MEGA JOURNAL OF CASE REPORTS

Case Presentation

Compiled Date: May 05, 2023

MICAL2 is Expressed in Lung Cystic Nodules of Lymphangioleiomyomatosis

Ivana Barravecchia PhD[1], Angela Pucci MD, PhD[2], Laura Tavanti MD[3], Gabriella Fontanini MD[4], Nefele Giarratana PhD[5], Maurilio Sampaolesi PhD[5] and Debora Angeloni PhD[1*]

[1]Scuola Superiore Sant'Anna, The Institute of Biorobotics, 56124 Pisa, Italy

[2]U.O.C. Anatomia Patologica, Azienda Ospedaliera Universitaria Pisana, 56100 Pisa, Italy

[3]U.O. Pneumologia, Dipartimento di Patologia Chirurgica, Medica, Molecolare e di Area Critica e Azienda Ospedaliero-Universitaria Pisana, 56100 Pisa, Italy

[4]Sezione di Anatomia Patologica, Dipartimento di Patologia Chirurgia, Medica, Molecolare e di Area Critica, University of Pisa, 56100 Pisa, Italy

[5]Department of Development and Regeneration, Stem Cell Institute, KU Leuven. B-3000,Leuven, Belgium

[*]**Corresponding author**: Debora Angeloni, Scuola Superiore Sant'Anna, The Institute of Biorobotics, Via G. Moruzzi, 1 - 56124 Pisa, AND Health Science Interdisciplinary Center, Scuola Superiore Sant'Anna, Via G. Moruzzi, 56124 Pisa, Italy, Tel: 39 050 315 3092; Fax: 39 050 315 2166; E-mail: angeloni@santannapisa.it

Abstract

Background: Lymphangioleiomyomatosis (LAM) is a rare multisystem disorder affecting about 1/500,000-1/125,000 adult, pre-menopausal women in Europe, characterized by cystic lung destruction and extrapulmonary disease comprising intra-abdominal angiomyolipomas, lymphatic tumors such as lymphangioleiomyomas, and chylous effusions. LAM is a low-grade, invasive and metastasizing neoplasia consisting of cells showing smooth muscle (SM) and melanocytic differentiation. MICAL2 is a cytosolic enzyme with redox, actin-modifying function, evolutionarily conserved in differentiation and function of smooth, cardiac and skeletal muscle cells. Previously we showed that MICAL2 essential for myogenic lineage commitment.

Methods: We report a 32-year-old Caucasian, smoker woman admitted to the emergency room because of sudden-onset dyspnea, with diagnosis of LAM. With Immunohistochemistry method, we analyzed the presence of sound and new molecular markers of LAM.

We also provide a mini-review of the literature on LAM.

Results: We show for the first time that MICAL2, a pro-metastatic and pro-angiogenic protein, recently

discovered to be essential for muscle cells differentiation, is expressed in LAM nodular cysts of the lung, at an early stage.

Conclusion: Our hypothesis-driven study identified an interesting potential new biomarker of LAM for early diagnosis, and possibly a target for early therapeutic approach of LAM. Since the study was performed on a single case of the rare disease, further research is needed to extend and validate the result.

Keywords: Lymphangioleiomyomatosis; MICAL2

Abbreviations: CT: Computed Tomography; HMB45: Human Melanoma Black 45; LAM: Lymphangioleiomyomatosis; PAH: Pulmonary Arterial Hypertension; PASMC: Pulmonary Arterial SM Cell; PCNA: Proliferating Cell Nuclear Antigen; PEComas: Perivascular Epithelioid Cell Tumor Family Members; SM: Smooth Muscle: Molecule Interacting with CasL 2; Microtubule Associated Monooxygenase, Calponin And LIM Domain Containing 2; TSC1: Tuberous Sclerosis Complex Gene 1; TSC2: Tuberous Sclerosis Complex Gene 2; VEGF: Endothelial Growth Factor

