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Immunohistochemical investigation of selected endothelial markers in pulmonary epithelioid haemangioendothelioma

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Epithelioid haemangioendothelioma (EH) is a very rare neoplasm. It is assumed that these tumours derive from vascular endothelial cells. The aim of the study was to identify the immunohistochemical profile of tumour cells in lung EH. The unique material consisting of eight cases of lung EH was collected and examined by immunohistochemistry with three antibodies: CD-31, CD105, both marking vascular endothelial cells, and D2-40, marking lymphatic endothelial cells.
In all cases, the cytoplasm of tumour cells showed widespread expression of CD-31. Reaction with CD-105 antibody gave a positive result mainly in the cytoplasm of tumour cells located at the periphery of tumours, especially in highly cellular neoplasms containing spindle cells. Reaction with D2-40 antibody in most cases was negative. The presence of a few capillary vessels with positive endothelial cells was revealed in two cases at the periphery of the tumour. Only in one case of a highly cellular tumour did a small portion of spindle cells show a positive reaction to D2-40.
The above studies confirmed the origin of EH mainly from vascular endothelial cells. Medications inhibiting vascular endothelial growth factors can be considered as a treatment option for multifocal EH that does not qualify for surgical intervention.

Key words: epithelioid haemangioendothelioma, lung, immunohistochemistry, anti-vascular treatment.

Introduction

Pulmonary epithelioid haemangioendothelioma (PEH) is a very rare pulmonary neoplasm. Usually it presents as multiple foci. A large proportion of these cases involve young women and a protracted development, usually over the course of many years, is characteristic [1, 2].

Some authors have divided PEH into a less malignant and a more malignant variant. The less malignant form of PEH has a protracted course. Histologically it is a paucicellular neoplasm with extensive areas of hyaline degeneration and occasional calcifications. Proliferating cells that are mainly found at the periphery of the neoplasm have an epithelial appearance and show evidence of vacuolization, which corresponds to early vessel formation (Fig. 1). The more malignant form is a highly cellular neoplasm with greater atypia and less extensive degenerative changes. This form typically follows a more rapid course, and pleural infiltration is frequent. Besides epithelioid cells, spindle cells may also be found in the more malignant variant of PEH. The presence of these two cell types in distinctly neighbouring zones can be confirmed in some neoplasms of the more malignant type (Fig. 2).

The confirmation of the vascular endothelial origins of PEH presents an opportunity for developing medications targeted and inhibiting vascular endothelial growth factors.

Materials and methods

The material for the study consisted of eight cases of PEH. Five patients were female and three male. Six

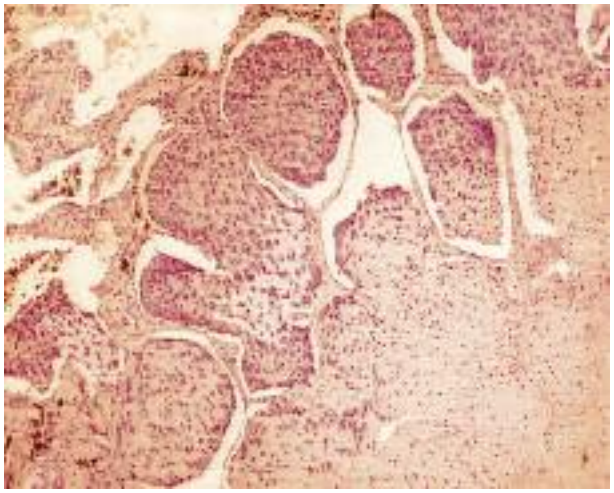


Fig. 1. Pulmonary epithelioid haemangioendothelioma with extensive hyaline degeneration. The tumour infiltrates the lung parenchyma without destroying its structure. (HE stain, 10×)

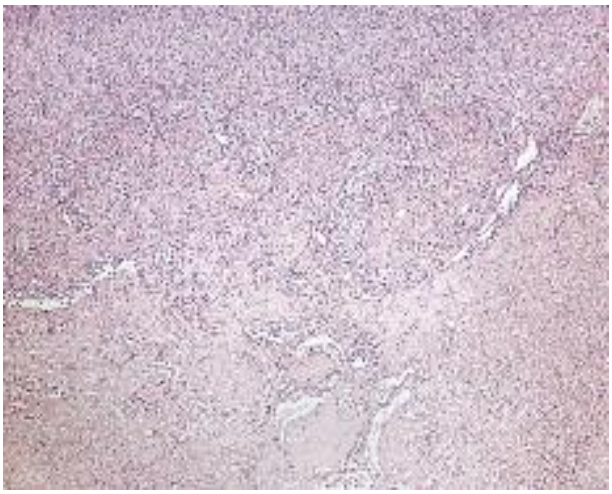


Fig. 2. Singular tumour infiltrating lung tissue in a similar manner as Fig. 1. The tumour is highly cellular, with multiple spindle cells. (HE stain, 10×)

of the cases presented as multiple foci; the remaining two cases were single lesions (Table I). A part of this material was previously described [2].

