Case Report
Malignant leiomyomatosis peritonei display inter-tumor genetic heterogeneity among synchronous and metachronous lesions: molecular evidence from a single case

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Abstract: Leiomyomatosis peritonei (LMP) is a rare entity characterized by multiple leiomyomata arising and recurring in the peritoneal cavity mostly in women of reproductive age. The reported cases in literature had lead to the assumption that LMP is a benign condition, with a few exceptions, where this condition was associated with aggressive behavior reminiscent of a leiomyosarcoma. Herein, we report a case of leiomyomatosis peritonei with leiomyosarcomatous transformation, in a patient with a previous hysterectomy for benign leiomyoma uteri. Comparative immuno-histochemical using Androgen Receptor and molecular analysis using Androgen Receptor's CAG repeats are performed and discussed on the leiomyoma uteri, benign LMP nodules, and synchronous and metachronous malignant LMP nodules, followed by systematic review of literature.

Keywords: Leiomyomatosis peritonei, malignant Leiomyomatosis peritonei, leiomyoma, leiomyosarcomatous transformation, androgen receptor, androgen receptor's CAG repeats

Case report

The patient is a 48 year old woman complaining of menorrhagia of one year. Upon pelvic examination and ultrasound imaging, she was found to have uterine fibroid, for which she underwent total abdominal hysterectomy (TAH) and unilateral salpingo oophorectomy (SO). Histopathological examination revealed one benign endometrial polyp and one leiomyoma uteri (4×4 cm; microscopically showing a mitotic count of 1 per 10 HPF, no evidence of coagulative necrosis or atypia).

6 months later, she came back to clinic with abdominal pain and distention, and had abnormal ultrasound findings, prompting further evaluation by abdomino-pelvic CT scan. The radiology report described the presence of 2 large intraperitoneal soft tissue masses. The largest lobulated mass (15×12×11 cm) seen in the left lumbar region, showed heterogeneous enhancement and a necrotic center. The other mass is smaller (8 cm in diameter), circumscribed and homogenous, reminiscent of a fibroid. The left ovary was reported as "normal". No ascites is seen. The patient then underwent laparatomy and excision of the masses, with 2 additional peritoneal lesions also identified (4 and 3 cm in diameters) and sent for histopathology as well. An intra-operative frozen section was performed. Frozen section of the well-circumscribed smaller masses showed smooth muscle tumors. The average mitotic count in all 3 lesions was less than 5 per 10 HPF. No atypia, coagulative necrosis or myxoid changes were seen in any of them. Thus, those masses were diagnosed as leiomyomatosis peritonei.

Frozen section of the largest mass, however, showed a spindle cell lesion with moderate to severe nuclear pleomorphism and brisk mitotic activity (average of 10 per 10 HPF). Multiple areas of coagulative necrosis and myxoid degeneration were present. Thus a diagnosis of

Table 1. A summary of previously reported malignant LMP

Year	Author	Age\sex	Hormonal Status	Follow Up Period	Outcome
1986	Rubin et al [25]	27\F	Ov.F.T	23 mo	DOD
1990	Akkersdijk et al [26]	25\F	Normal	22 mo	DOD
1990	Lausen et al [27]	41\M	Normal	4 mo	NR
1993	Abulafia et al [13]	20\F	Normal	24 mo	DOD
1994	Sanchez A et al [28]	?\F	?	?	?
1996	Raspagliesi et al [29]	26\F	Normal	36 mo	NR
		48\F	Normal	---	DOD
1999	Morizaki et al [30]	33\F	Normal	11 mo	DOD
2003	Yamaguchi et al [9]	77\M	Normal	12 mo	NR
2007	Yu RS et al [8]	42\M	Normal	8 mo	DOD
2011	Lamarca et al [31]	37\F	normal	24 mo	DOD
2014	Zyla MM et al [32]	26\F	OCP	18 mo	NR

List of Abbreviations: F: female; M: male; Ov.F.T: ovarian fibrothecoma; FUP: follow up period; mo: month; F.U.O: follow up outcome; DOD: died of disease; NR: no recurrence.