Introduction

Lymphangioleiomyomatosis (LAM) is a rare multisystem disorder affecting about 1/500,000- 1/125,000 adult, pre-menopausal women in Europe (ORPHA: 538, https://www.orpha.net/consor/cgi-bin/index.php), characterized by cystic lung destruction and extrapulmonary disease comprising intra-abdominal angiomyolipomas, lymphatic tumors such as lymphangioleiomyomas, and chylous effusions. LAM was recently reclassified as a low-grade, invasive and metastasizing neoplasia [1] consisting of cells showing Smooth Muscle (SM) and melanocytic differentiation [1]. Histologic features consist of nodular LAM cell proliferation associated to thin-walled cysts in the lungs. The nodular component comprises small, spindle- shaped central LAM cells, and a peripheral rim of epithelioid LAM cells. Both cell types react with antibodies against SM antigens such as α-actin, vimentin, and desmin. The epithelioid cells react also with anti-melanocytic antigen HMB45 [1]. Spindle-shaped cells also react to anti-Proliferating Cell Nuclear Antigen (PCNA) antibodies. In patients with declining lung function the modern treatment with mTORC1 inhibitors sirolimus or everolimus has made chronic a previously fatal disease. However, new diagnostic and prognostic tools, and treatment options are strongly needed for patients who experience disease progression despite mTOR inhibition [1]. MICAL2 (Microtubule Associated Monooxygenase, Calponin and LIM Domain Containing 2, HGNC) is a cytosolic enzyme with redox, actin-modifying function that regulates cell shape, adhesion and motility [2]. MICAL2 has an evolutionarily conserved role in differentiation and function of smooth, cardiac and skeletal muscle cells. Drosophyla MICAL is necessary for muscle organization, larval movements and fly [3,4]. In zebrafish embryo, MICAL2 is expressed in cardiomyocites and somites, i.e., mesodermal structures that will yield muscles later in development [5]. MICAL2 was found up-regulated among ten functionally linked genes involved in disease evolution of a mouse model of Duchenne muscular dystrophy [6]. MICAL2 is expressed in human Pulmonary Arterial SM Cells (PASMCs) that have cancer-like phenotype in Pulmonary Arterial Hypertension (PAH), a rare, progressive lung disease. In PASMCs, cell proliferation is inhibited by miR-205-5p that specifically targets MICAL2 ([7] and references therein). Previously, we showed that MICAL2 is expressed in vascular SM cells and in cardiac myocytes [2,8]. We also showed that MICAL2 essential for myogenic lineage commitment [9]. The histopathologic features of LAM prompted us to test whether MICAL2 is expressed in lung LAM nodules. With immunohistochemistry analysis, we found it highly expressed in LAM

cells.

