Human Pathology (2012) **43**, 1777–1784

ELSEVIER

Human PATHOLOGY

www.elsevier.com/locate/humpath

Case study

Uterine angiosarcoma associated with lymphangioleiomyomatosis in a patient with tuberous sclerosis complex: an autopsy case report with immunohistochemical and genetic analysis☆,☆☆

Takuo Hayashi MD, PhD[a,b,*], Kengo Koike MD, PhD[b,c], Toshio Kumasaka MD, PhD[b,d], Tsuyoshi Saito MD, PhD[a], Keiko Mitani MT, CT, (IAC)[a,b], Yasuhisa Terao MD, PhD[e], Daiki Ogishima MD, PhD[f], Takashi Yao MD, PhD[a], Satoru Takeda MD, PhD[e], Kazuhisa Takahashi MD, PhD[c], Kuniaki Seyama MD, PhD[b,c]

[a]*Department of Human Pathology, Juntendo University School of Medicine, 2-1-1, Hongo, Bunkyo-ku, Tokyo 113-8421, Japan*
[b]*The Study Group of Pneumothorax and Cystic Lung Diseases, Japan*
[c]*Department of Respiratory Medicine, Juntendo University School of Medicine, 2-1-1, Hongo, Bunkyo-ku, Tokyo 113-8421, Japan*
[d]*Department of Pathology, Japanese Red Cross Medical Center, 4-1-22, Hiroo, Shibuya-ku, Tokyo, Japan*
[e]*Department of Gynecology and Obstetrics, Juntendo University School of Medicine, Tokyo 150-8935, Japan*
[f]*Department of Gynecology and Obstetrics, Juntendo University Nerima Hospital, 3-1-10, Takanodai, Nerima-ku, Tokyo 177-8521, Japan*

Received 10 January 2012; revised 9 March 2012; accepted 9 March 2012

Keywords:
Lymphangioleiomyomatosis;
Angiosarcoma;
Tuberous sclerosis complex

Summary A 41-year-old woman carrying a germline tuberous sclerosis complex 2 (*TSC2*) mutation, whose regular medical follow-up for tuberous sclerosis complex and tuberous sclerosis complex–associated lymphangioleiomyomatosis had continued for 2 years, had uterine angiosarcoma concomitant with uterine lymphangioleiomyomatosis. Immunohistochemically, the uterine angiosarcoma cells showed an extremely skewed lymphatic differentiation; they were diffusely immunopositive for CD31 but negative for other vascular endothelial markers including factor VIII and CD34 yet strongly immunopositive for lymphatic endothelial markers including D2-40 and Prox-1. Loss of heterozygosity analysis demonstrated that not only lymphangioleiomyomatosis and renal angiomyolipoma but also the uterine angiosarcoma had loss of heterozygosity on *TSC2*. Furthermore, direct sequencing revealed a *TP53* mutation in the uterine angiosarcoma. Collectively, the findings suggest that combined dysfunction of the p53 and *TSC2* tumor suppressor proteins may contribute to the development of uterine angiosarcoma in this rare clinical setting.

☆ Disclosures: The authors have no conflicts of interest to disclose.
☆☆ This study was supported by a Grant-in-Aid for Scientific Research No. 19590406 (Dr Kumasaka), No. 23590434 (Dr Saito), and No. 18390243 (Dr Seyama); in part by the High Technology Research Center Grant from the Ministry of Education, Culture, Sports, Science, and Technology, Japan, and in part by a grant to the Respiratory Failure Research Group from the Ministry of Health, Labor, and Welfare, Japan.
* Corresponding author. Department of Human Pathology, Juntendo University School of Medicine, 2-1-1, Hongo, Bunkyo-Ku, Tokyo 113-8421, Japan.
E-mail address: tkhyz@juntendo.ac.jp (T. Hayashi).

0046-8177/$ – see front matter © 2012 Elsevier Inc. All rights reserved.
doi:10.1016/j.humpath.2012.03.020

1. Introduction

Angiosarcomas usually arise in skin and soft tissue as de novo or against a background of long-standing lymphedema, most commonly after a mastectomy. Other anatomical sites where angiosarcomas develop include breast, liver, bone, spleen, heart and great vessels, orbit, pharynx, and oral cavity. Radiation therapy and chemotherapy have been proposed as risk factors for angiosarcomas, and *TP53* mutations are identified in angiosarcomas of the liver [1]. In contrast, uterine sarcomas other than leiomyosarcomas and endometrial stromal sarcomas are uncommon. In particular, the uterine angiosarcoma is very rare, and fewer than 20 well-documented cases have been reported. Most patients were postmenopausal and generally presented with heavy uterine bleeding, weight loss, and pelvic mass. Uterine angiosarcomas are usually highly aggressive and extend beyond the uterus, sometimes recurring within weeks to months of initial diagnosis. Angiosarcoma cells are immunopositive for endothelial markers including CD31, CD34, and factor VIII, but the contributory molecular alterations are unknown. Here we present data from an autopsy of a patient with tuberous sclerosis complex (TSC)–associated lymphangioleiomyomatosis (LAM) complicated by a uterine angiosarcoma. In this rare clinical setting, we performed immunohistochemical and genetic analyses to elucidate possible mechanisms by which a uterine angiosarcoma developed during the course of TSC-associated LAM.

