Immunohistochemical Expression and Distribution of VEGFR-3 in Malignant Mesothelioma

Adhemar Longatto Filho, M.Sc., Ph.D., P.M.I.A.C.,[1,2] Fátima Baltazar, Ph.D.,[1] Carlos Bedrossian, M.D., Ph.D. (Hon), F.I.A.C.,[3] Claire Michael, M.D.,[4] and Fernando C. Schmitt, M.D., M.I.A.C.[5,6*]

Homogeneity of mesothelial and lymphatic endothelial cells, express some markers that are presumed to be exclusive of the endothelium was recently reported. This similarity is important to improve the diagnosis and prognosis of malignant mesothelioma (MM). Additionally, some of these markers provide the rationale for specific molecular-targeted novel therapies aimed at MM, an aggressive malignant neoplasm, with an usually dismal prognosis. The goal of our study was to determine the prevalence and expression pattern of VEGF receptor-3 (VEGFR-3) immunoreactivity in MM and whether this immunoreactivity occurs in different phenotypes of this neoplasm. Formalin-fixed and paraffin-embedded samples from 29 MM cases and 5 metastatic carcinomas were immuno-stained for VEGFR-3 according to the streptavidin–biotin–peroxidase complex technique using a primary antibody (Zymed Laboratories, CA, USA) diluted at 1:200. Lymphatic vessels (LV) were outlined mainly in the peripheral area surrounding the neoplasms. Blood vessels were only rarely positive for VEGFR-3 in a pattern easily distinguishable from LV. In 25 out of 29 cases (86.2%) LV were strongly positive for VEGFR-3: 14 cases (48.2%) exhibited positive VEGFR-3 reactivity in malignant cells. Epitheliod MM showed a moderate to intense VEGFR-3 positive reaction in LV from 8 out of 19 cases. Among the other histological subtypes, a positive VEGFR-3 reaction was noted in malignant cells from two cases of transitional and one case of pleomorphic MM. Malignant cells from two out of three biphasic and one out of three sarcomatoid MM were also positive for VEGFR-3. Interestingly, one case of the multicystic subtype was negative for VEGFR-3 in malignant cells and faintly positive in an occasional LV. All cases of metastatic carcinoma were negative for VEGFR-3 in the neoplastic cells. In conclusion, VEGFR-3 was expressed in malignant cells from different subtypes of MM, reinforcing the putative role of this marker as a potential therapeutic target in this group of neoplasia. Diagn. Cytopathol. 2007;35:786–791.

Key Words: lymphangiogenesis; angiogenesis; vascular endothelial growth factor receptor 3; mesothelioma

Cancer transformation of mesothelial cells gives rise to malignant mesothelioma (MM), an aggressive malignant neoplasia predominantly of the pleura and closely associated to asbestos exposure. Recently, simian virus (SV40) infection was also recognized as a causative agent of MM oncogenesis. In vitro evidence exists, showing that SV40-exposed mesothelial cells are particularly susceptible to malignant transformation; and in vivo observation showed that SV40 is able to produce MM when injected in rodent visceral cavities.[1]

MM incidence is increasing worldwide, which is believed to be a consequence of a more widespread exposure to asbestos. Because of the relentless, aggressive behavior of MM, there is a great interest among Public Health authorities and the public regarding this epidemic.[2] Prognosis is usually dismal, worse in male patients, with median survival around 12 months from the time of diagnosis and no measurable improvement with traditional forms of treatment.[2,3] Novel therapies with Alimta and Cisplatinum only marginally improve survival, but may improve the quality of life in terminal patients.