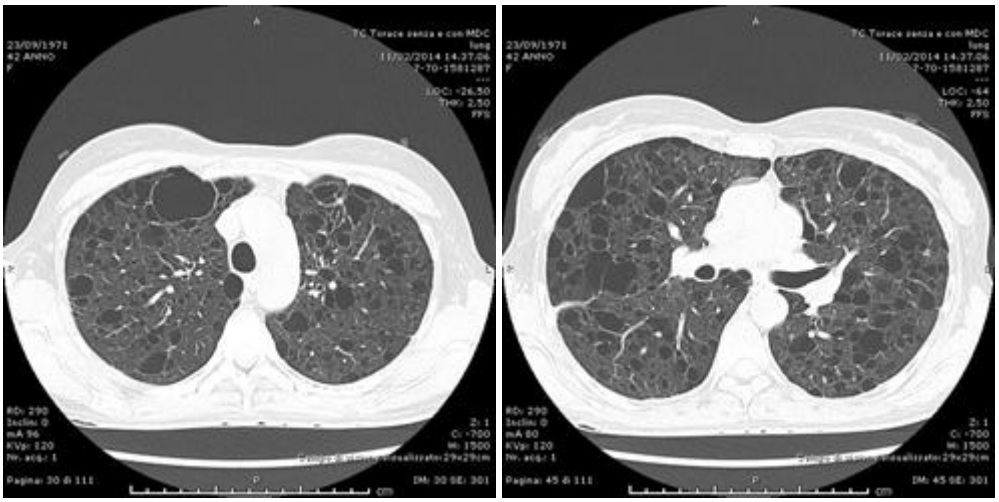
Methods

Immunohistochemistry was performed on adjacent serial sections as in **[2].** Briefly, slides were deparaffinized, rehydrated and treated with H_2O_2 before incubation with primary antibodies (rabbit polyclonal anti-MICAL2, generated in our laboratory **[2]:** anti-HMB45, anti-smooth muscle actin, Dako Laboratories). After incubation with secondary antibody (Vectastain, Vector Laboratories), immunoreactivity was revealed with 3'-5' diaminobenzidine peroxidase substrate (Thermo Scientific). Slides were counterstained (haematoxylin), and viewed under optical microscope (Olympus BX43) equipped with camera Olympus DV20. Images were analyzed with Cell Sens Dimension software.

Results

Case Presentation

A 32-year-old Caucasian, smoker woman was admitted to the emergency room because of sudden- onset dyspnea. Chest X-Ray showed a large pneumothorax on the left side and reticular micronodules on the right. She underwent Computed Tomography (CT) scan of the chest that showed bilateral, diffused bullous disease of the lung with multiple cysts **(Figure 1a-c),** angiomyolipoma in the left kidney and multiple **(Figure 1d and e),** small angiomyolipoma lesions in right kidney. The patient underwent video-assisted thoracoscopic surgery with wedge resections of a lung bleb and pleurodesis.



