Cancer Genetics 208 (2015) 537–544

Cancer Genetics

ORIGINAL ARTICLE

A translocation t(6;14) in two cases of leiomyosarcoma: Molecular cytogenetic and array-based comparative genomic hybridization characterization

Marieke A. de Graaff [a], Daniëlle de Jong [b], Inge H. Briaire-de Bruijn [a], Pancras C.W. Hogendoorn [a], Judith V.M.G. Bovée [a], Károly Szuhai [b,*]

[a] *Department of Pathology, Leiden University Medical Center (LUMC), Leiden, The Netherlands;* [b] *Department of Molecular Cell Biology, LUMC, Leiden, The Netherlands*

Leiomyosarcomas are malignant mesenchymal tumors that recapitulate smooth muscle cell differentiation. Tumors are characterized by a genetic heterogeneity with complex karyotypes without a tumor-specific genetic aberration. Their pathobiology is still poorly understood and no specific targeted treatment is currently available for these aggressive tumors. For six leiomyosarcomas, cells were cultured and analyzed by combined binary ratio labeling fluorescence in situ hybridization (COBRA-FISH) karyotyping. A t(6;14) was identified in two cases. FISH breakpoint mapping of case L1339 reveals a breakpoint at chromosome 6p21.31 close to *HMGA1*, and a small deletion was observed on the distal side of the gene. A small homozygous deletion was also found in the breakpoint region of chromosome 14q24.1 involving *ACTN1*. The second case revealed a der(6)t(6;14)(p21.1;q21.3), with a duplication adjacent to the breakpoint at chromosome 6. Confirmatory FISH revealed a second leiomyosarcoma with an aberration at 14q24.1. Alterations at this locus were found in 5% (2 of 39) of the leiomyosarcomas in this study. The other identified breakpoints appeared to be non-recurrent, because they were not detected in other leiomyosarcomas, uterine leiomyomas, undifferentiated spindle cell sarcomas, or undifferentiated pleomorphic sarcomas.

Keywords Leiomyosarcoma, soft tissue sarcoma, translocation, FISH, array-CGH, HMGA1

Leiomyosarcomas are malignant tumors originating from smooth muscle cells and are responsible for 10-15% of adult soft tissue sarcomas. Tumors show a varying degree of smooth muscle differentiation, which is inversely proportional to tumor grade. Histologically low grade tumors are composed of sharply demarcated bundles of spindle cells intersecting at right angles, whereas high grade lesions might contain poorly differentiated regions with nuclear pleomorphism and atypical mitoses. Areas of myxoid change, epithelioid morphology, fibrosis, hemorrhage, or necrosis can be present. Smooth muscle differentiation can be detected with desmin and h-caldesmon immunohistochemistry. The retroperitoneum is the most common location, but the large blood vessels, with a predilection for the vena cava inferior, the uterus, and other soft tissue sites, such as the lower extremities, are also frequently involved (1). Lesions of the retroperitoneum and blood vessels are encountered more frequently in women; no gender predilection is present for other soft tissue locations. Leiomyosarcomas are responsible for a substantial morbidity and mortality because they are characterized by aggressive behavior, with a 5-year overall survival (OS) of 64% (2,3). A high tumor grade, deep tumor location, and large tumor size (>5 cm) were independent adverse prognostic factors for metastatic disease and OS (2–4). Wide surgical excision remains the mainstay of treatment, often in combination with radiation or conventional chemotherapy. At the moment, no specific targeted treatment against leiomyosarcomas is available because the pathophysiology still remains poorly understood (5).

Received March 17, 2015; received in revised form June 29, 2015; accepted July 27, 2015.
* Corresponding author.
E-mail address: k.szuhai@lumc.nl

2210-7762/$ - see front matter © 2015 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.cancergen.2015.07.005

Genetically, leiomyosarcomas are characterized by complex karyotypes with numerous reported non-recurrent and non-specific aberrations that result from a high degree of genetic instability (1,6,7). Although gains and losses of many different chromosomes have been reported and the inter-tumor heterogeneity is extensive, some similarities are also described. Both cytogenetic and array-based comparative genomic hybridization (array-CGH) analyses have revealed recurrent involvement of losses of 1p, 2p, 10q, 11q, 13q, 16q, and 17p, and gains of 17q and 17p (2,8).

The main objective of this study is to investigate the genetic alterations in leiomyosarcomas and to search for a common molecular genetic aberration in order to improve the understanding of this malignant tumor and to identify prognostic factors or candidate targets for future therapeutic regimens. We identified two cases with a t(6;14) by using state-of-the-art molecular karyotyping tools, and fine mapped breakpoint regions were tested on a tissue microarray (TMA) that consisted of a large panel of leiomyosarcomas, uterine leiomyomas, undifferentiated spindle cell sarcomas, and undifferentiated pleomorphic sarcomas with interphase fluorescence in situ hybridization (FISH).

