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Lymphangioleiomyomatosis—presence of receptor tyrosine kinases and the angiogenesis factor VEGF-A as potential therapeutic targets

Lymphangioleiomyomatosis (LAM) is a rare systemic disorder in women occurring either sporadically (sporadic LAM) or in association with tuberous sclerosis (TS-LAM). It is caused by proliferating smooth muscle-like LAM cells, which lead to a progressive cystic destruction of the lungs and abdominal tumours (renal angiomyolipomas and/or axial lymph node lesions). LAM cells express receptors for oestrogen and progesterone and stain positive for HMB-45, an antibody against the melanoma-related antigen.[1] LAM fulfils the criteria of a neoplastic disease with enhanced proliferation,[2] metastasising processes,[3] increased migratory activity and invasiveness of LAM cells.[4] Currently, an effective treatment interfering with these processes does not exist. Growth factors such as platelet-derived growth factor (PDGF) and epidermal growth factor (EGF) have been identified to enhance LAM and renal angiomyolipoma cell proliferation in vitro.[2 5] Whether LAM cells express growth factor-associated receptor tyrosine kinases and the angiogenesis factor vascular endothelial growth factor-A (VEGF-A), which represent promising targets of small-molecule and antibody therapy in neoplastic diseases, is currently unknown.

We studied immunohistochemically the expression of the following proteins by LAM cells in 10 formalin-fixed and paraffin-embedded LAM specimens: epidermal growth factor receptor (EGFR; PharmDx Kit, Dako, Hamburg, Germany), platelet-derived growth factor receptor α (PDGFR-α; rabbit polyclonal, Dianova, Hamburg, Germany), human epidermal growth factor receptor-2 (HER2; HercepTest, Dako), VEGF-A (clone VG1 identifying the VEGF-A isoforms $VEGF_{121}$, $VEGF_{165}$ and $VEGF_{189}$, DCS, Hamburg, Germany) and c-KIT (CD117; rabbit polyclonal, Dako). Staining procedures were carried out according to the manufacturer's instructions, and appropriate positive and negative controls were used. A semiquantitative scoring system of the immunohistochemical reactions for all receptor tyrosine kinases, the hormone receptors and VEGF-A was applied as follows: negative, no reaction or percentage of positive cells <5%; 1, 5–25% positive cells; 2, 26–50% positive cells; 3, 51–75% positive cells; 4, >75% positive cells; +, weak staining intensity; ++, moderate staining intensity; +++, strong staining intensity. Histological severity of lung destruction was assessed using the LAM histological score.[6] The assessment of the LAM histological score and the immunohistochemical stainings was performed independently by two histopathologists (KE and MA). Only morphologically clear-cut, HMB-45 positive LAM lesions (nodules, cysts and diffuse LAM cell proliferations) were taken for analysis. All final decisions were made by consensus. Additionally, EGFR gene copy number per LAM cell nucleus was investigated by one histopathologist (SL) using fluorescence in situ hybridisation (FISH; LSI EGFR SpectrumOrange/CEP 7 SpectrumGreen probe, Vysis, Abbott Laboratories, Wiesbaden, Germany). The study was approved by the local ethics committee and written informed consent was obtained from all participants or their close relatives.

In all specimens, LAM lesions were consistently positive for PDGFR-α and VEGF-A. EGFR-positive LAM cells were observed in seven specimens. No amplification or higher polysomy of the EGFR gene was detected. In addition to c-KIT-positive mast cells, which were sporadically present in LAM lesions and the surrounding lung tissue, LAM cells themselves were found to be positive for c-KIT in six of the specimens. HER2 was negative in all specimens (fig 1). For details, see supplementary table available online at http://www.thorax.bmjjournals.com/supplemental.

We demonstrated that PDGFR-α, EGFR, c-KIT and VEGF-A as targets of currently available compounds are expressed by LAM cells. These findings imply further research in the field of small-molecule and antibody therapy in LAM.