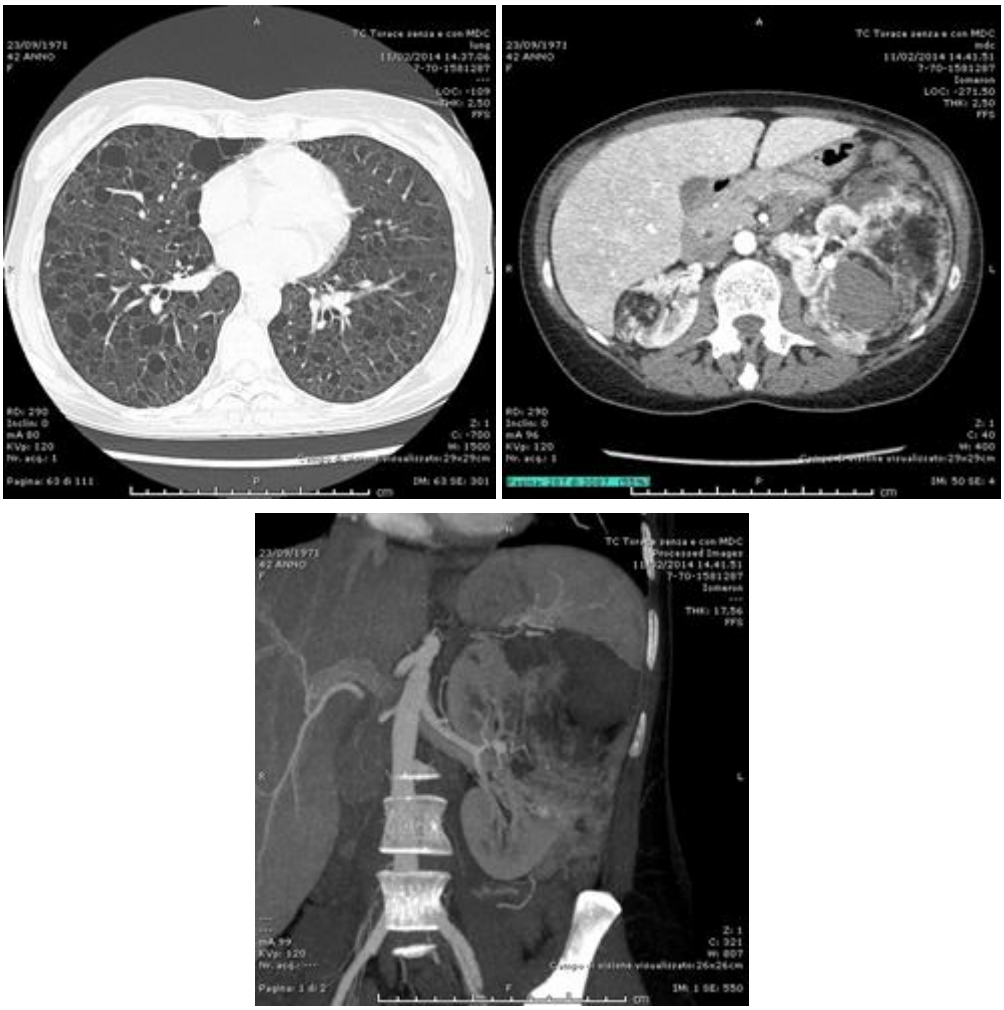


Figure 1: Computed tomography (CT) scan at presentation showed a-c): Multiple cysts, with ubiquitous distribution, within lung normal parenchyma. Signs of atypical resection are evident. d, e) Left kidney angiomyolipoma with past bleeding, along with pseudoaneurysms.

At follow up, CT of the chest and abdomen confirmed evidence of bilateral diffused bullous disease with cysts and signs of previous bleeding at the level of the left renal angiomyolipoma. In 2014 therefore the patient underwent surgery and renal artery embolization. Since 2015, the patient is on sirolimus associated to ramipril and amlodipin for systemic arterial hypertension. At two- and four-year follow-up she showed clinical, functional and radiological stability.

Immunohistochemistry analyses

Histology of the lung resection, performed and independently evaluated by two pathologists (A.P., G.F), showed small clusters and nests of SM-like cells along the alveolar walls, pulmonary blood vessels, lymphatics, and bronchioles **(Figure 1f and g).** Mitotic figures were absent or very rare. LAM cells expressed the melanocytic marker HMB-45 (Figure 1h), the immunoreactivity pattern confirming the lesion as lymphangioleiomyomatosis, belonging to the perivascular epithelioid cell tumor family members (PEComas). We found MICAL2 immunoreactivity in the HMB45- immunoreactive LAM cells **(Figure 1i-k).** Such lesions correspond to the early stage of the disease.

