:270-5.

[Podoplanin--a specific marker for lymphatic endothelium expressed in angiosarcoma]

Breiteneder-Geleff S, Soleiman A, Horvat R, Amann G, Kowalski H, Kerjaschki D.

AIMS: Angiosarcomas apparently derive from endothelia of the blood vasculature, however occasionally their histologic features suggest mixed origin from blood and lymphatic endothelia. In the absence of specific positive markers for lymphatic endothelia the precise distinction between these components was not possible so far. Here we provide evidence that **podoplanin**, a approximately 38 kD membrane glycoprotein of podocytes is a specific marker of lymphatic endothelium that was used to identify the relative fraction of tumor cells with lymphatic or blood vascular endothelial phenotype in vascular tumors. **METHODS:** Podoplanin was localized in normal human skin and kidney cortex by immunohistochemistry on paraffin sections, double immunofluorescence on frozen sections with PAL-E, immunoelectron microscopy and by immunoblotting. 45 vascular tumors (29 benign lesions, 11 angiosarcomas and 5 gastrointestinal Kaposi's sarcomas) were evaluated for podoplanin expression. Complementary staining was obtained with established endothelial markers (CD 31, CD 34, Factor VIII related antigen, UEA I) and with podocalyxin, another podocytic protein mainly present in endothelia of blood vessels. **RESULTS:** In human tissues podoplanin is specifically expressed in the endothelium of lymphatics, but not in blood vasculature or in hemangiomas. This expression is preserved in endothelia of all benign lymphatic tumorous lesions and all Kaposi's sarcomas examined. By contrast 10 out of 11 G3 angiosarcomas contained only variable fractions of podoplanin-expressing tumor cells. Most tumor cells coexpressed podoplanin and markers of blood vessel phenotype. **CONCLUSIONS:** (1) Podoplanin is a selective marker of lymphatic endothelium; (2) G3 angiosarcomas display a quantitative spectrum of podoplanin-expressing tumor cells; (3) In the majority of angiosarcomas

tumor cells coexpress podoplanin and endothelial markers of blood vessels; (4) All endothelial cells of Kaposi's sarcomas expressed the lymphatic marker podoplanin.

Lab Invest. 1999 Feb;79(2):243-51.

Expression of vascular endothelial growth factor receptor-3 and podoplanin suggests a lymphatic endothelial cell origin of Kaposi's sarcoma tumor cells.

Weninger W, Partanen TA, Breiteneder-Geleff S, Mayer C, Kowalski H, Mildner M, Pammer J, Sturzl M, Kerjaschki D, Alitalo K, Tschachler E.

Despite intensive research over the past decade, the exact lineage relationship of Kaposi's sarcoma (KS) tumor cells has not yet been settled. In the present study, we investigated the expression of two markers for lymphatic endothelial cells (EC), ie, vascular endothelial growth factor receptor-3 (VEGFR-3) and **podoplanin**, in AIDS and classic KS. Both markers were strongly expressed by cells lining irregular vascular spaces in early KS lesions and by tumor cells in advanced KS. Double-staining experiments by confocal laser microscopy established that VEGFR-3-positive and podoplanin-positive cell populations were identical and uniformly expressed CD31. By contrast, these cells were negative for CD45, CD68, and PAL-E, excluding their hemopoietic and blood vessel endothelial cell nature. Podoplanin expression in primary KS tumor lysates was confirmed by Western blot analysis. Both splice variants of VEGFR-3 were found in KS-tumor-derived RNA by RT-PCR. In contrast to KS tumor cells in situ, no expression of VEGFR-3 and podoplanin was detected in any of four KS-derived spindle cell cultures and in one KS-derived autonomously growing cell line (KS Y-1). Our findings that KS tumor cells express two lymphatic EC markers in situ strongly suggest that they are related to or even derived from the lymphatic EC lineage. Lack of these antigens on cultured cells derived from KS lesions indicates that they might not represent tumor cells that grow in tissue culture, but rather other cell types present in KS lesions.

