

PRIMARY LEIOMYOSARCOMA OF THE MESENTERY IN TWO SISTERS: CLINICAL AND MOLECULAR CHARACTERISTICS

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Mesenteric leiomyosarcoma (LMS) is a very rare malignancy whose familial occurrence has not yet been reported. We present two sisters who developed intestinal LMS. Pathological analysis of the tumor samples, including evaluation of smooth muscle actin+, desmin+, Myf4-, DOG-1-, S100-, CD34- and CD117- confirmed LMS diagnosis. Molecular analysis of the lesions, both primary tumors and a liver metastasis, revealed several genomic imbalances, with recurrent chromosomal aberration: interstitial gain at chromosome 17p11.2-13.1 with the minimal overlapping region of 9.2 Mb. Our study provides further evidence for the significant role of the genes located in this region in the early stage of carcinogenesis.

Key words: mesenteric leiomyosarcoma, familial occurrence, chromosome 17p gain, genomic rearrangements, array-CGH.

Introduction

Leiomyosarcoma (LMS) of the mesentery is a rare malignancy and its familial occurrence has never been reported. We describe an unusual case of two sisters who developed this tumor and present a molecular analysis of both cases.

Case 1. A 62-year-old woman underwent breast-conserving therapy for T1bN0 lobular carcinoma of the left breast. Six years later at routine abdomen CT an irregular tumor of 54 × 62 × 78 mm adherent to the jejunum loops and multiple hypodense liver lesions up to a diameter of 26 mm were noted. PET/CT confirmed increased tracer uptake. The patient underwent palliative resection of the abdominal tumor and a biopsy of liver lesions. The pathologic examination revealed the tumor composed of an expansile solid portion, with infiltration of the surrounding adipose tissue (Fig. 1A–C). The plexiform structure was composed of spindle cells with intermediate grade of atypia. The mitotic index averaged 6/10 high power fields (HPFs). Tumor

cells showed expression of smooth muscle actin (SMA) and desmin, whereas Myf4, S100, CD34, DOG-1 and CD117 were not identified. Final diagnosis of LMS, grade 2 was rendered. The patient received 3 cycles of palliative chemotherapy including doxorubicin and cisplatin; however, further therapy was discontinued due to the lack of response and poor tolerance. The patient died two years later due to disease progression.

Case 2, the younger sister, at the age of 46 years, was admitted to the hospital for extirpation of the myomatous uterus. On laparotomy a large mass was revealed in the sigmoid mesentery and was removed together with the uterus. The pathologic examination showed the tumor composed of long fascicles of spindle cells with areas of necrosis. Atypia of the cells was minimal, but the mitoses were multiple. The mitotic index was 16/10 HPFs. The tumor cells were strongly SMA-positive, with scattered cells expressing desmin, while CD34, CD117, DOG-1 and Myf4 were not identified (Fig. 1D–F). The tumor was diagnosed as LMS grade 2. No malignancy was found in the uterus,