2. Case report

This 39-year-old woman was admitted to a local hospital because of severe lower abdominal pain and vaginal discharge. She had a family history of TSC because her father had facial angiofibromas and underwent a nephrectomy due to renal angiomyolipomas. Her facial angiofibromas had been recorded since age 10 years, and a right pneumothorax occurred when she was 33 years old. Thereafter, she gradually experienced exertional dyspnea. Thorough examinations using computed tomography and magnetic resonance imaging resulted in a diagnosis of TSC with LAM as well as renal and hepatic angiomyolipomas, but no abnormalities appeared in her abdomen or pelvic cavity. The diagnosis of LAM was established from pathologic analysis of a transbronchial lung biopsy. Monthly subcutaneous injections of gonadotropin-releasing hormone analogue (leuprorelin acetate 1.88 mg) followed in the hope of stabilizing the LAM.

At the age of 41 years, she experienced abdominal distention, lower abdominal pain, and uterine bleeding. A computed tomographic scan of her lower abdomen and pelvic cavity revealed an enlarged uterus and swelling of the retroperitoneal lymph nodes from her lower abdomen to the pelvic cavity. An endometrial biopsy resulted in a diagnosis of high-grade sarcoma. Her uterus and bilateral adnexa were subsequently resected. Histopathologic and immunohistochemical examinations of those resected specimens revealed uterine angiosarcoma and LAM. Neither chemotherapy nor radiation therapy was performed after the surgery. The angiosarcoma recurred locally and then metastasized to her liver and lungs 6 months after surgery, followed by her death. An autopsy was performed with a written informed consent.

3. Materials and methods

3.1. Histopathologic and immunohistochemical examination

All tissues were fixed in 10% buffered formalin and embedded in paraffin after routine processing, sectioning, and staining with hematoxylin and eosin (H&E). Immunostaining was performed with antibodies directed to the following: vimentin (dilution 1:500; Dako Cytomation, Carpinteria, CA), CD31 (dilution 1:100; Dako Cytomation), CD34 (dilution 1:200; Novocastra Laboratories Ltd, New Castle, UK), factor VIII (dilution 1:300; Dako Cytomation), D2-40 (dilution 1:200; Dako Cytomation), Prox-1 (dilution 1:750; AngioBio, Del Mar, CA), VEGF-A (dilution 1:200; R&D Co Ltd, Minneapolis, MN), VEGF-C (dilution 1:200; R&D Co Ltd), VEGF-D (dilution 1:200; R&D Co Ltd), VEGFR-3 (dilution 1:50; R&D Co Ltd), c-kit (dilution 1:200; Dako Cytomation), p53 (dilution 1:200; Dako Cytomation), α smooth muscle actin (αSMA) (dilution 1:200; Dako Cytomation), HMB45 (dilution 1:50; Dako Cytomation), estrogen receptor (ER) (dilution 1:50; Novocastra Laboratories Ltd), and progesterone receptor (PgR) (dilution 1:50; Novocastra Laboratories Ltd). An EnVision kit (Dako Cytomation) was used for the immunostaining of vimentin, CD31, CD34, factor VIII, D2-40, Prox-1, VEGF-A, p53, c-kit, αSMA, HMB45, ER, and PgR to detect binding of the first antibodies according to the manufacturer's instructions, and 3,3′-diaminobenzidine tetrahydrochloride was used as the chromogen. For immunostaining of VEGF-C, VEGF-D, and VEGFR-3, biotinylated antigoat rabbit antibody (Dako Cytomation) and alkali phosphatase-conjugated streptavidin (Dako Cytomation) were used to detect antibody binding. Fast red was used as the chromogen.

3.2. Loss of heterozygosity analysis of the *TSC1* and *TSC2* gene-associated regions

The angiosarcoma, LAM, and angiomyolipoma were analyzed for loss of heterozygosity (LOH) on chromosome 9q (including the *TSC1* gene-associated region) and 16p (including the *TSC2* gene-associated region). Genomic DNA was extracted from the following tissues: angiosarcoma in the uterus, LAM in the retroperitoneal lymph node, and angiomyolipoma in the kidney. All tissues were fixed in 10%