The aim of the study was to examine the expression of endothelial markers in PEH using immunohistochemical methods. Three antibodies were used: CD-31 and CD-105, both marking vascular endothelial cells, and D2-40, marking lymphatic endothelial cells (Table II).

The tumour tissue was fixed in formalin, routinely processed and embedded in paraffin. From these, 4 μm sections were prepared. A representative section from each tumour was selected for immunohistochemical investigation. The immunohistochemistry was performed by the standard method. Briefly, the slides were dewaxed, rehydrated and incubated in 3% peroxide solution for 10 minutes to block the endogenous peroxidase activity. Antigen retrieval was carried out by microwaving in citrate buffer (for CD-105 and D2-40) and EDTA (for CD-31) for 5 minutes at 700 W, then for 5 minutes at 600 W. The primary antibodies used are listed in Table II. The Lab-Vision detection system was used.

Table I. Clinicopathological features of PEH

No.	Histological No.	Sex	Age	Clinical Findings	CD-31	CD-105	D2-40
1.	1719424	F	34	multiple, bilateral tumours	+++	++	negative
2.	133271/133246	M	57	single tumour in upper left lobe	+++	++	positive in single spindle cells
3.	105302	F	19	multiple, bilateral tumours	+++	++	single vessels positive
4.	1515641	F	44	multiple, bilateral tumours	+++	++	negative
5.	122735	F	65	multiple, bilateral tumours	+++	++	negative
6.	927128	F	18	multiple, bilateral tumours	+++	++	negative
7.	187048	M	33	multiple, bilateral tumours	+++	++	negative
8.	1457626	M	75	single tumour in upper left lobe	+++	++	single vessels positive

Table II. Primary antibodies used for immunohistochemical reactions

Antibody	Manufacturer	Pre-Treatment	Dilution/Incubation Time	Detection Method
CD-31	DAKO	EDTA, pH = 8	1 : 20 (60 min)	Lab-Vision
CD-105	Novocastra	citrate buffer, pH = 6	1 : 50 (60 min)	Lab-Vision
D2-40	Polgen	citrate buffer, pH = 6	"ready to use" (30 min)	Lab-Vision

Results

In all cases, the cell membrane and cytoplasm of tumour cells showed strong and widespread expression of CD-31. Areas of hyaline change and calcification were negative for CD-31 (Fig. 3 and 4). The reaction for CD-105 gave a positive result mainly in the cytoplasm of neoplastic cells located at the periphery of tumours, especially in highly cellular neoplasms containing spindle cells, as well as in the endothelium of newly formed vessels, and was similar to CD-31 (Fig. 5 and 6). Areas of hyalinization and calcification were negative in CD-105.

The reaction with D2-40 antibody in most cases was negative. A few capillary vessels positive for D2-40 at the periphery of the tumour were present in two cases. We cannot rule out the possibility that some of them were remnants of normal pulmonary vessels (Fig. 7). In one case of a highly cellular tumour in a 57-year-old male (case no. 2) a small portion of spindle cells showed a positive reaction for D2-40 (Fig. 8).

Discussion

CD-31 antigen is present in the cell membrane of vascular endothelium in vessels and is characterized by linear endothelial staining. It promotes endothelial and leukocyte adhesion in the vascular endothelium. CD-31 is also present in neoplasms derived from endothelial cells [3, 4]. Among neoplasms of vascular origin spindle cells found in Kaposi sarcoma represent an exception, as they do not react with CD-31 antibody [3]. In addition, megakaryocytes, platelets, cells of myeloid origin, as well as lymphocytes and their precursors may show a heterogeneous immunoreaction for CD-31 [4, 5]. Currently, this marker is used for investigating