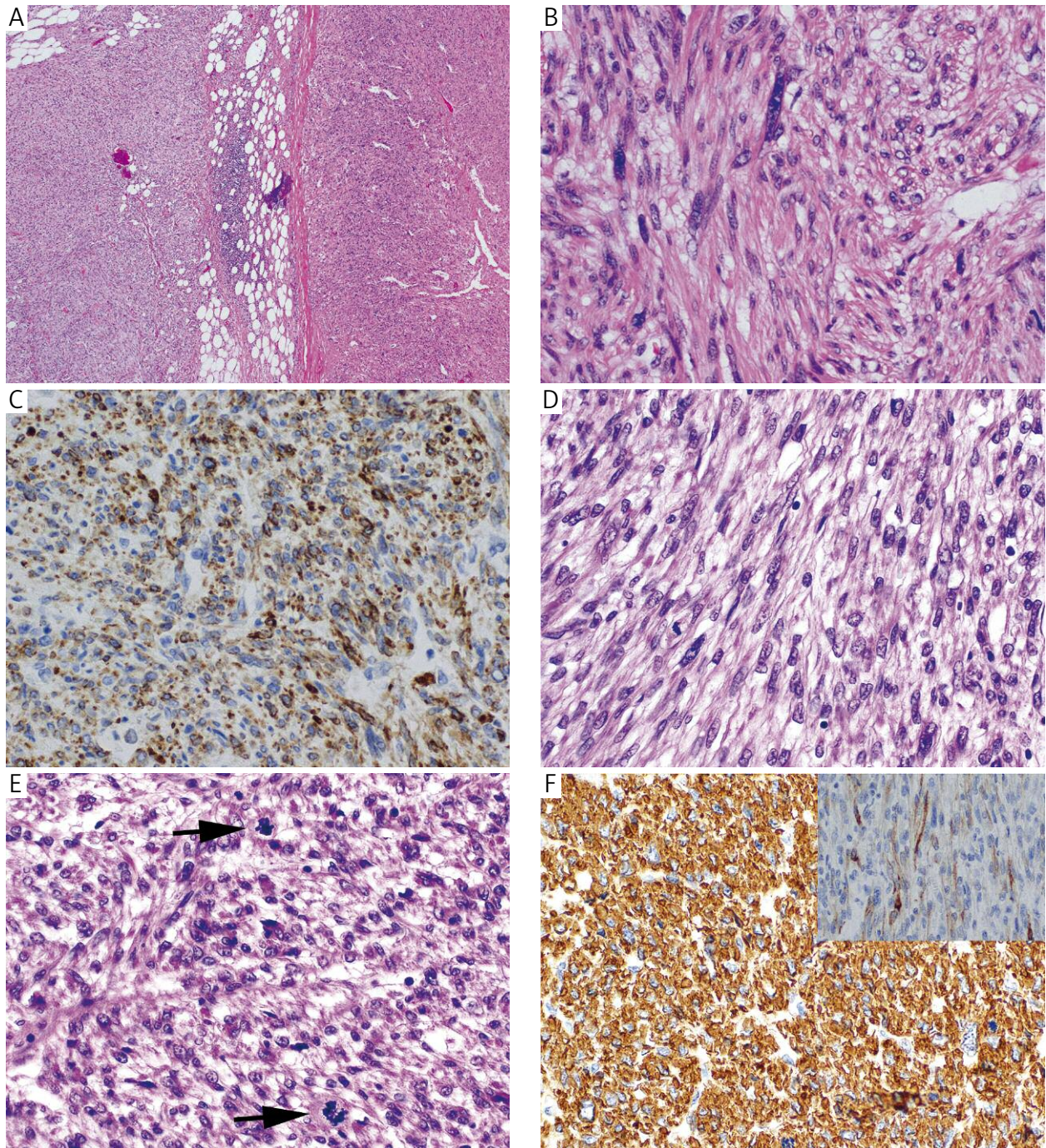


Fig. 1. A–C – patient 1. At the periphery of the main tumor bulk (A, on the right) infiltrating LMS nodules in the adipose tissue (A, on the left, magnification 20×). The short fascicles of the elongated tumor cells contained scattered pleomorphic cells (B, magnification 400×). Cytoplasmic expression of desmin was identified (C, magnification 400×). D–F – patient 2. The tumor was composed of long fascicles with scattered mitoses (D, magnification 400×). Some of the mitoses were atypical (E, magnification 400×, arrows). Smooth muscle actin was detected in all tumor cells (F, magnification 200×), whereas desmin was expressed by a portion of tumor cells (F, magnification 100×, inset)

apart from intramural and subserous leiomyomas. Six months later, due to extensive local recurrence, the patient underwent resection of the right colon, sigmoid, ileum and rectum. After another nine months she underwent removal of the recurrent retroperitoneal tumors. Pathologic examination in both instances sho-

wed LMS. Due to further progression, the patient received two cycles of chemotherapy consisting of doxorubicin and dacarbazine, followed by one cycle of doxorubicin and cisplatin. Owing to poor tolerance and lack of response, chemotherapy was withheld. At four years from initial diagnosis she underwent chemoemboliza-

tion of liver metastases, but eventually she died ten months later with signs of disease progression.