Table 2. Androgen receptor expression and cag repeat analysis in the different tumors from the same patient

Specimen	AR Expression by IHC				AR Expression by molecular analysis			
	SI	PP	HS	IHC	DNA conc. (ng/ul)	CAG repeats	Active allele	Clonality
ULM	0	0	0	neg	134.2	-	-	-
b-LMP	2	3	6	pos	No band	-	-	-
m-LMP 1	2	4	8	pos	98.1	18, 19	19	Monoclonal
m-LMP2a	3	4	12	pos	106.5	19, 25	25	Monoclonal
m-LMP2b	3	4	12	pos	151.0	20, 26	20	Monoclonal

List of Abbreviations. ULM: uterine leiomyoma; b-LMP: benign LMP; m-LMP1: malignant LMP; m-LMP2 a and b: synchronous recurrent malignant LMP nodules; SI: stain intensity; PP: positivity percentage; HS: histologic score; conc.: concentration; neg: negative; pos: positive.

leiomyosarcoma was given. Immunohistochemical staining (IHC) was then performed on formalin-fixed tissue blocks to confirm the nature of the largest mass. The neoplastic cells were immuno-reactive diffusely for Smooth Muscle Actin (SMA), and focally to estrogen receptors (ER); while they were negative for CD10, HMB-45, WT-1, Inhibin, pan CK, CD31, or CD117 (c-kit). Considering the lack of leiomyosarcoma in her uterus or other pelvic organs, the clinical, radiological and histopathological features were consistent with the extremely rare but characteristic condition known as Leiomyomatosis peritonei with malignant (leiomyomsarcomatous) change.

4 months following her laparatomy, the patient came back with increasing abdominal discomfort. Radiological CT scan studies reveals recurrent abdominal masses in multiple locations, including right lumbar, pelvic, left upper quadrant, and at Douglas pouch. The features were highly suggestive of recurrence. No ascites, para-aortic or pelvic lymph node enlargement, nor masses in other organs were seen. Chest CT scan was unremarkable. The patient underwent excision of five peritoneal masses (largest 10 cm in max. dimension). All five masses had similar appearances and were consistent with leiomyosarcoma with a similar IHC profile to the previous masses. Follow up of the patient revealed that she had received multiple chemotherapy sessions after her second and third surgeries for another pelvic recurrence. Unfortunately, only 3 months following the last surgery, the patient succumbed to death due to her disease 15 month of the initial diagnosis of LMP.

IHC and molecular analysis

IHC analysis using antibodies for androgen receptors (AR) was performed on Formalin-fixed paraffin-embedded representative tissue blocks from the different tumors; namely- the previous benign uterine leiomyoma (ULM), benign nodules of LMP, and the malignant LMP masses (one diagnosed with other benign LMP and another 2 recurrent malignant lesions). The aim was to investigate any differential expression of AR among those tumors. Unstained microscopic slides of the corresponding blocks were taken for molecular analysis of clonality using HUMARA gene, and AR CAG repeat structure.

