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CASE REPORT

Well-Differentiated Liposarcoma of the Oesophagus: Clinicopathological, Immunohistochemical and Array CGH Analysis

Jae Kyung Myung · Ji Bong Jeong · Daehee Han · Chi Sung Song · Hyeon Jong Moon · Young A. Kim · Ji Eun Kim · Sun-ju Byun · Woo Ho Kim · Mee Soo Chang

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Abstract Liposarcoma develops extremely rarely in the oesophagus. Microscopically, it exhibits subtle atypia of H&E-stained features. Accordingly, immunohistochemical features and chromosomal alterations are used for its confirmatory diagnosis. However, cytogenetic analysis has not been performed for oesophageal liposarcoma. We studied chromosomal alterations using array comparative genomic hybridization (CGH), as well as endoscopic, radiological, H&E-stained and immunohistochemical features in the oesophageal well-differentiated liposarcoma of a 67-year-old man. Array CGH analysis revealed the presence of high-level amplifications at chromosomal locations 1p12–1q21.2, 12q13.2–12q15 and 12q21.33–12q23.1. At least 29 genes were highly amplified ($\log_2$ ratio >2), among which *CDK4* and *MDM2* were the most highly amplified ($\log_2$ ratio >4) and were accepted as major target genes. Moreover, the amplification of *AMDHD1, HAL* and *LTA4H* ($\log_2$ ratio=3.153) was a novel finding. This case suggests the presence of a characteristic profile of gene amplification in well-differentiated liposarcoma of the oesophagus. The amplified genes may be of pathogenic importance for primary oesophageal well-differentiated liposarcoma.

Keywords Oesophagus · Well-differentiated liposarcoma · Array comparative genomic hybridization · Chromosome · *CDK4* · *MDM2*

Myung, J.K. and Jeong, J.B. contributed equally to this study.

J. K. Myung · Y. A. Kim · J. E. Kim · S.-j. Byun · M. S. Chang (✉)
Department of Pathology, Seoul National University Boramae Hospital, Seoul National University College of Medicine, 425 Shindaebang-dong, Dongjak-gu, Seoul 156-707, Korea
e-mail: meesooch@snu.ac.kr

J. K. Myung · Y. A. Kim · J. E. Kim · S.-j. Byun · W. H. Kim · M. S. Chang
Department of Pathology, Seoul National University College of Medicine, Seoul 110-779, Korea

J. B. Jeong
Department of Internal Medicine, Seoul National University Boramae Hospital, Seoul 156-707, Korea

D. Han
Department of Radiology, Catholic University Seoul St. Mary's Hospital, Seoul 137-701, Korea

C. S. Song
Department of Radiology, Seoul National University Boramae Hospital, Seoul 156-707, Korea

H. J. Moon
Department of Thoracic Surgery, Seoul National University Boramae Hospital, Seoul 156-707, Korea

Introduction

Liposarcoma develops rarely in the gastrointestinal tract. In particular, primary oesophageal liposarcoma is exceedingly rare [1–6]. Liposarcoma itself is one of the most common sarcomas of soft tissue in adult life and occurs most frequently in the deep soft tissue of the limbs [7]. To the best of our knowledge, 20 cases of primary oesophageal liposarcoma have been reported [1–6] since the first

report by Mansour et al. in 1983 [1] (Table 1). Well-differentiated liposarcoma exhibits subtle cytoarchitectural atypia on H&E staining. Therefore, auxiliary findings, such as endoscopic and immunohistochemical features, can be crucial to establish a diagnosis. However, the cases published previously seem to manifest insufficient endoscopic and immunohistochemical features.

Recently, cytogenetic analysis has been established as an essential tool for the confirmation of the diagnosis and elucidation of the genetic aetiology of well-differentiated liposarcoma. In soft-tissue neoplasm, the presence of supernumerary circular ("ring") and giant linear rod chromosomes, which are composed of 12q13–15 amplicons with amplification of *MDM2* and, frequently, *CDK4*, are cytogenetic hallmarks of well-differentiated liposarcoma [8–10]. However, chromosomal analysis of oesophageal liposarcoma has not been performed to date. Herein, we report the chromosomal composition and amplified genes of a primary oesophageal well-differentiated liposarcoma, as assessed using array comparative genomic hybridization (CGH); we also describe its clinicopathological findings, including endoscopic, radiological and immunohistochemical features.