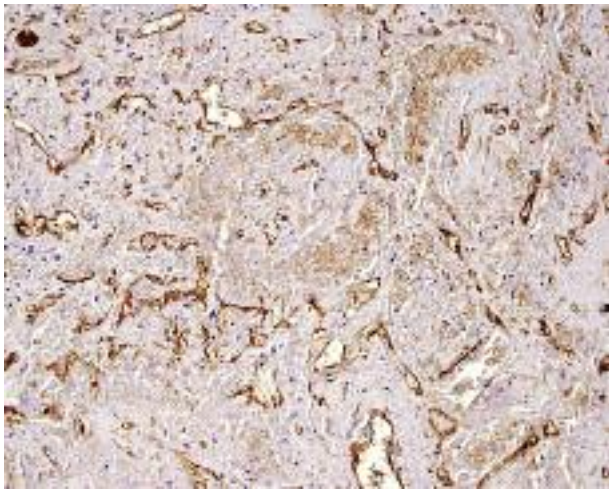


Fig. 3. Immunohistochemical reaction with CD-31 antibody: positive in majority of neoplastic cells. Areas of hyaline degeneration are negative. Vacuoles corresponding to early capillary formation are visible

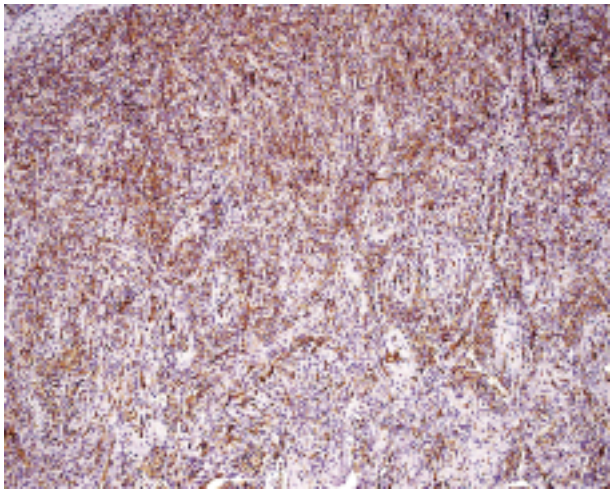
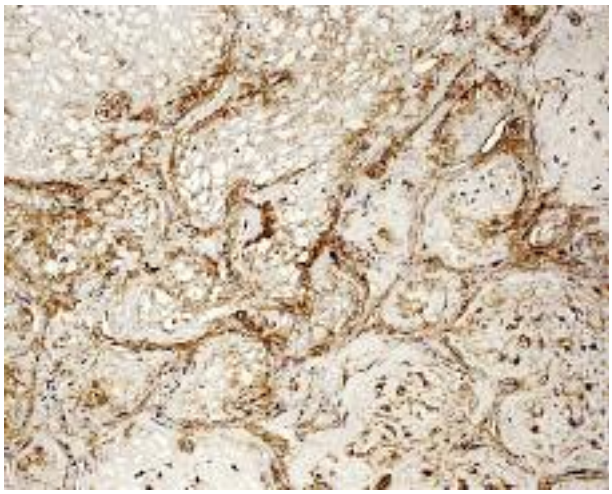
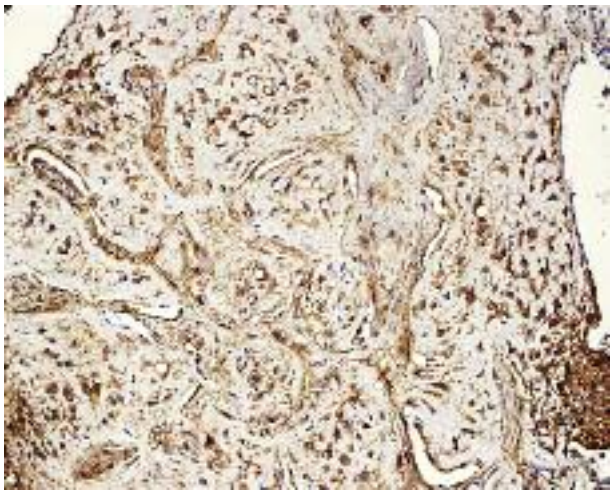


Fig. 4. Immunohistochemical reaction with CD-31 antibody: part of tumour consisting of multiple spindle cells positive for CD-31





Figs. 5, 6. Immunohistochemical reaction with CD-105 antibody similar to CD-31 in a biopsy of a classical PEH. Cells at the periphery of the tumour are strongly positive. Areas of hyaline change are negative

angiogenesis in neoplastic processes [6]. CD-105 (endoglin) belongs to a family of integrins involved with adhesion receptors found in capillaries, arterioles and venules. It is preferentially expressed on endothelia of newly formed vessels, and may thus be used in neoangiogenesis investigation. This is in accordance with our results, as CD-105 positive cells were seen at the periphery of the lesions, supposedly their growing front. Reactions for the aforementioned vascular antigens are thought to be highly specific, staining for certainly more than reactions for CD-34 and von Willebrand factor used in the past.