Anti-AR IHC stain with 1:25 dilution (Leica Biosystems Newcastle Ltd, UK) was performed. Results were interpreted and scored by 2 pathologists. Only nuclear stain was regarded positive. The staining was evaluated semi-
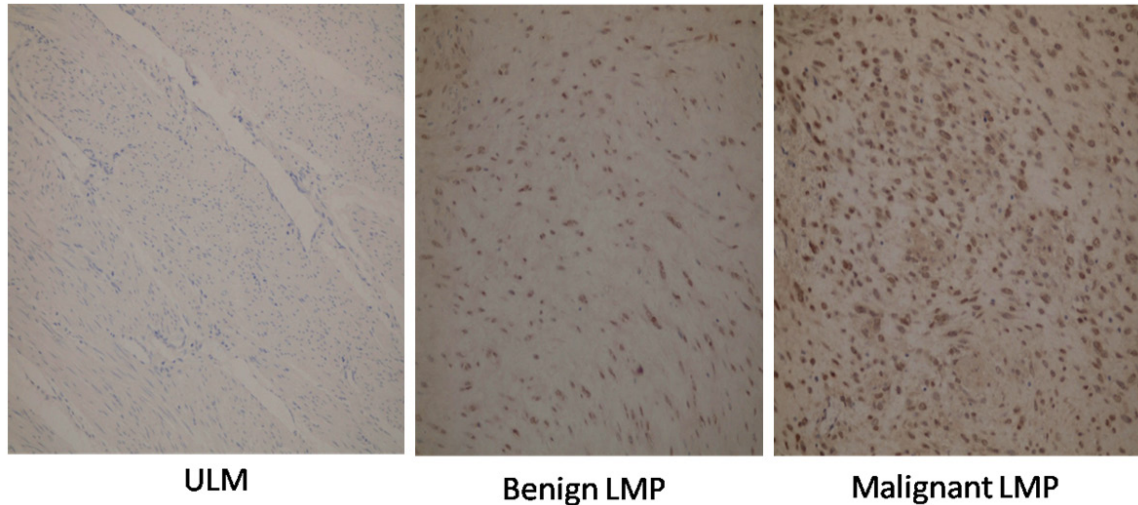
Figure 1. Differential AR expression in different tumors from the same patient. ULM: uterine leiomyoma; LMP: Leiomyomatosis peritonei. (Original magnification 200×).

quantitatively by stain intensity (SI) and percentage of positive tumor cells (PP) both considered. SI was graded from 0 (no stain); 1 (weak); 2 (moderate) ; and 3 (strong), and PP from 0, 1 (< 10%), 2 (10-50%), 3 (> 50%), and 4 (> 75%). Androgen receptor histologic score (HS) was then calculated as the average product of SI and PP on 5 random high power fields at 200× magnification. For HS = 0 was considered negative, and HS 1-12 positive. HS 1-5 was defined as low expression and 6-12 as high expression.

IHCs results (**Table 2**) was interesting in that there was a significant difference in IHC of AR scores with a gradual increase of HS score from ULM (negative) to benign LMP, and to malignant LMP nodules, where the strongest stain was obtained (**Figure 1**).

As for molecular analysis, DNA extraction performed for 5 tissue samples, Using ZR FFPE DNA MiniPrep Kit (Zymo ResearchCorp, USA). The extracted samples were amplified by the PCR reaction for AR CAG repeats using the following primers (Integrated DNA Technologies Inc, USA): TCCAGAATCTGTTCCAGAGCGTGC (forward) and ATGAGGAACAGCAACCTTCACAGC (reverse).

Samples were sent for sequencing (Genewiz Inc, USA) and chromatograms were analyzed manually using bioEdit (IBIS Biosciences, Carlsbad, CA, ISA). The sequences were compared to AR reference sequence obtained from NCBI (GenBankAH002624.2). Since the alleles could be heterozygous consisting of different number of CAG repeats resulting in peak doubling, the number of CAG repeats of each allele was determined after marking distinguishable DNA sequences as landmarks. Two CAG repeats were examined, CAG1 and CAG2, within AR exon 1. CAG2 consisted of six repeats, whereas CAG1 starting earlier was the polymorphic sequence of interest. Some previous studies used 21CAG repeat length as cutoff to differentiate between long and short length [1].