Case Report

Clinical Features

A 67-year-old man was admitted to our hospital with weight loss, vomiting and progressive dysphagia. The patient had a history of diabetes mellitus, hypertension and seizures associated with a head trauma suffered 20 years prior to admission. A diffuse submucosal elevated lesion of the oesophagus was observed on the first endoscopic examination and the lumen was almost obstructed, precluding the passage of the endoscope (Fig. 1a). The second endoscopic examination, which was performed using a baby scope, revealed that the oesophageal lesion was a huge vertically elongated submucosal mass with a broad basal stalk (Fig. 1b and c), arising at a level located 20 cm from the incisors and extending into the distal oesophagus, 35 cm from the incisors. The hardness of the mass was soft-to-rubbery on palpation using forceps. The histopathological diagnosis of an oesophageal mucosal biopsy was unremarkable.

Three-dimensional (3D) reformatted images acquired using multidetector CT (Lightspeed 16, GE Healthcare,

Table 1 Overview of the literature on primary oesophageal liposarcoma

Source, year	Nationality	Age(yr) /Sex	Type of lesion	Tumour size (cm)	Location	Diagnosis	Treatment	Follow-up
Mansour et al. 1983	USA	53/M	Polyp	4	CE	ML	P	12 mo DF
Bak et al. 1989	Korea	49/F	Polyp	20	CE	WDL	TE	7 mo DF
Yates et al. 1990	UK	49/M	Polyp	NA	CE	ML	P	6.5 y R
Baca et al. 1991	Germany	66/F	Polyp	12	CE	ML	P	30 mo DF
Masumori et al. 1991	Japan	46/F	Polyp	11	CE	WDL	P	24 mo DF
Cooper et al. 1991	UK	68/M	Polyp	7	DE	ML	SE	12 mo DF
Boggi et al. 1997	Itally	50/M	Polyp	NA	CE	ML	P/TGR	12 mo DF
Salis et al. 1998	Argentina	73/M	Polyp	15	CE	WDL	P	NA
Mandell et al. 1999	USA	62/F	Polyp	9	CE	WDL	P/PH	4 mo DF
Rupper et al. 1999	Austria	72/F	Polyp	23	CE	WDL	P	NA
Beaudoin et al. 2002	Canada	68/F	Polyp	8.5	CE	WDL	P	25 y R
Chung et al. 2003	Korea	56/M	Polyp	21	CE	WDL	TE	NA
Brehant et al. 2004	France	70/M	Polyp	20	CE	WDL	P	16 mo DF
Garcia et al. 2004	USA	42/M	Transmural	10.5	DE	PL	TE	2 mo DF
Liakakos et al. 2006	Greece	72/M	Polyp	5	DE	WDL	P	6 mo DF
Yang et al. 2006	China	49/M	Polyp	8	CE	WDL	TE	NA
Di Mascio et al. 2006	UK	44/M	Polyp	NA	DE	WDL	P	36 mo DF
Maruyama et al. 2007	Japan	50/M	Polyp	18.5	CE	WDL	P	NA
Will et al. 2007	Germany	60/M	Polyp	20	CE	DDL	P	NA
Xu et al. 2008	China	50/M	Polyp	11	CE	WDL	P	36 mo DF
The present case	Korea	67/M	polyp	12	CE	WDL	P	15 mo DF

NA not available, *CE* cervical oesophagus, *DE* distal oesophagus, *ML* myxoid liposarcoma, *WDL* well-differentiated liposarcoma, *PL* pleomorphic liposarcoma, *DDL* dedifferentiated liposarcoma, *P* polypectomy, *SE* subtotal oesophagectomy, *TE* total oesophagectomy, *TGR* transgastric removal, *PH* pharyngotomy, *DF* disease free, *R* recurrence