Accurate diagnosis is crucial for appropriate therapeutic intervention, but, not infrequently, patients with MM are misdiagnosed at the time of their first consultation, such delays generally occurring in centers where MM is uncommon.[4] Cytologic diagnosis can be achieved rapidly and accurately depending on the experience of the cytopathologist. Nevertheless, negative cytological results of

[1]Life and Health Sciences Research Institute, Health Sciences School, University of Minho, Braga, Portugal
[2]Pathology Division, Adolfo Lutz Institute, São Paulo, Brazil
[3]Department of Pathology, Norwegian American Hospital, Chicago, Illinois
[4]Department of Cytopathology, University of Michigan, Ann Arbor
[5]Department of Pathology, University of Porto, Porto, Portugal
[6]IPATIMUP – Institute of Molecular Pathology and Immunology of University of Porto, Porto, Portugal
*Correspondence to: Fernando C. Schmitt, M.D., Ph.D., M.I.A.C., IPATIMUP, Rua Roberto Frias s/n, 4200 Porto, Portugal.
E-mail: fschmitt@ipatimup.pt
Received 29 July 2007; Accepted 26 September 2007
DOI 10.1002/dc.20767
Published online in Wiley InterScience (www.interscience.wiley.com).

pleural effusion do not exclude the possibility of MM.[5] Video-assisted toracoscopic (VATS) biopsy can often establish the diagnosis of malignancy, but sometimes do not allow a definitive diagnosis of MM.[3] Histopathological diagnosis can be difficult because MM exhibits a number of morphological appearances. Additionally, the serous membranes of the three body cavities are commonly invaded by metastasis, and often display mesothelial hyperplasia and florid reactive fibrosis.[6] Rarely primary pleural sarcomas, can mimic MM.[5,6] The histological type of MM seems to be related to the prognosis, i.e., the tubulopapillary variant of epithelial type has a more favorable prognosis than sarcomatoid type.[7] As both the cytological and histological diagnosis of MM can be difficult when based only in routine staining, immunohistochemical markers should be considered in most instances.[8–10] A number of studies have suggested different panels of immunohistochemical markers, which are useful in routine practice, to distinguish MM from metastatic carcinoma, but less effective in separating benign, reactive conditions from malignant lesions.[11–13]

MM cases have a tendency to remain within the framework of the serosa, a thin, expandable membrane, rich in lymphatics. Metastases are less common than in lung carcinomas, including pleurotropic tumors growing along the subserosal layer, the so- called pseudo- mesotheliomatous carcinoma.[14] When MM metastasize, they usually follow the lymphatic pathway, but rarely they may give distant metastasis, via the blood vessels. The underlying factors for these preferential routes are not known, but may be related to the ability of MM tumor cells to survive in different microenvironments or their ability to interact with the stroma.[15] In 1999, Ohta et al. showed that lymphangiogenesis was an important feature in MM and it was related to the production of VEGF-C and its receptor: VEGFR-3.[16] More recently, Ando et al.[17] described the homogeneity of mesothelial and lymphatic endothelial cells in expressing some markers originally believed to be exclusive of endothelium. These authors, with an elegant in vitro study, reported that cultured mesothelial and lymphatic endothelial cells expressed VEGFR-3, LYVE-1, and Prox-1 lymphatic-specific markers. Additionally, D2-40 and Podoplanin, two other specific lymphatic markers were also reported to be highly sensitive and specific to recognize epithelioid mesothelioma.[18–20] In a study of Nerve Growth Factor Receptors, Davidson et al.[21] found that p-trK-a receptor and to a less extent p75, distributed preferentially along the MM blood vessels, including lymphatics.

These new molecular players in the mesothelioma scenario certainly increased the expectations in the diagnosis and prognosis arenas, but also open an important route for emerging strategies in the translational therapy of MM.[22] Consider for example the vascular endothelial growth factor (VEGF) family, which is involved in the angiogenesis and lymphangiogenesis switch in health and disease. Preliminary results of growth inhibitors agents targeting VEGF members, ligands and their receptors, are encouraging as therapeutic option for MM.[23]

The purpose of our study was to determine the immunohistochemical reactivity of VEGF receptor-3 (VEGFR-3) in human MM cases and find out whether its prevalence and distribution pattern is similar among the different histological subtypes of MM.