Three samples were successfully sequenced (**Figure 2**). The results were remarkable for the presence of different CAG repeat frequencies among the different tumors that were successfully sequenced (**Table 2**), including metachronous and synchronous malignant LMP nodules, indicating inter-tumor genetic heterogeneity and that each tumor mass represents a unique proliferation (multi-centric) rather than uni-centric origin of these lesions.
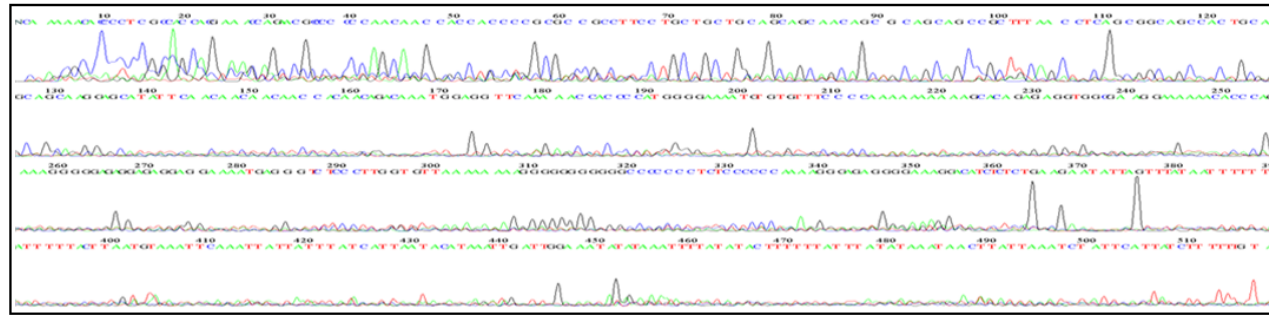
Discussion

LMP is an unusual peritoneal condition featuring proliferation of smooth muscle fibers similar to that seen in ordinary uterine leiomyomas (ULM). Although this condition may clinically and radiographically mimic peritoneal carcinomatosis, it is thought to have a benign behavior [2]. Most cases are diagnosed in reproductive
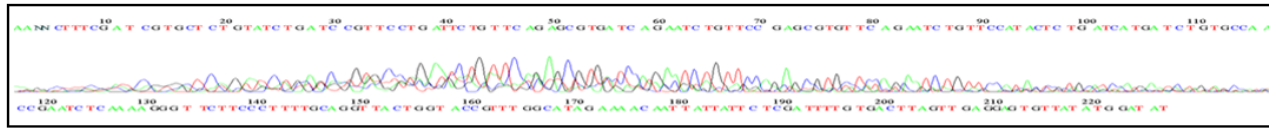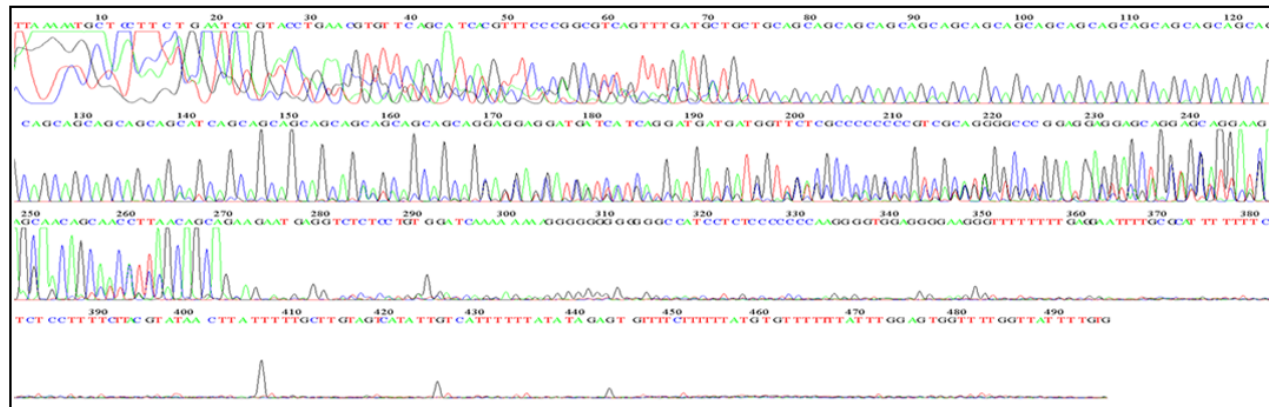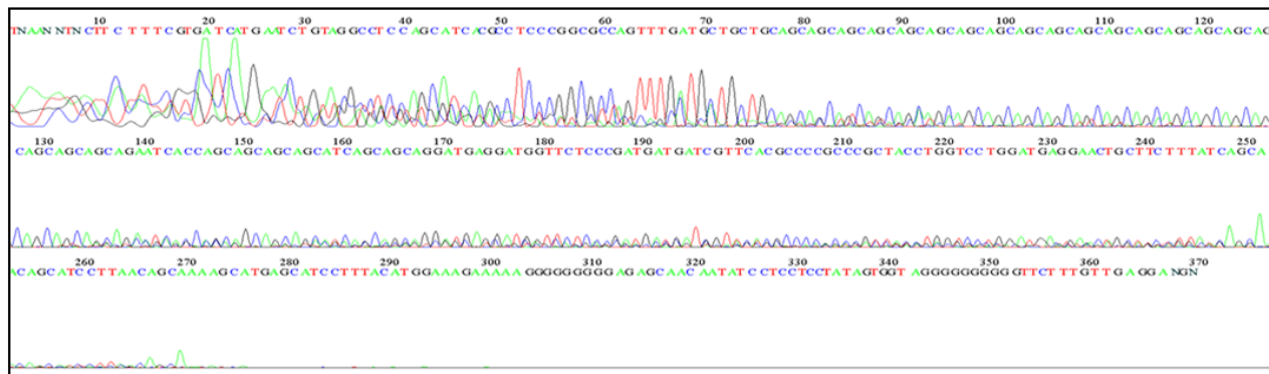
Figure 2. AR CAG repeats sequencing data in the different tumors. Only 3 samples (m-LMP 1, 2a, and 2b) were successfully sequenced and analyzed manually using AR CAG repeat reference.