Material and methods

5- μ m paraffin-embedded sections of primary tumors (two distinct samples from each lesion) and a liver metastasis of patient 1 were subject to molecular evaluation. DNA was extracted using a standard isolation protocol with proteinase K digestion, phenol-chloroform extraction and ethanol precipitation. Additionally, constitutional DNA of patient 1, as well as reference DNA for array-CGH (aCGH) and quantitative real time polymerase chain reaction (qPCR) experiments from 9 anonymous healthy female volunteers, was isolated from peripheral blood leukocytes using QIAamp DNA (Qiagen). Selected exons of *KIT* (exons 9, 11, 13, 17) and *PDGFRA* (exons 12, 14, 18) genes were sequenced in DNA samples extracted from all lesions. Also, *TP53* mutation status was checked in the constitutional DNA sample.

Array-CGH analysis was at first performed using $3 \times 720\text{K}$ WG-T v.3.0 Arr Del array, and later expanded using high-resolution 2.1M WG-T v2.0D Arr Del array (NimbleGen, Roche). Arrays were scanned with an MS200 Microarray Scanner and analyzed using NimbleScan and SignalMap (NimbleGen, Roche). All identified genomic imbalances were verified in the data-

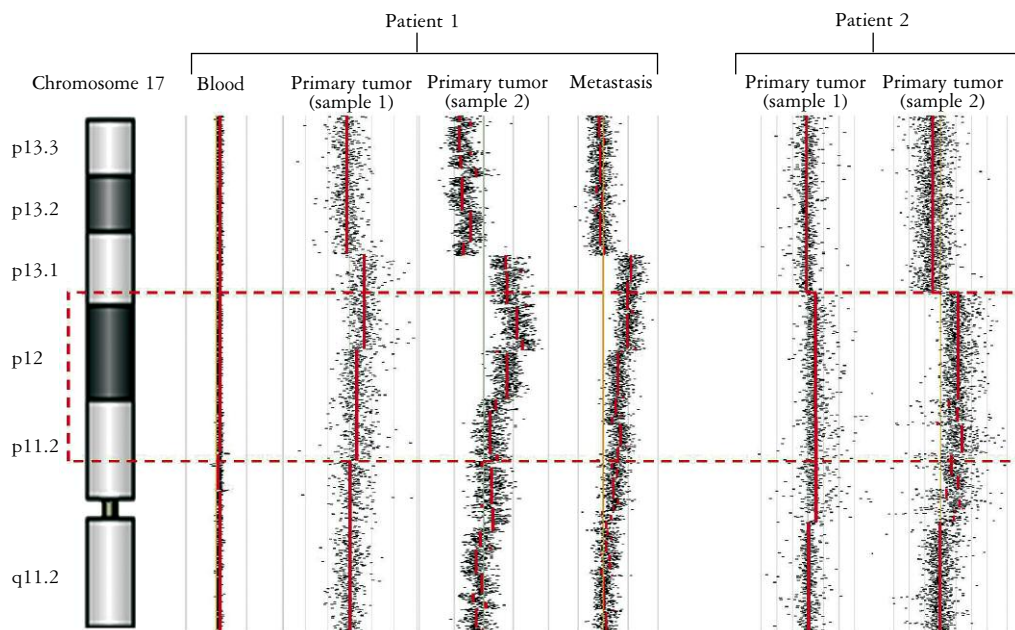
base of genomic variants (DGV; <http://projects.tcag.ca/variation>; last accessed September 2012).