Materials and Methods

Patients and Tumor Samples

Tissue samples were retrospectively obtained from the Department of Pathology, Norwegian American Hospital, Chicago, IL and the University Michigan Hospital and Clinics, Ann Arbor, MI. Formalin-fixed and paraffin-embedded samples of 29 MM cases and 5 pleurotropic metastatic adenocarcinomas were recovered. All cases were reviewed by two of us (CB and CM), without prior knowledge of the original treating physician diagnosis or classification, according to histological, immunocytochemical and ultrastructural criteria previously described.[24] Tumors selected for this study were further classified following the WHO recommendations for certain recognizable patterns such as tubulo-papillary, small cell, clear cell, deciduoid, transitional, pleomorphic, lympho-histiocytoid, and microglandular.[25]

A total of 29 cases were obtained: 21 from pleura (3 women and 18 men) and 6 from peritoneum (3 women and 3 men). The age of the patients with pleural MM ranged from 19 to 85 years old (mean 62.4) and from peritoneal MM from 17 to 78 (mean 50.2). Five cases of pleurotropic metastatic carcinoma of the lung were also included for comparison with MM.

VEGFR-3 Immunohistochemical Procedure

Immunohistochemical reaction was performed according to streptavidin–biotin–peroxidase complex technique using a primary antibody raised against VEGFR-3 (Zymed Laboratories, CA) diluted 1:200. In brief, deparaffinized and rehydrated sections were immersed in 0.01*M* citrate buffer (pH 6.0) and microwaved at 700 W for 15 min; then the slides were incubed with 3% hydrogen peroxide in methanol for 10 min and with Ultravision Block solution (Neomarkers, Freemont, CA) for 10 min also, at room temperature, before incubating 30 min with the primary antibody. Sections were sequentially washed in PBS 1× with 0.02% Tween 20 and incubated with biotinylated goat anti-polyvalent antibody for 10 min, streptavidin peroxidase for 10 min, and developed with 3,3′-diamino-benzidine for 15 min. The slides were counterstained with Mayer hematoxylin and mounted with Entellan (Merck, Dermstadt, Germany). Negative controls of

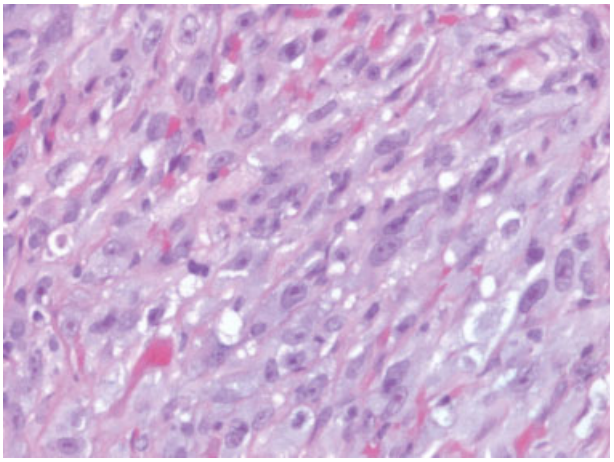

Fig. 1. Transitional MM, with no readily apparent lymphatic vessels (LV)-H&E (×400). [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

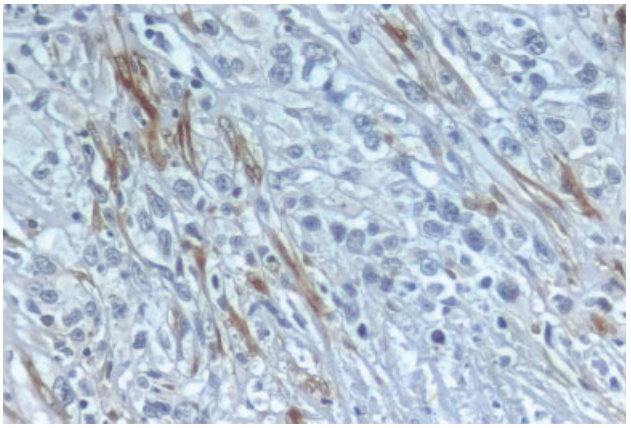

Fig. 2. Closely compressed VEGFR-3-positive intratumoral lymphatics in transitional MM-VEGFR-3 (×400). [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

reactions were performed with omission of primary antibody; as positive controls we have used invasive ductal breast carcinoma tissue as advised by the manufacturer.