aged females, usually as an incidental finding during gynecologic or non-gynecologic surgery. When symptomatic, the main symptoms are abdominal pain and increased waist girth.

The most common sites affected by LMP are the omentum, mesentery, and parietal peritoneum, followed by broad ligaments, ovarian and uterine serosa. Grossly, the lesions appear as nodular peritoneal whitish masses that vary in number from a few to numerous. Histologically, LMP tend to display bland features; along with high expression levels of estrogen and progesterone receptors [3].

The etiology of LMP is uncertain, however, most researchers believe that LMP is influenced by increased estrogens, similar to the hormonal influence seen in uterine LM [4, 5], including endogenous and exogenous sources of elevated estrogens (Oral Contraceptive Pills, ovarian estrogen-secreting tumors, pregnancy, etc). Other researchers had proposed another approach based on molecular cytogenetic analyses, considering it the result of implantation and evolution of ULM cells, following morcellation for ULM [6].

A thorough literature search has revealed over a hundred LMP, however, only 12 Cases were with a proof of a malignant behavior; the first report of a malignant LMP was in 1986 [7]. Except for three male cases (aged 41, 77 and 42 years) [8-10], all of other reports were diagnosed in females. The females were of reproductive age group except for one [11]. The age of the female patients ranged from 20 to 48 years. One case was from a pregnant woman [12].

The clinical presentation was usually a rapidly increasing abdominal mass and pain, with abnormal findings on abdomino-pelvic imaging studies, including Abdominal computed tomography (CT) and magnetic radiogram imaging (MRI) [13]. The radiographical findings included a large single mass, several masses with a dominant one, or numerous intraperitoneal small nodules and ascites. Such radiologic features maybe misinterpreted as peritoneal carcinomatosis or disseminated lymphoma [9].