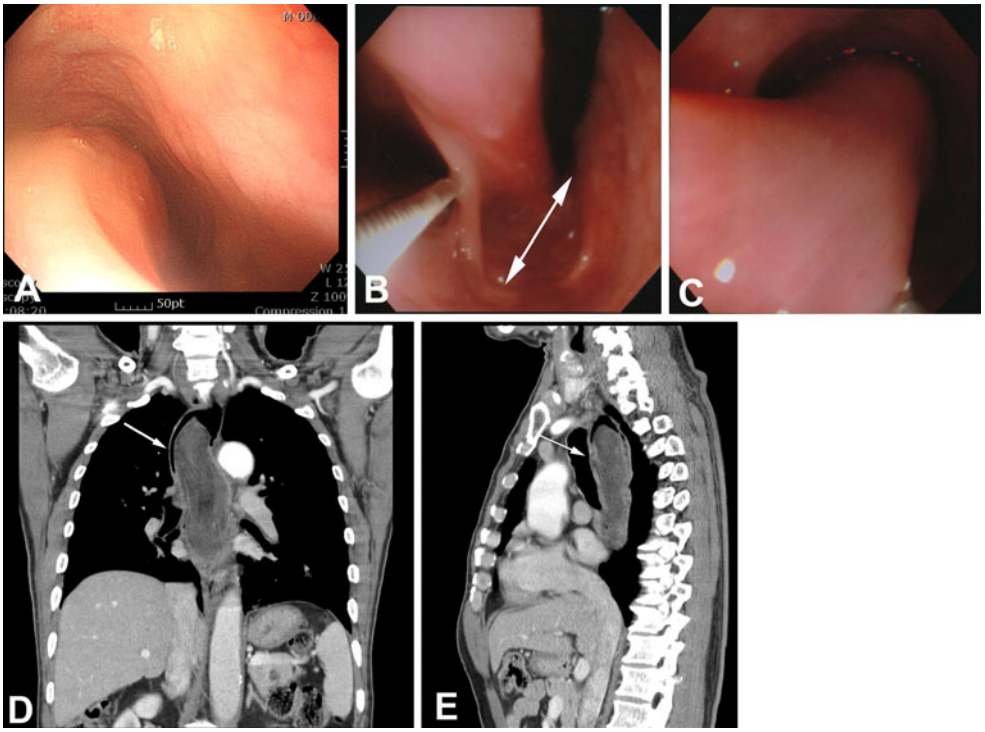

Fig. 1 Oesophageal endoscopic view (**a–c**) and reformatted post-contrast CT scan (**d–e**). **a**, An elevated submucosal lesion almost obstructs the lumen. **b** and **c**, Note a huge vertically elongated submucosal mass in the oesophagus, with a broad stalk (arrow in **b**). **d** and **e**, Coronal (**d**) and sagittal (**e**) views on CT scan show the presence of a large sausage-shaped mass (arrow), which was composed of fatty tissue, within the dilated oesophageal lumen

Milwaukee, WI, USA) revealed the entire length of the lesion, and showed a relatively broad base at the level of the thyroid cartilage and was connected to a vast sausage-like mass that extended to the lower third of the oesophagus (Fig. 1d and e). The oesophageal lumen was dilated by the bulky mass. Pre-contrast images revealed the presence of a fat density throughout the entire lesion, which was interspersed with areas of soft-tissue attenuation. Post-contrast images revealed the presence of a patchy, heterogeneous enhancement within the mass and a couple of arterial branches that originated from the base and made their course through the stalk and main portions of the mass.

Surgical resection was performed. We used an approach via the right side of the neck with a low cervical oesophagostomy; this was followed by delivery from the oesophageal lumen of a giant sausage-shaped pedunculated mass, which was 12 cm in longitudinal length and had a broad stalk (3 cm at the base; 2 cm of pedicle length), and transection of its stalk adjacent to the mucosal wall.