Evaluation of VEGFR-3 Positive Reactions

Immunohistochemical VEGFR-3 expression was considered positive on cytoplasm of the endothelial lymphatic cells and malignant cells.

We semi-quantitatively assessed the distribution of the marker in decorated cells according to the following grading system: negative (−), absence of expression; slight positive staining (+), expression in up to 10% of cells; moderate positive (++), when the positive reaction was observed in 10% and until 50% of cells; strongly positive (+++), when the positive reaction was expressed in more than 50%. The assessment of positive reactions was performed in hot spot areas where proliferating vascular structures and epithelial malignant cells were present and stained, accordingly previously reported.[26]

Correlation of VEGFR-3 Expression and Mesothelioma Phenotype

VEGFR-3 results were correlated with the different subtypes of MM, age, sex, and localization.

Results

The majority of LV from MM cases were strongly positive for VEGFR-3, including 25 out of 29 (86.2%) cases. VEGFR-3 positive LV were more prevalent and more intensily outlined in tumor with dense desmoplastic stroma, including abundant collagen separating the malignant cells. Conversely, LV were lesser prominent in well-differentiated epithelioid MM, including tubulopapillary tumors rich in fibrovascular cores, supporting outcrops of cuboidal neoplastic cells. Indeed, the negative and slightly positive VEGFR-3 reactions were for the most part observed in these cases. Just one of three sarcomatoid MM showed a VEGFR-3 reaction that was faintly positive. The lymphatics in this case appeared either as large and tangled vessels, or as small slit-like spaces, difficult to discern with the H&E stain.

Once they had been clearly outlined with the positive VEGFR-3 reaction, LV appeared as microvascular tufts without RBCs in their lumina, both at the periphery of neoplasms and as part of their supporting stroma (Fig. 1) Peritumoral and intratumoral LV outlined by VEGFR-3 occurred as closely stretched linear structures, with different dimensions, predominantly elongated in shape and containing few RBCs in their collapsed lumina (Fig. 2). Fourteen cases (48.2%) exhibited positive VEGFR-3 staining in the malignant cells (Fig. 3); in seven cases the VEGFR-3 expression was focal: three epithelioid, two transitional, one sarcomatoid and one biphasic MM. The epitheliod subtype of MM showed VEGFR-3 positive reaction in malignant cells from 8 out of 19 cases (42%). These cases showed negative to slight Immunoreactivity in their LV outlined by VEGFR-3. From the others MM cases, positive VEGFR-3 reactions in malignant cells were observed in the two cases of transitional and one case of anaplastic subtype (Fig. 4). Two out of three biphasic and one out three sarcomatoid cases also exhibited VEGFR-3 positivity in malignant cells. Interestingly, the case of multicystic subtype was negative for VEGFR-3 in malignant cells and faintly positive in few LV (Fig. 5).

Table I depicts the scores of all VEGFR-3 reactions, correlating the histological subtype of MM with VEGFR-3 expression in both malignant cells and lymphatic endothelial cells. Epithelloid MM was the most common histological type observed, occurring in 19 out of 29 (65.5%)

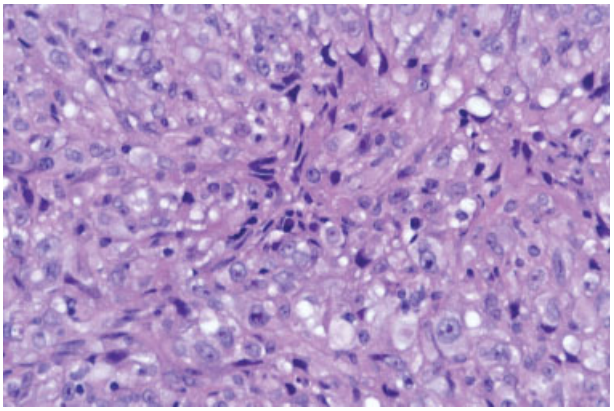

Fig. 3. Epithelioid MM, with poorly formed septa, outlined by compressed tumor cells-H&E (×400). [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