Am J Pathol. 1999 Feb;154(2):385-94.

Angiosarcomas express mixed endothelial phenotypes of blood and lymphatic capillaries: podoplanin as a specific marker for lymphatic endothelium.

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

Angiosarcomas apparently derive from blood vessel endothelial cells; however, occasionally their histological features suggest mixed origin from blood and lymphatic endothelia. In the absence of specific positive markers for lymphatic endothelia the precise distinction between these components has not been possible. Here we provide evidence by light and electron microscopic immunohistochemistry that **podoplanin**, a approximately 38-kd membrane glycoprotein of podocytes, is specifically expressed in the endothelium of lymphatic capillaries, but not in the blood vasculature. In normal skin and kidney, podoplanin colocalized with vascular endothelial growth factor receptor-3, the only other lymphatic marker presently available. Complementary immunostaining of blood vessels was obtained with established endothelial markers (CD31, CD34, factor VIII-related antigen, and Ulex europaeus I lectin) as well as podocalyxin, another podocytic protein that is also localized in endothelia of blood vessels. Podoplanin specifically immunolabeled endothelia of benign tumorous lesions of undisputed lymphatic origin (lymphangiomas, hygromas) and was detected there as a 38-kd protein by immunoblotting. As paradigms of malignant vascular tumors, poorly differentiated (G3) common angiosarcomas (n = 8), epitheloid angiosarcomas (n =

3), and intestinal Kaposi's sarcomas (n = 5) were examined for their podoplanin content in relation to conventional endothelial markers. The relative number of tumor cells expressing podoplanin was estimated and, although the number of cases in this preliminary study was limited to 16, an apparent spectrum of podoplanin expression emerged that can be divided into a low-expression group in which 0-10% of tumor cells contained podoplanin, a moderate-expression group with 30-60% and a high-expression group with 70-100%. Ten of eleven angiosarcomas and all Kaposi's sarcomas showed mixed expression of both lymphatic and blood vascular endothelial phenotypes. By double labeling, most podoplanin-positive tumor cells coexpressed endothelial markers of blood vessels, whereas few tumor cells were positive for individual markers only. From these results we conclude that (1) podoplanin is a selective marker of lymphatic endothelium; (2) G3 angiosarcomas display a quantitative spectrum of podoplanin-expressing tumor cells; (3) in most angiosarcomas, a varying subset of tumor cells coexpresses podoplanin and endothelial markers of blood vessels; and (4) all endothelial cells of Kaposi's sarcomas expressed the lymphatic marker podoplanin.

J Am Soc Nephrol. 1998 Nov;9(11):2013-26.

Epitope-specific antibodies to the 43-kD glomerular membrane protein podoplanin cause proteinuria and rapid flattening of podocytes.

Matsui K, Breiteneder-Geleff S, Kerjaschki D.

The 43-kD integral membrane protein **podoplanin** is localized on the surface of rat podocytes, and transcriptionally downregulated in rat puromycin nephrosis. In this study, a single intravenous injection of polyclonal rabbit anti-podoplanin IgG resulted in selective binding of IgG to the entire podocyte's surface. Some IgG produced by different rabbits rapidly induced transient proteinuria (approximately 350 mg/24 h at day 1, normal levels around day 5), whereas other IgG were ineffective. All anti-podoplanin IgG shared a common binding site at amino acids 39 to 47 (DDMVNPGLE), whereas IgG inducing glomerular damage specifically bound to an additional epitope at amino acids 74 to 79 (PIEELP), as observed by a SPOTs analysis on overlapping synthetic peptides. Proteinuria was not prevented by complement depletion or by treatment with the oxygen radical scavenger dimethylthiourea. Injection of Fab fragments failed to induce glomerular pathology, indicating that dimerization of podoplanin by divalent IgG was required. Proteinuria was paralleled by extensive flattening of foot processes that was also induced by blood-free perfusion of isolated rat kidneys with anti-podoplanin IgG. Thus, glomerular changes were due to direct interaction of distinct epitope(s) of podoplanin and divalent IgG. These results provide evidence that podoplanin plays a role in maintaining the unique shape of podocyte foot processes and glomerular permeability.