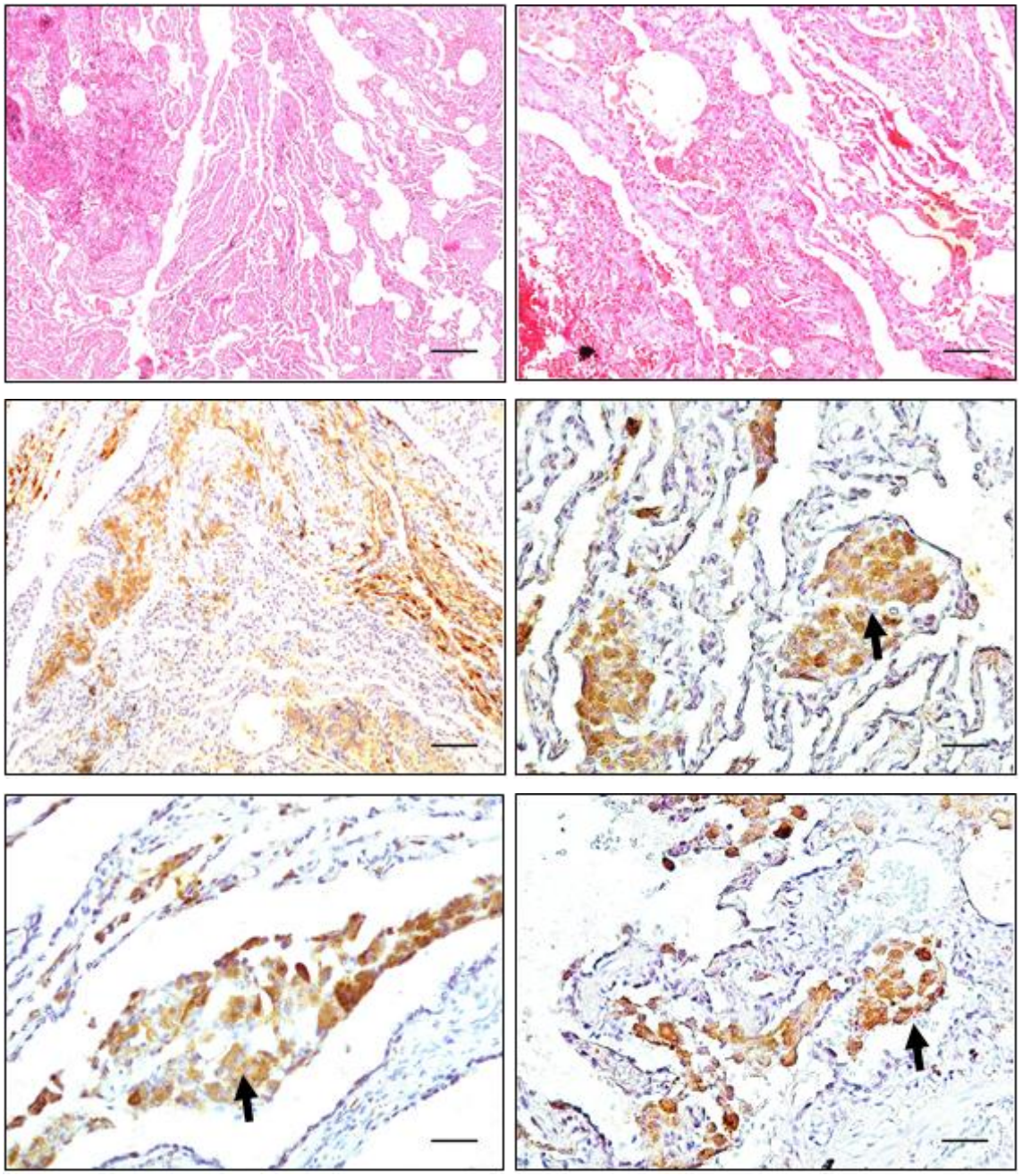


Figure 1: Histopathology sections of lung parenchyma stained with Hematoxylin/Eosin: f) 5X; g) 10X. Immune histochemistry staining with anti-HMB45: h) 10X; anti-MICAL2: i) 10X, j) 20X, k) 20X.

Discussion

Anti-Vascular Endothelial Growth Factor (VEGF) therapies are among treatment options for LAM **[1].** Interestingly, MICAL2 knock-down inhibits VEGF signaling, in vitro **[8].** Further, TSC2 mutations increase RhoA GTPase activity and cell survival **[1].** Rho GTPases influence the activation of SRF/MRTF-A-dependent gene transcription pathway, through which MICAL2 regulates epithelial to mesenchymal transition (**[1]** and references therein). Tissue inflammation might drive MICAL2 overexpression in LAM cells. Although LAM is not classified as an inflammatory disease, the high expression of inflammatory chemokines in bronchoalveolar

lavage and nodules from LAM patients strongly suggests that inflammation is involved of in its pathogenesis **[9].** Further, a TSC2-null murine LAM model showed alveolar macrophage accumulation, recruitment of immature multinucleated cells, increased induction of proinflammatory genes, nitric oxide synthase 2 and several other inflammatory cytokines and chemokines **[10].** Finally, proliferation of both LAM cells and lymphatics commonly interest the bronchi of late-stage LAM patients, showing that chronic inflammation is a **[11]** to what observed in other cell types **[2,8],** in LAM cells such complex inflammatory context might up-regulate MICAL2 transcription, leading to cell activation, acquisition of motility and invasiveness.

Conclusion

Here, we show for the first time that MICAL2, a pro-metastatic and pro-angiogenic protein, recently discovered to be essential for muscle cells differentiation, is expressed in LAM nodular cysts of the lung, at an early stage. A specific MICAL2 inhibitor exists, CCG-1423, and others are under synthesis and characterization in our laboratory. Our study was hypothesis-driven and, although performed on a single case of the rare disease, it identified an interesting potential new biomarker of LAM for early diagnosis or a target for early therapeutic approach. Future studies will shed light on the biological role of MICAL2 in LAM, with possible important implications for future patient care of this invalidating disease.