A positive reaction with CD-31 antibody in all cases of PEH neoplasms tested in this study confirms their relationship to endothelium. Other authors have also shown the endothelial quality of such neoplasms using various immunohistochemical and ultrastructural methods [1, 7-9]. This theory is supported by the epithelial appearance of the tumour cells. However, the sporadic presence of cytokeratin immunoreactivity that has been described within cells of some PEH tumours can be the source of diagnostic errors [3].

D2-40 is an antibody directed against M2A oncofetal antigen, present on the endothelia of lymphatic vessels [10-13]. The majority of our samples were D2-40 negative; a slight positive reaction in the vessels at the border of two tumours may have represented residual lymphatics from the proper lung parenchyma. The singular case of an anaplastic tumour showed the presence of spindle cells which were weakly positive for D2-40. Pleural invasion was also observed in this case. It is worth mentioning here that PEH neoplasms of the more malignant type, especially those with pleural infiltration, have spindle cells similar to those seen in malignant mesothelioma. The presence of such cells represents a potential source of diagnostic confusion, especially on examination of small pleural biopsies. Interestingly, mesothelium also shows D2-40 reactivity. Furthermore, D2-40 is used for the study and diagnostic workup of mesothelium, as well as neoplastic processes of mesothelial origin [14]. Khan *et al*. confirmed the presence of D2-40 in angiosarcoma and Kaposi sarcoma [11].

In conclusion, PEH is a rare, usually multifocal neoplasm that may be found in the lungs. Such multiplicity, along with a good clinical status of those affected, is the basis of the unsettled debate whether PEH is a primary, multifocal neoplasm or rather the lung metastasis of tumours located in other organs (mainly the liver). Currently, most authors suggest the metastatic theory of origin of multiple PEH. The bilateral and multifocal presence of these tumours in patients excludes the possibility of surgical intervention. Primary, unifocal PEH neoplasms are seldom described – in these cases the possibility of successful surgical treatment exists. Taking into account the results of the present study, and the conclusion that the pulmonary epithelioid haemangioendotheliomas are of blood vessel endothelial and not lymphatic endothelial origin, medications inhibiting vascular endothelial growth factor can be considered as a treatment option for multifocal PEH that does not qualify for surgical intervention. A report of chemotherapy with an anti-VEGF agent (bevacizumab) in primary pleural epithelioid haemangioendothelioma recently appeared [15]. Although that antivascular treatment in the two cases described so far was not successful, this approach warrants further investigation.

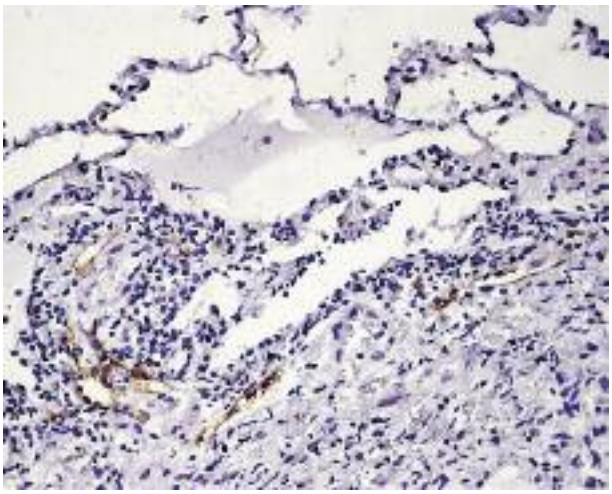


Fig. 7. Reaction with D2-40 antibody: only a few, small vessels at the periphery of the tumour show a positive reaction in the cytoplasm of cells forming their walls. These may be lymphatic vessels of residual lung parenchyma

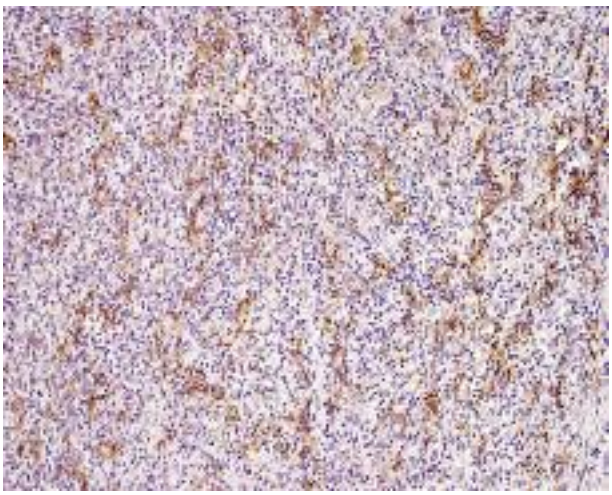


Fig. 8. Positive reaction for the presence of D2-40 in some cells of the tumour invading pleura

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