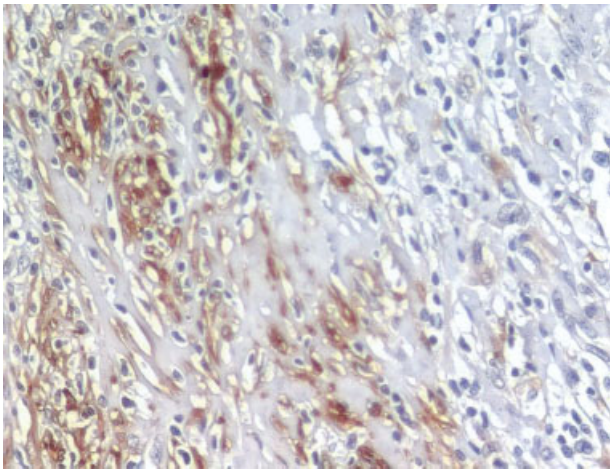

Fig. 4. Peritumoral VEGFR-3 positive lymphatics surrounding epithelioid MM tumor cells negative for VEGFR-3-VEGFR-3 (×400). [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

cases. These tumors exhibited remarkably bland cytomorphologic characteristics, although their architectural pattern included a wide spectrum of solid, papillary, tubulopapillary, and adenomatoid areas. Vascularity of these neoplasms was extremely variable, including LV channels that could not be readily recognizable, without the help of the VEGFR-3 immunostain. VEGFR-3 clearly outlined lymphatic vessels (LV) mainly in areas surrounding these neoplasms, including adipose tissue. Intra-tumoral LV in epithelioid MM were predominantly represented by compressed structures stained positively for VEGFR-3 (Fig. 6). Thick wall blood vessels were only rarely positive for VEGFR-3. All malignant cells from metastatic pleurotropic adenocarcinomas were negative for VEGFR-3.

The LV density showed a positive correlation between VEGFR-3 stained cases and MM subtypes as a whole:

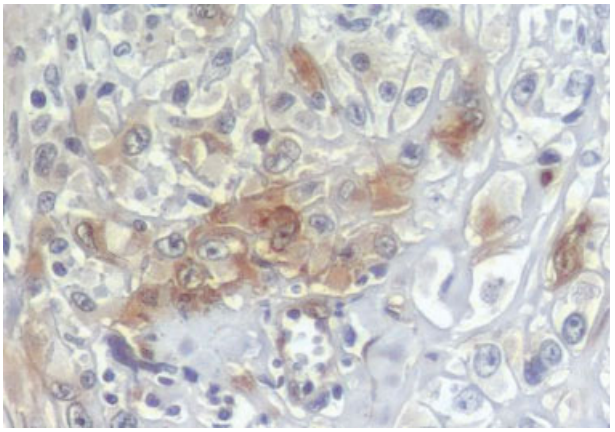

Fig. 5. VEGFR-3-positive tumor cells in pleomorphic MM-VEGFR-3 (×400). [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

only 4 out of 29 cases (13.8%) were negative for VEGFR-3. Except for epitheliod MM, the other subtypes were represented by very few cases, which limited the correlation between VEGFR-3 expression and histological differentiation. Despite this limitation, sarcomatoid, transitional, biphasic, and pleomorphic MM subtypes displayed a rich lymphatic vasculature strongly reactive for VEGFR-3.

Discussion

The involvement of vascular growth factor family (VEGF A, -B, -C, -D, and placenta growth factor – PIGF) in angiogenic and lymphangiogenic phenomena is well established and crucial for the proliferation of mesothelial cells and the growth of MM.[16–21] The potential anti-cancer therapy using inhibitors targeted to VEGF family appears as a rational tool for several tumors including MM.[23] VEGF, the best known angiogenic factor plays a role in the progression of MM since it is implicated in tumor-associated microvascular hyperpermeability and carcinogenesis, which results in malignant effusions.[27] Heparanase, a 65 KDa active precursor, after proteolytic cleavage yields an 8 and a 50 kDa subunit, which heterodimerize to form an active endogycosidase, capable of releasing VEGF-sulfate bound to the extracellular matrix (ECM). This enzyme not only plays a significant role in cancer metastasis and angiogenesis, but is also believed to play a role in malignant effusions caused by MM.[28]