Acknowledgments and Funding

Contributions: Conceptualization and overall supervision: D.A.; methodology: D.A., I.B., A.P.; investigation and validation, data collection, image, clinical case: I.B., A.P., L.T, G.F., N.G; writing—original draft preparation: D.A., I.B., M.S.; funding acquisition and project administration:

D.A. All authors have read and agreed to the published version of the manuscript.

DA is grateful for support of the study to Mr. and Mrs. Rosario Leonardis; to Mr. and Mrs.Vito Mingolla (A.N.I.E., Livorno, Italy); to Lions Club International, District 108LA, Toscana, Italy, in particular to Carlo Bianucci and Giovanna Ciampi, and to the following Clubs: Fiesole, Firenze Amerigo Vespucci, Firenze Arnolfo di Cambio, Firenze Brunelleschi, Firenze Cosimo de' Medici, Firenze Poggio Imperiale, Firenze Ponte Vecchio, Poggio a Caiano, Prato Curzio Malaparte; to the Associazione in Memoria di Errico Camarda, Teramo.

The authors have not any conflict of interests to declare.

References

1. McCarthy C, Gupta N, Johnson SR, Yu JJ, McCormack FX. Lymphangioleiomyomatosis: pathogenesis, clinical features, diagnosis, and management. Lancet Respir Med. 2021;9(11):1313-27.
2. Mariotti S, Barravecchia I, Vindigni C, Pucci A, Balsamo M, Libro R, et al. MICAL2 is a novel human cancer gene controlling mesenchymal to epithelial transition involved in cancer growth and invasion. Oncotarget. 2014;7(2):1808–25.
3. Beuchle D, Schwarz H, Langegger M, Koch I, Aberle H. Drosophila MICAL regulates myofilament organization and synaptic structure. Mech Dev. 2007;124(5):390–406.
4. Schnorrer F, Schönbauer C, Langer CCH, Dietzl G, Novatchkova M, Schernhuber K, et al. Systematic genetic analysis of muscle morphogenesis and function in Drosophila. Nature. 2010;464(7286):287–91.
5. Xue Y, Kuok C, Xiao A, Zhu Z, Lin S, Zhang B. Identification and expression analysis of mical family

genes in zebrafish. J Genet Genomics. 2010;37(10):685–93.

6. Marotta M, Ruiz-Roig C, Sarria Y, Peiro JL, Nuñez F, Ceron J, et al. Muscle genome-wide expression profiling during disease evolution in mdx mice. Physiol Genomics. 2009;37(2):119–32.
7. Tao W, Sun W, Zhu H, Zhang J. miR-205-5p suppresses pulmonary vascular smooth muscle cell proliferation by targeting MICAL2-mediated Erk1/2 signaling. Microvasc Res. 2019;124:43–50.
8. Barravecchia I, Mariotti S, Pucci A, Scebba F, De Cesari C, Bicciato S, et al. MICAL2 is expressed in cancer associated neo-angiogenic capillary endothelia and it is required for endothelial cell viability, motility and VEGF response. Biochim Biophys Acta - Mol Basis Dis. 2019;1865(9):2111–24.
9. Giarratana N, Conti F, La Rovere R, Gijsbers R, Carai P, Duelen R, et al. MICAL2 is essential for myogenic lineage commitment. Cell Death Dis. 2020;11(8):654.
10. Atochina-Vasserman EN, Guo C-J, Abramova E, Golden TN, Sims M, James ML, et al. Surfactant dysfunction and lung inflammation in the female mouse model of lymphangioleiomyomatosis. Am J Respir Cell Mol Biol. 2015;53(1):96–104.
11. Hayashi T, Kumasaka T, Mitani K, Okada Y, Kondo T, Date H, et al. Bronchial involvement in advanced stage lymphangioleiomyomatosis: histopathologic and molecular analyses. Hum Pathol. 2016;50:34–42.

Citation of this Article

Barravecchia I, Pucci A, Tavanti L, Fontanini G, Giarratana N, Sampaolesi M and Angeloni D. MICAL2 is Expressed in Lung Cystic Nodules of Lymphangioleiomyomatosis. Mega J Case Rep. 2023; 6: 2001-2007.

Copyright