Pathological Features

On gross examination, the mass was 12×6×6 cm in size and was similar in shape to an eggplant with a stalk. The mass was covered with oesophageal mucosa, which exhibited multiple ulcerations (Fig. 2a). Tissue sectioning revealed that the mass was poorly demarcated beneath the mucosa of the oesophagus. The cut surface was grey or grey/yellow, soft/gelatinous, homogeneous glistening and myxoid (Fig. 2b). On microscopic examination, the superficial area located beneath the non-neoplastic squamous epithelium was fibrotic with scattered and slightly atypical spindle cells and lipocytes. The inner part of the mass was composed of slightly atypical lipocytes because of a significant size variation and was admixed with some slightly atypical stromal cells exhibiting an irregular shape and nuclear hyperchromasia in a fibrillary collagenous stroma background (Fig. 2c). High-magnification examination revealed the presence of a few lipoblasts containing a multivacuolated cytoplasm and hyperchromatic nucleus in a rich vascular network background (Fig. 2d). Immunohistochemistry demonstrated the presence of S-100-positive (1:300, Novocastra) and vimentin-positive (1:500, Dako) adipocytes, including lipoblasts (Fig. 2e), whereas the tumour cells were negative for c-kit (1:200, Dako), HMB 45 (1:500, Dako), desmin (1:200, Dako) and CD68 (1:50, Novocastra). Moreover, atypical stromal cells were positive for CD34 (1:300, Novocastra) and vimentin (Fig. 2f).

Array CGH Analysis

Array CGH was carried out according to the manufacturer's protocols (Macrogen, Inc., Seoul, Republic of Korea) using commercially available genomic DNA microarray slides (MacArray Karyo 4500, Macrogen). Briefly, tumour DNA and reference DNA were labelled with Cy3- or Cy5-labelled dCTP (NEL; PerkinElmer, Boston, MA, USA)