Results

No germline mutation in *TP53*, *KIT* and *PDGFRA* genes was found. Although extraction of DNA from paraffin samples yielded genetic material of fair average quality, we were able to screen also the most common sites of somatic mutations (coverage ca. 80%) and no mutation was detected.

Array-CGH analysis of the tumors revealed a number of aberrations, mainly terminal gains (13p, 14p) and losses (chromosomes 1p36; 4p16; 19q13), and a few interstitial deletions (3q26; 6p22, 10q22, 11q21, Xq25), with the size ranging up to 61 Mbp. Only two aberrations were present in all samples: a known 2 Mb CNV at 5q13.2 (detected also in the control sample of constitutional DNA) and a duplication on the short arm of chromosome 17. The latter was found to be a recurrent somatic lesion, present in all of the analyzed neoplastic lesions, both primary and metastatic, but not in the constitutional DNA (Fig. 2). The minimal overlapping region of 9.2 Mb covered a total of 57 genes. The *TP53* gene, also located on the chromosome 17p arm, lay outside of the common region of aberration.

Even though the resolution of aCGH experiments was 5 kbp, only gross aberrations (> 1 Mbp) could be



The region of gain at chromosome 17p detected using 720K array (primary tumor sample 1 of patient 1 and primary tumor sample 1 of patient 2) and 2.1M array (the rest of the hybridizations). For patient 1, DNA extracted from neoplastic lesions (both primary and metastatic) was hybridized against constitutional DNA of the same patient. For patient 2, DNA was hybridized against a pool of control reference DNA. Log 2 ratio values for all probes are plotted as a function of their chromosomal position.

Red-dashed area: minimal overlapping region (17: 9581249–18818749 bp; GRCh36/hg18).

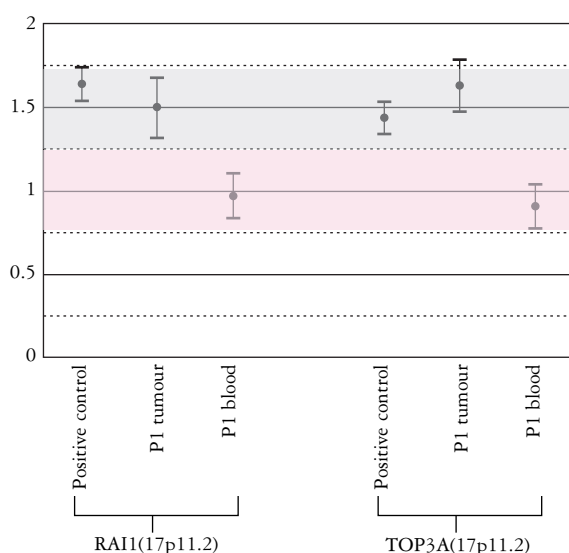
Fig. 2. Array-CGH analysis showing aberration on chromosome 17

positively detected because of the aforesaid fair average quality of DNA extracted from paraffin-embedded tissue samples. The results of microarray studies were validated by quantitative real-time PCR (Fig. 3).

Discussion

Mesenteric LMS is a rare malignancy and, since its first description in 1963 [1], only a few dozen patients have been reported [2, 3]. LMS requires differentiation from gastrointestinal stromal tumor (GIST), the most common mesenchymal malignancy of the GI tract. The older reports on mesenteric smooth muscle tumors cannot be translated into the current classification, especially those that were not analyzed regarding CD117 expression typical for GIST. Most of the cases previously diagnosed as leiomyomas, LMS and leiomyoblastomas would now be reclassified into the realm of GIST. Hence, true malignant smooth muscle tumors are currently very uncommon.

The only effective therapy for mesenteric LMS is surgery, as these tumors are notoriously resistant to radiotherapy and chemotherapy. Of the two cases reported here, one was diagnosed at a locally advanced stage and the other at dissemination; therefore neither could be effectively managed with surgery. The tumors did not show an immunophenotypic profile typical for GIST (lack of CD34, DOG1 and CD117 and expression of desmin); nor did they carry mutations in the *KIT* and *PDGFRA* genes. Thus, in neither case was imatinib therapy attempted.