The few informative studies in series of human tumors encouraged us to evaluate the expression of VEGFR-3 in MMs. Our findings reinforce previous studies that had demonstrated lymphangiogenesis in MM.[13] VEGFR-3 expression in lymphatic vessels was demonstrated in 86.2% of our cases and in 10 out of 25 (40%) cases with lymphangiogenesis we observed a high number of LV. These findings corroborate the aggressive biological

Table I. Correlation Between the Mesothelioma Subtypes and VEGFR-3 Expression in Malignant Cells and Lymphatic Vessels

	VEGFR-3 reaction malignant cells		*VEGFR-3 reaction lymphatic vessels*			
Histologic subtype	*Negative*	*Positive*	*Negative*	*1+*	*2+*	*3+*
Epithelioid ($n = 19$)	11	8	4	6	6	3
Sarcomatoid ($n = 3$)	2	1	0	1	0	2
Biphasic ($n = 3$)	1	2	0	0	0	3
Transitional ($n = 2$)	0	2	0	1	0	1
Pleomorphic ($n = 1$)	0	1	0	0	0	1
Multicystic ($n = 1$)	1	0	0	0	1	0

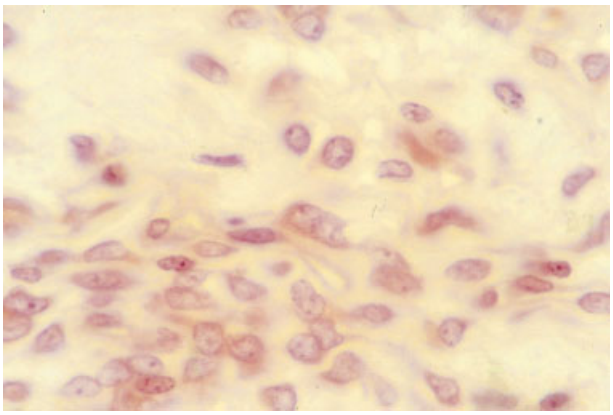

Fig. 6. Desmoplastic region from sarcomatoid MM, without positivity for VEGF-3-VEGFR-3 (×400). [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

behavior of this group of neoplasias as previously demonstrated in the literature.[2,3]

Additionally, recent publications describing the expression of lymphatic endothelial cell markers by the mesothelium constitute an exciting trend to be explored.[18–20] Nonetheless, we did not investigate the VEGFR-3 expression with the purpose to add one more marker to the already extensive panel aiming to distinguish MM from its mimics. Our goal was to ascertain the VEGFR-3 expression in MM, including its different subtypes, in order to establish the relationship between tumor differentiation and this marker. This is of interest because VEGF-C and its receptor VEGFR-3 are coexpressed in mesothelial cell lines. VEGF-C acts as a potent mitogen, which stimulates lymphangiogenesis. In addition, a functional VEGF-C autocrine growth loop exists in mesothelial cells and is considered a promising therapeutic target.[29,30] However, it is well known that the specificity of various markers in malignant MMs should be assessed according to histological subtypes because the antigenicity can be severely affected by heterogeneity and dedifferentiation, consequent to overexpression or underexpression of certain antigens.[4,11]

Molecular signaling pathways in MM are nowadays a complex and motivating area of research with clear usefulness in routine conditions. The rising incidence and consistent aggressiveness of MM needs urgent improvement to patients' therapy. The available staging scores have limited the prognostic value of these molecular markers, and the main application of these molecular systems, e.g., the selection of patients for clinical trials.[31] This is the most compelling reason to study alternative options linked to molecular pathways, such as research on VEGF family, including KDR; heparanase (HPSE-1), basic fibroblastic growth factor (bFGF), epidermal growth factor receptor, cell cycle control proteins, insulin growth factor, cyclooxyenase-2, and other molecules. These studies should provide rationale for better informed MM therapeutic decisions in the near future as is already the case in more common neoplasms, such as breast carcinoma.[32]