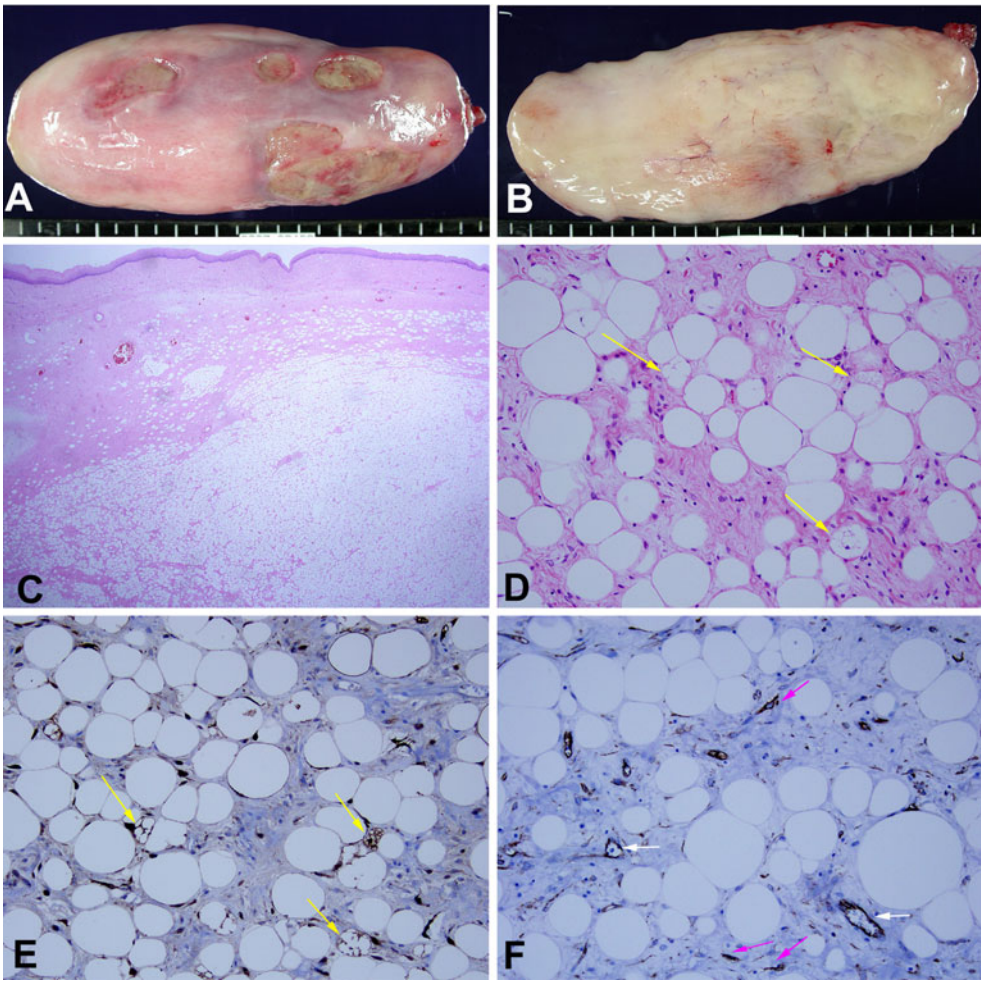

Fig. 2 Macroscopic (**a–b**) and microscopic (**c–f**) features of the resected oesophageal mass. **a**, Multiple ulceration on the mucosal surface. **b**, The cut surface of the mass is greyish yellow, soft and glistening. **c,** A poorly circumscribed tumour is composed of fibrotic and adipocytic tissue, and a squamous epithelium covering. **d**, Tumour cells consist of variable-sized lipocytes and multivacuolated lipoblasts (arrows), admixed with atypical spindle cells. **e**, Immunohistochemistry for S-100 accentuates multivacuolated lipoblasts (arrows). **f**, Some atypical spindle cells are positive for CD34 (pink arrows). Note the good vascularization with CD34-positive endothelial cells and the presence of RBCs in their lumina (white arrows)

using the BioPrime® Array CGH Genomic Labeling System (Invitrogen, Carlsbad, CA, USA). DNA samples labelled with fluorochromes were mixed with Cot-1 DNA (Invitrogen), denatured at 72°C for 10 min, and incubated at 37°C for 1 h. The hybridization mixture was then introduced into the hybridization chamber of the microarray slide, which was incubated at 37°C for 48 h. After a post-hybridization wash, the slides were rinsed, dried by spinning, scanned using GenePix4200A (Axon Instrument, Foster City, CA, USA) and analysed with an analysis software that was developed specifically for the present array-based CGH (ArrayAnalysis, Macrogen). Data processing included the calculation of the average ratio of the two replicate spots for each clone. A total of 3,775 different BAC clones were used in the final analysis. The test (Cy3): reference (Cy5) fluorescence ratio of each sample was determined automatically using the same software. Increases and decreases in sample DNA copy number were determined as thresholds set at $\log_2$ ratios of 0.25 and −0.25, respectively, according to the standard protocol provided by the manufacturer (Macrogen). High-level amplification was defined by a $\log_2$ ratio threshold >2, as described previously [11].

We identified high-level amplifications at 1p12–1q21.2, 12q13.2–12q15 and 12q21.33–12q23.1 chromosomal locations (Fig. 3). Detailed analysis of the genes located within these amplicons revealed that at least 29 genes were highly amplified (Table 2). Among the various amplicons, the 12q14.1 amplicon ($\log_2$ ratio=4.193) was the most highly amplified chromosomal region and comprised at least 10 genes, including *cyclin-dependent kinase 4* (*CDK4)*. The next most highly amplified region, the 12q15 amplicon ($\log_2$ ratio=4.144) contained at least two genes, including *murine double minute-2* (*MDM2*).

Clinical Follow-up

This patient did not receive any further treatment, such as chemotherapy or radiotherapy. In addition, there was no sign of clinical symptoms, local recurrence or metastasis during the postoperative follow-up period of 15 months.

Fig. 3 Array CGH in primary oesophageal well-differentiated liposarcoma. **a**, Whole genomic profile. Gains/amplifications are indicated to the left by "+" and losses are indicated by "−". Note the prominent amplifications on the 1q and 12q regions. **b**, Detailed profile of chromosome 12 reveals the presence of significant gains on chromosomal regions 12q13.2–12q15 and 12q21.33–12q23.1

Discussion

The differential diagnosis of well-differentiated liposarcoma and lipoma may be challenging for pathologists. Well-differentiated liposarcoma is well vascularized, but does not exhibit an antler-mimicking arborizing vascularization pattern, which is typical of other subtypes of liposarcoma [7]. The presence of atypical stromal cells and multivacuolated lipoblasts contributes to the morphological diagnosis; however, these morphological changes are often subtle. Thus, immunohistochemistry can be useful to highlight such cells. The present case was the first report of the presence of CD34-positive atypical stromal cells. Additionally, S-100-positive multivacuolated lipoblasts were delineated clearly in this paper. Immunohistochemistry was mentioned in four cases reported previously [2–6] and only one paper depicted S-100-positive cells; however, there was no description of multivacuolated lipoblasts [6].