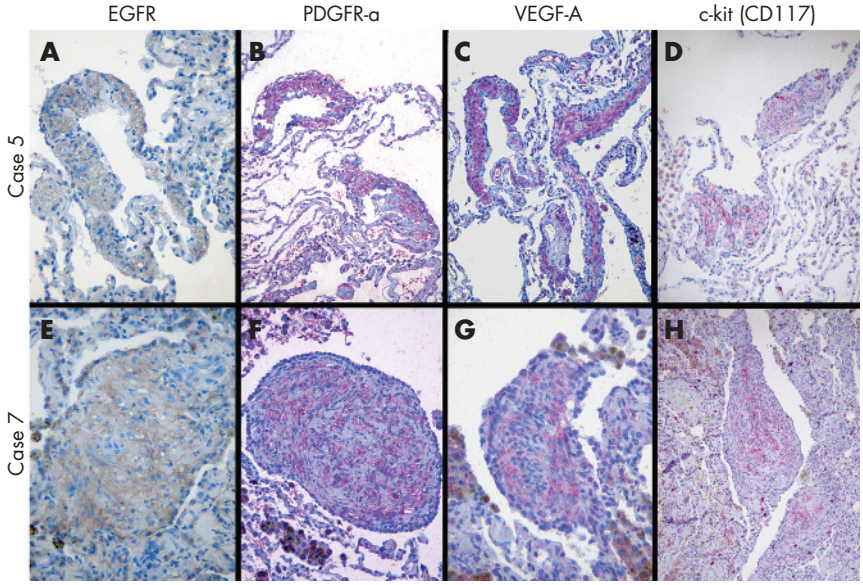

Figure 1 Expression of epidermal growth factor receptor (EGFR), platelet-derived growth factor receptor α (PDGFR-α), vascular endothelial growth factor-A (VEGF-A) and c-KIT (CD117) in lung lymphangioleiomyomatosis (LAM) lesions. The panel shows two pulmonary LAM specimens (case 5, A–D; case 7, E–H). Case 5 (A–D) represents predominant cystic and diffuse proliferating LAM lesions, whereas case 7 (E–H) represents predominant nodular growth pattern. The cases show a variable expression of EGFR, PDGFR-α, VEGF-A and c-KIT (CD117).

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Table 1

Study population and results of immunohistochemistry (IHC) and fluorescence in situ hybridisation (FISH; EGFR gene only)

Case-No	Subtype	Organ	LHS	OR*	PR*	VEGF-A*	PDGFR-α*	CD117*	HER2*	EGFR-IHC*	EGFR-FISH
1	S-LAM	Lung	2	4 +	1 +	4 ++	4 ++	negative	negative	negative	disomy
2	S-LAM	LALM	n. a.	1+	1+	4 +	4 ++	4 +	negative	4 +	no results
3	TS-LAM	Pleura	n. a.	1 +	2 ++	4 ++	4 ++	negative	negative	negative	disomy
4	TS-LAM	Lung	2	1 +	4 ++	4 +	2 ++	negative	negative	negative	disomy
5	S-LAM	Lung	1	1 +	1 ++	4 +	3 +++	4 ++	negative	4 +	no results
6	S-LAM	Lung	2	2 +	4 ++	4 +	2 ++	4 +++	negative	1 +	monosomy
7	TS-LAM	Lung	1	4 +	3 +	2 +	2 +	3 ++	negative	2 +	trisomy
8	S-LAM	Lung	1	2 +	4 ++	4 +	1 +	negative	negative	1 +	disomy
9	S-LAM	Lung	3	2 ++	1 +	4 +	4 ++	4 ++	negative	1 +	disomy
10	S-LAM	LN	n. a.	4 +	1 +	4 ++	4 ++	4 +	negative	1 +	tetrasomy

S-LAM, sporadic LAM; TS-LAM, tuberous sclerosis associated LAM; LALM, lymphangioleiomyoma; LN, lymph node; LHS, pulmonary LAM histologic score ranging from 1 to 3; OR, oestrogen receptor; PR, progesterone receptor; VEGF-A, vascular endothelial growth factor A; PDGFR-α, platelet derived growth factor receptor alpha; CD117, c-KIT receptor; HER2, human epidermal growth factor receptor-2; EGFR, epidermal growth factor receptor;

*semiquantitative scoring of immunohistochemical reactions (percentage positive cells and staining intensity); negative, no reaction or percentage positive cells < 5 %; 1, 5 % to 25 % positive cells; 2, 26 % to 50 % positive cells; 3, 51 % to 75 % positive cells; 4, > 75 % positive cells; +, weak staining intensity; ++, moderate staining intensity; +++, strong staining intensity;

n. a., non applicable