Human Pathology (2016) **55**, 143–150

ELSEVIER

Human PATHOLOGY

www.elsevier.com/locate/humpath

Original contribution

Oncogene mutation profiling reveals poor prognosis associated with *FGFR1/3* mutation in liposarcoma☆,☆☆

Chengfang Li MD[a,b], Yaoyuan Shen MD[a], Yan Ren PhD[a], Wei Liu PhD[a], Man Li MD[a,c], Weihua Liang BS[a], Chunxia Liu MD, PhD[a,c,*], Feng Li MD, PhD[a,c,*]

[a]*Department of Pathology, Shihezi University School of Medicine, Shihezi, Xinjiang 832002, China*
[b]*Department of Pathology, The Affiliated Hospital of Zun Yi Medical College, Zunyi, Guizhou 563000, China*
[c]*Department of Pathology, The First Affiliated Hospital, Shihezi University School of Medicine, Shihezi, Xinjiang 832002, China*

Received 8 January 2016; revised 3 May 2016; accepted 12 May 2016

Keywords:
Liposarcomas;
Mutations;
Oncogenes;
MassARRAY technology;
FGFR1/3;
Prognosis

Summary Liposarcoma (LPS) is one of the most prevalent soft tissue sarcomas. LPS shows a poor response to radiation and chemotherapy. The causes of death in patients with LPS include locally recurrent and metastatic disease. We sought to examine novel gene mutations and pathways in primary and matched recurrent LPSs to identify potential therapeutic targets. We conducted a high-throughput analysis of 238 known mutations in 19 oncogenes using Sequenom MassARRAY technology. Nucleic acids were extracted from 19 primary and recurrent LPS samples, encompassing 9 dedifferentiated LPSs (DDLPS), 9 myxoid/round cell LPSs, and 1 pleomorphic LPS. Mutation screening revealed missense mutations in 21.1% (4/19) of the LPS specimens, including 4 different genes (*FGFR1*, *FGFR3*, *PIK3CA*, and *KIT*). Based on histologic subtypes, 22.2% DDLPS (2/9) and 22.2% myxoid cell LPS (2/9) contained gene mutations. Specifically, 3 (23.1%) of 13 primary tumors harbored mutations. Furthermore, although gene mutations were identified in 1 (11.1%) of 9 recurrent LPS samples, the difference between the primary and the recurrence was not statistically significant. Analysis of patient survival data indicated that patients harboring *FGFR1/3* mutations experienced reduced overall survival ($P < .05$). Despite the limited number of samples, our findings provide the first evidence of *FGFR1/3* mutations in DDLPS, which were associated with poor clinical outcomes. The *FGFR* pathway may play an important role in the development and progression of DDLPS and warrants further investigation; moreover, *PIK3CA* mutation is a common event (11.1%) in myxoid cell LPS.

1. Introduction

Liposarcomas (LPSs) constitute a heterogeneous group of distinctive lesions. Per the current World Health Organization and other guidelines, LPS is currently subclassified into 4 categories based on the distinctive morphologies and unique genetic profiles thereof: atypical lipomatous tumor/well-differentiated LPS (WDLPS), dedifferentiated LPS (DDLPS), myxoid/round cell LPS (M/RCLPS), and pleomorphic LPS

☆ Competing interests: We confirm that all authors fulfill all conditions required for authorship. We also confirm that there is no potential conflict of interest or financial dependence regarding this publication, as described in the Instruction for Authors.

☆☆ Funding/Support: This work was supported by grants from the National Natural Science Foundation of China (grant nos. 81160322 and 81460404).

* Corresponding authors.
E-mail addresses: liuliu2239@sina.com (C. Liu), lifeng7855@126.com (F. Li).

http://dx.doi.org/10.1016/j.humpath.2016.05.006
0046-8177/

(PLS). Cytogenetic characterization, which involves amplification of the 12q13-15 chromosomal region, has identified several genes that are implicated in WDLPS and DDLPS, most notably murine double minute 2 (*MDM2*), cyclin-dependent kinase 4 (*CDK4*), high-mobility group AT-hook 2 (*HMGA2*), and, recently, factor receptor substrate 2 (*FRS2*) [1–3]. M/RCLPS is characterized by reciprocal gene fusion involving the more frequent t(12;16) (*FUS-DDIT3*) or the less common t(12;22) (*EWSR1-DDIT3*) chromosomal translocations [4,5]. In contrast, PLS shows complex structural and numerical chromosomal abnormalities [6] and cannot be characterized by any specific molecular features. Previous studies have indicated that a mutation in the phosphoinositide-3-kinase, catalytic, α (*PIK3CA*) gene occurs in 14% to 18% of myxoid LPS [7,8]. Other studies have established that 18.2% (2/11 cases) of myxoid LPS (MLPS) harbored mutations in the *TP53* gene and that the methylation frequency of the $p14^{ARF}$ gene is 83.3% (10/12 cases) [9]. Recently, mutations were identified in the telomerase reverse transcriptase (*TERT*) promoter in 80% of MLPS [10].