Currently, cytogenetic analysis provides a confirmation of diagnosis, as well as evidence for the pathogenic mechanisms involved in tumour development. To our knowledge, this is the first array CGH study performed for primary oesophageal well-differentiated liposarcoma. These amplicons showed similar regions to the 1q21–25, 12q14–q15, and 12q21–22 locations detected in well-differentiated liposarcoma of soft tissue [8–10]; however, the 12q23.1 amplicon was found exclusively in the oesophageal well-differentiated liposarcoma case described here. Among the most highly amplified genes located in the

Table 2 Highly amplified chromosomal regions and corresponding genes in a primary oesophageal well-differentiated liposarcoma

Chromosome region	$\log_2$ ratio	Highly amplified genes ($\log_2$ ratio >2)
1q21.1	3.137	*BCL9*
	3.008	*GJA8, GPR89B, LOC728905*
	2.89	*LOC391092*
	2.7	*OR13Z2P*
	2.28	*ZNF364, CD160, PDZK1*
12q14.1	4.193	*CDK4, OS9, CENTG1, TSPAN31, MARCH9, CYP27B1, METTL1, FAM119B, TSFM, AVIL*
	4.082	*CTDSP2, MIRN26A2*
12q15	4.144	*MDM2, CPM*
	2.373.	*RAP1B, NUP107, SLC35E3*
12q21.33	2.778	*unnamed*
12q23.1	3.153	*AMDHD1, HAL, LTA4H*

12q region identified for oesophageal well-differentiated liposarcoma, the most conspicuous genes were *CDK4* and *MDM2*, which were amplified in well-differentiated liposarcoma of soft tissue [8–10]. In addition, several highly amplified genes, such as *amidohydrolase domain containing 1* (*AMDHD1*), *histidine ammonia lyase, or histidase* (*HAL*) and *leukotriene A4 hydrolase* (*LTA4H*) were located in the 12q23.1 amplicon but were not previously implicated in well-differentiated liposarcoma of soft tissue. To date, there have been few studies addressing the roles of *AMDHD1*, *HAL* and *LTA4H* in oncogenesis. *AMDHD1* is overexpressed (fivefold change) in adrenal adenoma compared with adrenal carcinoma, as assessed using quantitative analysis of gene expression [12]. This is consistent with the fact that well-differentiated liposarcoma shows a generally benign behaviour [1–7]. *AMDHD1* is involved in the histidine metabolism pathway [12]. *HAL* is a histidine-degrading enzyme. According to a study performed using human hepatoblastoma cells, all of the amino-acid-degrading enzymes, including HAL, may control the body's nitrogen balance in a high-protein diet [13]. *LTA4H* converts LTA4 to LTB4, which exerts its biological effect in inflammatory cells [14]. Moreover, the 5-lipoxygenase-LTA4H pathway may play roles in the proliferation of human glioma cells [15].

CDK4 and *MDM2* were regarded as two major target genes in this primary oesophageal well-differentiated liposarcoma case. CDK4 complexes with D-type cyclins (i.e., cyclin D1) regulate the progression from the G1 to the S phase of the cell cycle. In addition, MDM2 binds to, and inhibits transactivation by the tumour suppressor protein p53, as part of an autoregulatory negative feedback loop [16, 17]; thus, amplification of *MDM2* may result in excessive inactivation of p53 [17]. Amplification of *cyclin D1* or *MDM2* has been found in various types of human malignant tumour [17, 18].

In conclusion, we presented an extraordinarily rare case of primary oesophageal well-differentiated liposarcoma. This report represented a comprehensive clinicopathological study including the assessment of cytogenetic alterations and clinical follow-up data. The present case suggests that the detection of gene amplification is relevant for the confirmation of the diagnosis of well-differentiated liposarcoma of the oesophagus. The genetic aberrations underlying the pathogenesis of oesophageal well-differentiated liposarcoma may be akin to those identified in soft-tissue well-differentiated liposarcoma, in terms of the amplification of MDM2 and CDK4, although they did not correspond regarding AMDHD1, HAL and LTA4H amplification.

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