Materials and methods

Cell culture and combined binary ratio labeling FISH (COBRA-FISH)

Tumor specimens were collected directly after surgery and manually dissected in small fragments. After the addition of IMDM, supplemented with 10% FBS and 1% penicillin/streptomycin (Gibco; Life Technologies, Bleiswijk, The Netherlands), the specimens were placed in a humidified 5% CO_2 incubator at 37°C. Cells were cultured for several passages and harvested after 25 min of incubation with Calyculin A (Sigma-Aldrich, Zwijndrecht, The Netherlands). Subsequently, cells were placed in a hypotonic 0.075 M KCl solution followed by three fixation steps of methanol/glacial acetic acid (4:1 ratio). Slides with metaphase spreads were made in a conditioned chamber (25°C, 50% humidity; Thermotron, Holland, MI).

COBRA-FISH karyotyping was performed on slides with metaphase spreads of six leiomyosarcomas; no conventional G-banding studies were performed. A detailed protocol on the pre- and post-hybridization treatments was published previously (9). A DMRA fluorescence microscope (Leica Microsystems, Rijswijk, The Netherlands) was used to capture images, which were analyzed with the ColorProc software tool that was developed in-house (Leiden University Medical Center (LUMC), Leiden, The Netherlands). Karyotypes were described according to the International System for Human Cytogenetic Nomenclature (ISCN 2013) (10). In three leiomyosarcomas, no detectable cytogenetic abnormalities were found in the analyzed cells. Tumor cells and fibroblasts show a similar, spindle cell morphology in vitro, thereby making it difficult to discriminate them morphologically. The leiomyosarcomas with a seemingly normal karyotype might have contained a mix in which fibroblasts have overgrown the tumor cells. Therefore, these three cases were excluded from further analysis.

FISH breakpoint mapping

Two-color FISH was performed to investigate the exact breakpoints of chromosomes 6p and 14q of two selected leiomyosarcoma cases. To detect possible rearrangements of the region of *HMGA1* on chromosome 6p21 bacterial artificial chromosome (BAC) clones, RP3-349A12, proximal to the breakpoint, and RP11-936A3, distal to the breakpoint, were selected. BAC clones used in the fine mapping of the deletion breakpoints on chromosome 6 were RP11-175A, RP5-1077I5, RP3-468B3, and RP11-974K1. On region 14q24, around the *ACTN1* gene, RP11-204K16 was selected proximally and BAC clone RP11-486O13 was located distally from the breakpoint. Other BAC clones used in the fine mapping of the breakpoint on chromosome 14 were RP11-226F19 and RP11-565I3. All BAC clones were obtained from Wellcome Trust Sanger Institute (Cambridge, UK). Probes were directly labeled with fluorescein isothiocyanate (FITC) or Cy3 using a nick translation labeling reaction. Probes were hybridized overnight on metaphase slides using a protocol otherwise identical to the COBRA-FISH protocol as described previously.

Array-CGH analysis

Genomic DNA was isolated from frozen tumor sections of the two selected cases with a Nucleospin DNA extraction protocol (Macherey-Nagel, Düren, Germany). Histologic evaluation confirmed that the tumor content exceeded 80-90%. DNA labeling and purification were done according to the BioPrime Total Genomic Labeling System (Invitrogen, Bleiswijk, The Netherlands); labeling efficiency was calculated with a Nanodrop ND-1000 spectrophotometer (Thermo Scientific, Wilmington, DE) measuring A_{260} (DNA), A_{550} (Cy3), and A_{649} (Cy5). Array-CGH was performed with an oligonucleotide-based human genome CGH microarray 4×44 k (Agilent Technologies, Santa Clara, CA) at 65°C for 40 h. Slides were washed according to the manufacturer's protocols and scanned using an Agilent microarray scanner with a 5-μm scan resolution. Images were imported to Feature Extraction Software and Genomic Workbench (Agilent) with a human reference genome and standard settings for analysis. Commercially available healthy male or female DNA (Promega, Madison, WI) was used as reference DNA. Test and reference samples were hybridized as a gender mismatch to illustrate the dynamic range of hybridization at the X and Y chromosomes.

Tissue microarray

A TMA with a broad selection of soft tissue tumors, which were diagnosed at the Department of Pathology of the LUMC, was used for confirmatory FISH analysis (11). In this study, the evaluable cases included 37 leiomyosarcomas, 17 undifferentiated pleomorphic sarcomas, 4 undifferentiated spindle cell sarcomas, and 4 uterine leiomyomas. The histologic diagnosis of all samples was confirmed upon review of the H&E-stained slides by a specialized bone and soft tissue tumor pathologist (J.V.M.G.B. and P.C.W.H.). At least one of the smooth muscle markers—h-caldesmon or desmin—was positive in the leiomyosarcomas. The included leiomyosarcomas were negative for HMB45, which is a marker positive in perivascular epithelioid cell tumors (PEComas). Soft tissue