Dosage of the two genes localized in minimal overlapping region 17p11.2: *RAI1* and *TOP3A* relative to two reference genes: *GPR15* (3q11.2) and *ERMP1* (9p24.1) and normalized to reference DNA. Patient's constitutional DNA and tumor DNA were analyzed against a positive control DNA sample extracted from a case with constitutional duplication at 17p11.2 (Potocki-Lupski syndrome). All samples were run in triplicate.

The diploid (two copies of a gene) samples have the normalized ratio = 1 (rose-colored band), whereas for triploid the normalized ratio = 1.5 (grey-colored band).

Fig. 3. Normalized gene copy number ratios measured by qPCR

The uniqueness of our case was the occurrence of apparently identical malignancy of the small intestine mesentery in two sisters. This prompted us to perform molecular analysis of both tumors and constitutional DNA analysis. To start with, we excluded Li-Fraumeni syndrome as no *TP53* germline mutation was detected. Subsequently, a search for a common genomic aberration was undertaken. Even though genomic imbalances are common in LMS, with the estimated occurrence exceeding 90%, no consistent, recurrent aberrations have yet been observed at the chromosomal level [3-6]. The majority of tumors have complex karyotypes, presenting with numerous gains and losses, including an amplification at 17p [3-6]. Usually, numerous chromosomal aberrations are detected for a single tumor. In line with the foregoing, in the presented cases, a number of gains and losses were detected (mean 9 aberrations per sample). Nevertheless, partial 17p gain was the only gross somatic chromosomal anomaly detected in all lesions and, what is more, it shared ~80% similarity (Fig. 2).

Chromosome 17p gains are presumed to be relatively common in LMS, with the estimated frequency of 33-55% [4]. Proximal 17p gains frequently occur also in other sarcomas, including osteosarcoma, chondrosarcoma and malignant fibrous histiocytoma [3, 5]. Besides, constitutional aberrations in the 17p11.2 region cause four well-known constitutional genomic syndromes: Potocki-Lupski, Smith-Magenis, Charcot-Marie-Tooth type 1 and hereditary neuropathy with liability to pressure palsies (HNPP). The region is one of the genomic "recombination hotspots", as it is particularly prone to rearrangements due to distinct redundancy of large, complex and highly identical low-copy repeats, which are prone to mediate nonallelic homologous recombination.

The fact that 17p aberration was present in all lesions, both primary and metastatic, provides further evidence for the significant role of the genes located in this region in the early stage of carcinogenesis. Accordingly, we have attempted to further define the minimal overlapping region at 17p through comparison with previously reported genomic imbalances. Disappointingly, most of the published studies were performed with very low resolution, usually by means of classical karyotyping or comparative genomic hybridization; hence only large aberrations were described, making precise genotype-phenotype correlation studies almost impossible.

In the identified minimal overlapping region a total of 57 genes are located. Of these, a few have already been proposed as candidate genes in the pathogenesis of sarcoma. Two large expression profiling studies performed on LMS series identified *SREBF1* [6] and *MYOCD* [7] genes to be highly overexpressed. *SREBF1* codes for a transcription factor that regulates fatty acid and cholesterol synthesis, inhibition of which sensitizes

resistant cells to death apoptosis [8]. *MYOCD* encodes transcriptional serum response factor essential for cardiac and smooth muscle development and differentiation, and its expression is typical for well-differentiated LMS [7, 9].

The development of LMS in both sisters is suggestive of an autosomal recessive trait. However – in line with Knudson’s “two-hit” hypothesis – an autosomal dominant trait due to gonadal mosaicism cannot be excluded. Notwithstanding the above, further studies are needed to definitively identify the yet unknown gene responsible for cancer development.

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