Am J Pathol. 1997 Oct;151(4):1141-52.

Podoplanin, novel 43-kd membrane protein of glomerular epithelial cells, is down-regulated in puromycin nephrosis.

Breiteneder-Geleff S, Matsui K, Soleiman A, Meraner P, Poczewski H, Kalt R, Schaffner G, Kerjaschki D.

Puromycin aminonucleoside nephrosis (PAN), a rat model of human minimal change nephropathy, is characterized by extensive flattening of glomerular epithelial cell (podocyte) foot processes and by severe proteinuria. For comparison of expression of glomerular membrane proteins of normal and PAN rats, a membrane protein fraction of isolated rat glomeruli was prepared and monoclonal

antibodies were raised against it. An IgG-secreting clone designated LF3 was selected that specifically immunolabeled podocytes of normal but not of PAN rats. The antigen of LF3 IgG was identified as a 43-kd glycoprotein. Molecular cloning of its cDNA was performed in a delta gt11 expression library prepared from mRNA of isolated rat glomeruli. The predicted amino acid sequence indicated a 166-amino-acid integral membrane protein with a single membrane-spanning domain, two potential phosphorylation sites in its short cytoplasmic tail, and six potential O-glycosylation sites in the large ectodomain. High amino acid sequence identities were found to membrane glycoproteins of rat lung and bone and mouse thymus epithelial cells as well as to a phorbol-ester-induced protein in a mouse osteoblast cell line and to a canine influenza C virus receptor. In PAN, expression of this 43-kd protein was selectively reduced to < 30%, as determined by quantitative immunogold electron microscopy, immunoblotting, and Northern blotting. These data provide evidence that transcription of the 43-kd transmembrane podocyte glycoprotein is specifically down-regulated in PAN. To indicate that this protein could be associated with transformation of arborized foot processes to flat feet (Latin, pes planus) we have called it podoplanin . <http://www.researchd.com/miscabs/102pa40.htm>

Rabbit Anti-human Podoplanin

Cat#RDI-102PA40 \$250.00/100ug

Cat#RDI-102PA40X \$450.00/200ug

Description: Produced from sera of rabbits immunized with the recombinant ectodomain of Podoplanin (gp36). Podoplanin is a highly O-glycosylated integral membrane protein that is specifically expressed in the endothelium of lymphatic capillaries but not in the blood vasculature. In normal skin and kidney, podoplanin colocalized with VEGFR-3/FLT-4, another marker for lymphatic endothelial cells.

Host species:	Rabbit
Antigen:	Recombinant ectodomain of human gp36 (podoplanin)
Purification:	Protein A chromatography
Stabilizer:	none
Buffer:	lyophilized from PBS, pH 7.4 w/o preservative
Formulation:	lyophilized rabbit IgG
Reconstitution:	The lyophilized IgG is stable at 4°C. for at least one month and for more than a year when kept at -20°C. When reconstituted in sterile water to a concentration of >0.5 mg/ml the antibody is stable for at least six weeks at 2-4°C in the presence of a preservative. Reconstituted antibody can also be aliquotted and stored at -20°C to -70°C for at least 6 months without detectable loss of activity. Avoid repeated freeze-thaw cycles.

Applications

Immunoprecipitation:	For IP, the antibody should be used at a concentration of 2-4 µg/sample.
Western Analysis:	For Western blot analysis, the antibody should be used at a concentration of 1-2 µg/ml with the appropriate secondary reagents.
Immunofluorescence:	For immunofluorescence, the antibody should be used at a concentration of 1-2 µg.

Optimal dilutions should be determined by each laboratory for each application.

Literature:

[Breiteneder-Geleff et al., Am. J. Pathol. 151:1141,1997; Zimmer et al., Biochem. J. 341:277, 1999; Schacht et al., EMBO J. 22:3546-56, 2003]

**** please note : always centrifuge vials before opening ****

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