WDLPS is a typical example of a tumor that may become more aggressive with time [11]. DDLPS is traditionally defined as a nonlipogenic high-grade sarcoma originating from WDLPS, which confers metastatic potential. Coexpression of genes within the *MDM2*/*CDK4*/*TP53* cluster and alterations of chromosome 12 that characterize DDLPS suggest a link with dedifferentiation [12]. In DDLPS, loss of 11q23-24 is associated with genomic complexity and is characterized by a distinct morphology [13]. Taylor et al [14] suggested that methylation of the CCAAT/enhancer-binding protein α promoter played an important role in the progression from WDLPS to DDLPS; however, exome sequencing revealed a modest rate of point mutations (*HDAC1* mutations occurred in only 8.3% of DDLPS). Moreover, *H-ras* mutations were exclusively observed in DDLPS (4/19; 21%) [15]. In a rare study on gene mutations in PLS, a large-scale analysis of sarcomas revealed frequent gene mutations, including the genes for tumor protein p53 (*TP53*) in 17% and neurofibromin 1 (*NF1*) in 8% of PLS [7]. A recent study showed that 33.3% of PLS contained *TP53* mutations and that 50% (3/6) had $p14^{ARF}$ gene methylation [9].

With the gradual increase in our current understanding of genetic alterations, there has been a remarkable increase in the information about LPS. However, there remain many unanswered questions regarding LPS development and progression. The ultimate goal is to develop drugs that can specifically eliminate LPS cells while sparing normal tissues. Recent large-scale mutation analyses have established a standard for cancer genome studies [7,16,17]. However, to date, few such studies have focused on LPS. In the present study, we conducted a high-throughput analysis of 238 known mutations in 19 oncogenes from primary and matched recurrent LPS using Sequenom's MassARRAY technology (Sequenom, San Diego, CA) to evaluate novel gene mutations and pathways for potential therapeutic targets. We hypothesized that LPS may harbor mutations in a subset of these oncogenes, which would be revealed through profiling.

2. Materials and methods

2.1. Patients and samples

Samples were collected from 19 LPS patients (13 primary and 6 pairwise recurrent tumor tissue samples) who were diagnosed at the Department of Pathology of the First Affiliated Hospital, Shihezi University School of Medicine (Xinjiang, China) between June 1989 and October 2013. The institutional review board and ethics committee approved the study, and informed consent was obtained from each patient.

For mutation analysis, 19 formalin-fixed, paraffin-embedded (FFPE) primary and recurrent tumor tissue samples were selected from the 13 patients with primary LPS. LPS diagnoses were corroborated by the results of histologic and immunohistochemical analyses. The clinical courses of all patients were monitored prospectively and reviewed retrospectively. Follow-up examinations were performed for all patients until death or the latest status.

2.2. Genomic DNA extraction

Genomic DNA was extracted from 10 consecutive 5-μm FFPE tissue sections from 19 LPS samples using the DNeasy FFPE kit (Qiagen, Hilden, Germany). The extraction procedure was performed according to the manufacturer's protocol, with the following exceptions: the protease K digestion was extended overnight, and an additional digestion step was performed for samples that showed incomplete digestion after overnight protease K treatment.

2.3. Mutation analysis using MassARRAY (Sequenom) genotyping

Mutation analysis was performed using the OncoCarta panel version 1.0 (Sequenom) and Sequenom MassARRAY technology (Sequenom), which is based on matrix-assisted laser desorptionionization time-of-flight mass spectrometer and is a simple method for the detection of clinically relevant mutation "hotspots" in cancer. It was essential that the methodology would work with degraded DNAs isolated from FFPE. The OncoCarta Panel version 1.0 consists of 24 pools of polymerase chain reaction (PCR) primer pairs and 24 pools of extension primers and detected 238 mutations in 19 genes, which are listed in Table 1.

Approximately 2 μL of genomic DNA (10-15 ng/μL) was required per pool. The procedure comprised the following 3 steps: PCR was performed using the OncoCarta PCR primer pools; PCR products were then processed with shrimp alkaline phosphatase to inactivate any unincorporated nucleotides; and finally, a single-base extension reaction was performed by using extension primers. Salts were removed by the addition