Our results have corroborated, in part, the observations of Ando et al. who demonstrated the similarities between mesothelial and endothelial cells with regards to their antigenicity.[17] The usefulness of this parallel immediately endorsed its application in routine conditions to optimize the discrimination between MM and adenocarcinoma, a puzzling point in daily routine of surgical pathology practice.[15–17] More than the differential diagnostic approach, the correspondence between mesothelium-originated malignant neoplasia and their LV in regards to endothelial molecular markers may provide a useful target for novel cancer therapies.[19] Indeed, this would be a welcome development, given MM's grim prognosis and the dismal results with existing treatment protocols, based on various combinations of surgery, radiation and chemotherapy.

MM is one of numerous malignant neoplasms where VEGF receptors' expression has been demonstrated.[16,23] Our results further reinforce these observations, since VEGFR-3 was identified in most cases of MM independently of the histological subtype. We recently reported the expression of VEGFR-3 in lymphatic and blood vessel cells, and myoepithelial cells from ductal invasive breast carcinoma, but not in malignant cells.[26,32] This is quite provoking, because it reinforces the hypothesis that VEGFR-3 signaling could act by a paracrine and also an autocrine pathways in MM.[30] Moreover, these previous reports are in accordance with the present finding of absence of VEGFR-3 expression in all cases of metastatic carcinomas studied as controls. Although not a purpose of our study, elucidation of common factors inducing lymphangiogenesis separately or in combination with micro-

vasculature angiogenesis, seems to be another emerging area, ripe for investigation. In conclusion, the demonstration that VEGFR-3 expression in MM is not restricted to the epithelioid variant, but it is expressed also in sarcomatoid and biphasic types, renders VEGFR-3 as a potential therapeutic target in this very aggressive human cancer, thus justifying its further investigation.