Intra-operative examination of the LMP with malignant transformation typically detected focal necrosis and hemorrhage. On rare occasions, LMP was diagnosed in concurrence with other conditions including ovarian cysts, borderline serous cyst, and endometriosis [14]. Some female patients had previous or synchronous uterine LM as well [6]. A history of prior hormonal treatment was confirmed in 1 case (Gonadotropin-releasing hormone analogue) [15]. Of the reported cases, only a recent one examined the molecular profile of LMP [16]. The clinico-pathological features of the previous malignant LMP cases are summarized in **Table 1**.

Histopathological inspection of those malignant LMP shows spindle tumor cells with moderate to severe nuclear atypia, frequent coagulative necrosis, hemorrhagic foci, and possibly myxoid changes [3]. The smooth muscle origin was confirmed using IHC, as they show reactivity to SMA, and/or Desmin. Because of the rarity of the condition, the differential diagnosis of LMP is broad and can be tricky. As mentioned earlier, clinical presentation of LMP is not specific and can be indistinguishable from symptoms of other gynecological conditions, including uterine fibroids [17]. Radiologically, LMP nodules can be easily mistaken for pelvic carcinomatosis or even peritoneal spread of lymphoma/leukemia [13]. Thorough examination of the female genital tract, namely, the uterus is needed to exclude those nodules being a metastasis from a uterine LMS. Histologically, LMP are smooth muscle tumors, as confirmed by IHC. These nodules must be differentiated, however, from other mesenchymal neoplasms like endometrial stromal sarcomas, rhabdomyosarcoma, poorly differentiated sarcomas, carcinosarcomas, and undifferentiated carcinomas.

The molecular backgrounds of LMP are not well understood, however, there had been approaches using similar methods to those used for the more common uterine *smooth muscle tumor:*

X chromosome inactivation. Quade et al [18] analyzed X chromosome inactivation in 44 LMP

from 4 patients by the methylation status of AR (HUMARA), and found that the same parental X chromosome was non-randomly inactivated in all LMP lesions, consistent with a metastatic uni-centric neoplasm. This contrasts with the previously observed random distribution of inactivated X chromosomes among multiple ULMs in an individual with respect to parental origin [19], consistent with a hypothesis of independent origin of each ULM.

Chromosomal abnormalities in DPL. A few molecular cytogenetic studies to assess the role of chromosomal abnormalities in DPL had been recently carried on. Morton et al [20], had found that all of the chromosomal abnormalities detected using FISH in a case of LMP, including r (1) (p34.3q41), del (3) (q23q26.33), and t (12; 14) (q14.3; q24.1), are distinctive chromosomal abnormalities detected in Uterine LM as well, signifying that functional genetic modification in this chromosomal region may play a role in LMP development from uterine LM.

MED12 mutations. Mutations in the mediator complex subunit 12 (MED12), has recently been identified as the most frequent genetic aberration in uterine LM, mostly in the form of frequent somatic mutations by exome sequencing [21]. Heterozygous mutations in intron 1 and exon 2 of the gene were detected in a high percentage of LMS as well [22]. Although it is believed that most LMS do not originate from pre-existing LMs, in one study, the two LMSs harboring the MED12 mutation also showed a benign component, with identical mutation, the authors suggested that a small subgroup of LMS may develop from MED12-mutated LMs [23]. It was suggested that MED12 has oncogenic function in a wide variety of smooth muscle tumors, including tumors arising in extra-uterine locations [22]. However, the definite role of MED complex in tumorigenesis or functional impact in uterine smooth muscle tumors is not fully clear. Similar gene alterations were also detected in some concurrent and metachronous extra-uterine LMs, particularly LMP [24].

Finally, LMP is a rare entity with only little knowledge of its pathogenesis and evolution in medical literature. Although we present a single patient here, the presence of this spectrum of smooth muscle tumors with some differential IHC and molecular profile is interesting. Whether the current results are of clinical significance is in need of further and larger scale studies.

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Disclosure of conflict of interest

None.

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