References

1. Mutsaers SE. The mesothelial cell. Int J Biochem Cell Biol 2004; 36:9–16.
2. Robinson BWS, Richard AL. Advances in mesothelioma 2005; 353:1591–1603.
3. Sterman DH, Albelda SM. Advances in the diagnosis, evaluation, and management of malignant pleural mesothelioma. Respirology 2005;10:266–283.
4. Bedrossian CWM. Introduction: Malignant mesothelioma: New frontiers, more questions. Sem Diagn Pathol 2006;23:1–4.
5. Roberts F, McCall AE, Burnett RA. Malignant mesothelioma: A comparison of biopsy and postmortem material by light microscopy and immunohistochemistry. J Clin Pathol 2001;54:766–770.
6. Bedrossian CW. Diagnostic problems in serous effusions. Diagn Cytopathol 1998;19:131–137.
7. Baker PM, Clement PB, Young RH. Malignant peritoneal mesothelioma in women. Am J Clin Pathol 2005;123:724–737.
8. Miettinen MM, Sarlomo-Rikala M. Expression of calretinin, thrombomodulin, keratin 5, and mesothelin in lung carcinomas of different types: An immunohistochemical analysis of 596 tumors in comparison with epithelioid mesotheliomas of the pleura. Am J Surg Pathol 2003;27:150–158.
9. Llinares K, Escande F, Aubert S, et al. Diagnostic value of MUC4 immunostaining in distinguishing epithelial mesothelioma and lung adenocarcinoma. Mod Pathol 2004;17:150–157.
10. Trupiano JK, Geisinger KR, Willingham MC, Manders P, Zbieranski N, Case D, Levine EA. Diffuse malignant mesothelioma of the peritoneum and pleura, analysis of markers. Mod Pathol 2004; 17:476–481.
11. Bedrossian CWM. Special stains; the old and the new- impact of immunocytochemistry in effusion cytology. Diagn Cytopathol 1998;18:141–149.
12. Ponjanski N, Crote HJ, Doganay P, Schmiemann V, Buckstegge B, Bocking A. Immunocytochemical identification of carcinomas of unknown primary in serous effusions. Diagn Cytopathol 2005;33: 309–315.
13. Politi E, Kandaraki C, Apostolopoulou C, Kyritsi T, Koutselini H. Immunocytochemical panel for distinguishing between carcinoma and reactive mesothelial cells in body cavity fluids. Diagn Cytopathol 2005;32:151–155.
14. Hartmann CA, Schultze H. Mesothelioma-like tumors of the pleura: A review of 72 autopsy cases. Cancer Res Clin Oncol 1004;120: 331–347.
15. Kassis J, Klominek J, Kohn E. Tumor Microenvironment: What can effusions teach us? Diagn Cytopathol 2005;33:316–319.
16. Ohta Y, Shridhar V, Bright RK, et al. VEGF and VEGF type C play an important role in angiogenesis and lymphangiogenesis in human malignant mesothelioma tumours. Br J Cancer 1999;81:54–61.
17. Ando T, Jordan P, Wang Y, Harper MH, Houghton J, Elrod J, Alexander JS. Homogeneity of mesothelial cells with lymphatic endothelium: Expression of lymphatic endothelial markers by mesothelial cells. Lymphat Res Biol 2005;3:117–125.
18. Ordonez NG. D2-40 and podoplanin are highly specific and sensitive immunohistochemical markers of epithelioid malignant mesothelioma. Hum Pathol 2005;36:372–380.
19. Chu AY, Litzky LA, Pasha TL, Acs G, Zhang PJ. Utility of D2-40, a novel mesothelial marker, in the diagnosis of malignant mesothelioma. Mod Pathol 2005;18:105–110.
20. Kimura N, Kimura I. Podoplanin as a marker for mesothelioma. Pathology Int 2005;55:83–86.
21. Davidson B, Reich R, Lazarovici P, et al. Expression of the nerve growth factor receptors TrkA and p75 in malignant mesothelioma. Lung Cancer 2004;44:159–165.
22. Pass HI, Robinson BW, Testa JR, Carbone M. Emerging translational therapies for mesothelioma Chest 1999;116:455S–460S.
23. Masood R, Kundra A, Zhu S, Xia G, Scalia P, Smith DL, Gill PS. Malignant mesothelioma growth inhibition by agents that target the VEGF and VEGF-C autocrine loops. Int J Cancer 2003;104:603–610.
24. Bedrossian C, Bonsib S, Moran C. Differential diagnosis between mesothelioma and adenocarcinoma: A multimodal approach on ultrastructure and immunocytochemistry. Semin Diagn Pathol 1992; 9:124–140.
25. Travis JNWD, Müller-Hermelink HK, Harris CC, Brambilla E. Pathology and Genetics of Tumours of the Lung, Pleura, Thymus and Heart. Lyon, France: IARC Press; 2004.
26. Longatto Filho A, Martins A, Costa SMA, Schmitt FC. VEGFR-3 expression in breast cancer tissue is not restricted to lymphatic vessels. Pathol Res Pract 2005;201:93–99.
27. Zebrowski BK, Yano S, Liu W, et al. Vascular endothelial growth factor levels and induction of permeability in malignant pleural effusions. Clin Cancer Res 1999;5:3364–3368.
28. Davidson B, Vintman L, Zcharia E, et al. Heparanase and basic fibroblast growth factor are co-expressed in malignant mesothelioma. Clin Experiment Metastasis 2004;21:469–476.
29. Hess-Stumpp H, Haberey M, Thierauch KH. PTK 787/ZK 222584, a tyrosine kinase inhibitor of all known VEGF receptors, represses tumor growth with high efficacy. Chembiochemistry 2005;6:550–557.
30. Strizzi L, Catalano A, Vanalo G, et al. Vascular endothelial growth factor is an autocrine growth factor in human malignant mesothelioma. J Pathol 2001;193:468–475.
31. Kumar P, Kratzke RA. Molecular prognostic markers in malignant mesothelioma Lung Cancer 2005;49 (Suppl 1):S53–S60.
32. Schmitt FC, Longatto Filho A. Controversial insights for lymphangiogenesis in breast cancer. J Clin Pathol 2004. e.Letter online.