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Case series

Impact of lower uterine segment involvement in type II endometrial cancer and the unique mutational profile of serous tumors

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ABSTRACT

Objective: Evaluation of the impact of lower uterine segment involvement (LUSI) in type II endometrial cancer, and mutational profile of uterine papillary serous carcinomas (UPSC).
Methods: Retrospective cohort study comparing patients with type II endometrial cancer with LUSI to patients without LUSI. Genes commonly implicated in carcinogenesis were analyzed in a subgroup of 42 patients with UPSC using next generation sequencing.
Results: 83 patients with type II endometrial cancer were included in the study, of these, LUSI was diagnosed in 31.3%. During a median follow-up of 45.5 months, patients with LUSI developed more local and distant recurrences (local: 19.2% vs. 3.5%, $P = .03$; distant: 50% vs. 17.5%, $P = .004$) and progression events (73.1% vs. 26.3%, $P < .001$), with shorter mean progression-free survival (16 months compared to 26.5 months, $P < .01$). In a multivariate analysis, LUSI was the only significant pathological factor, associated with a 2.9-fold increase in the risk of progression ($P = .007$), and a 2.6-fold increase in the risk of death ($P = .02$). In the subgroup of patients with UPSC, mutations were identified in 54 genes, including *TP53* (80%), *PPP2R1A* (40%), and *PTEN* (22.5%). Frequent mutations in the PTEN-PI3K-AKT signaling pathway were found in patients with tumor in the upper uterine segment only ($P = .04$), with *PTEN* being mutated in 29% of the samples ($P = .07$).
Conclusion: Type II endometrial cancers presenting in the LUS have a significantly worse prognosis and this might be associated with a unique mutational profile.

1. Introduction

Type II endometrial carcinomas, including uterine papillary serous carcinomas (UPSC) and clear cell carcinomas (CC), are generally associated with aggressive clinical behaviors (Moore and Fader, 2011). As with colorectal cancer, tumor location has been proposed as a prognostic factor in EC (Liu et al., 2017). While some studies have analyzed the importance of lower uterine segment involvement (LUSI), they primarily focused on patients with low grade endometrioid tumors (Masuda et al., 2011). The aim of this study was to evaluate the importance of lower uterine segment involvement in type II EC and to determine whether tumor location is correlated with a distinctive molecular profile.

2. Materials and methods

2.1. Study population

The study was conducted at the Jewish General Hospital, a tertiary care hospital in Montreal, Canada and approved by Institutional Review Board, protocol #03-041.

The study cohort included 83 consecutive patients with type II EC

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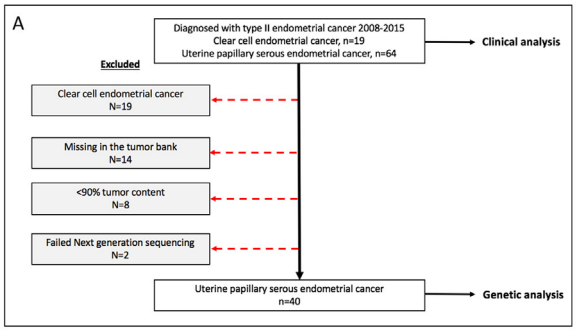

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characteristics	Tumor location: LUS N=26	Upper N=57	P-value	Tumor size: >2cm N=64	≤2cm N=19	p-value	LVSI: LVSI+ N=38	LVSI- N=45	P-value
Median age	72(53-84)	68(41-85)	0.71	71(41-85)	65(43-85)	0.29	72(41-85)	67(43-85)	0.38
BMI	27.2(18.7-44.6)	27.2(17-53)	0.87	27.2(17-53)	27.2(20-42.6)	0.68	26(18.7-44.6)	27.9(17-53)	0.25
ASA score			0.59			0.77			0.84
1	5 (19.2%)	8(14%)		10(15.6%)	3(15.8%)		7(18.4%)	6(13.3%)	
2	12(46.2%)	29(50.9%)		31(48.4%)	10(52.6%)		12(31.6%)	24(53.3%)	
3	8(30.8%)	18(33.3%)		21(32.8%)	5(26.3%)		17(44.7%)	14(31.1%)	
4	1(3.8%)	0		1(1.6%)	0		1(2.6%)	0	
missing	0	2(3.5%)		1(1.6%)	1(5.3%)		1(2.6%)	1(2.2%)	
Histology type			0.58			0.76			0.20
UPSC	19(73.1%)	45(78.9%)		50(78.1%)	14(73.7%)		32(84.2%)	32(71.1%)	
Clear cell	7(26.9%)	12(21.1%)		14(21.9%)	5(26.3%)		6(15.8%)	13(28.9%)	
FIGO 2009 Stage			<0.01			0.03			0.009
1	4(15.4%)	35(61.4%)		24(37.5%)	14(73.7%)		10(26.3%)	28(62.2%)	
2	6(23.1%)	3 (5.2%)		9(14.1%)	1(5.3%)		6(15.8%)	4(8.9%)	
3	14(53.8%)	13(22.8%)		25(39.1%)	2(10.5%)		16(42.1%)	11(24.4%)	
4	2 (7.7%)	6(10.5%)		6(9.4%)	2(10.5%)		6(15.8%)	2(4.4%)	
Retrieved nodes	11(2-18)	14(0-33)	0.11	13(0-33)	9(2-30)	0.55	12.5(2-18)	14(0-33)	0.10
Positive nodes									
Pelvic	9(34.6%)	14(24.6%)	0.43	21(32.8%)	2(10.5%)	0.08	13(34.2%)	10(22.2%)	0.33
Para-aortic	2 (7.7%)	5 (8.8%)	1.0	7(10.9%)	0	0.32	6(15.8%)	1(2.2%)	0.01
Recurrence	15(60%)	11(19.6%)	0.001	21(33.9%)	5(26.3%)	0.59	16(43.2%)	10(22.7%)	0.06
Distant	13(50%)	10(17.5%)	0.004	19(29.7%)	4(21.1%)	0.57	14(36.8%)	9(20%)	0.14
Local	5(19.2%)	2(3.5%)	0.03	5(7.8%)	2(10.5%)	0.66	3(7.9%)	4(8.9%)	1.00
Death	16(61.5%)	12(21.1%)	<0.001	25(39.1%)	3(15.8%)	0.06	21(55.3%)	7(15.6%)	<0.001
Adjuvant treatment	25(96.1%)	49(86%)	0.26	59(92.2%)	15(78.9%)	0.20	37(97.4%)	37(82.2%)	0.035
Radiation	22(84.6%)	42(73.7%)	0.4	54(84.4%)	10(52.6%)	0.01	33(86.8%)	31(68.9%)	0.07
Chemotherapy	25(96.1%)	46(80.7%)	0.09	57(89.1%)	14(73.7%)	0.13	37(97.4%)	34(75.6%)	0.005

Data are median (range) or n (%). ASA = American Society of Anesthesiologists. FIGO=International Federation of Gynecology and Obstetrics.

Fig. 1. Study population: A. Selection criteria. B. Patient characteristics, histology, staging and outcomes by tumor location, size and LVSI.

(64 patients with UPSC and 19 patients with clear cell carcinoma) out of 544 fully staged patients with EC between the years 2008–2015 (Fig. 1A). All cases were originally evaluated by a gynecologic pathologist and re-evaluated independently by 2 gynecologic pathologists for this study. A tumor originating in the uterine isthmus was classified as LUS.

The surveillance period includes routine follow-up examinations every 4 months during the first two years, followed by every 6 months for up to 5 years, and then yearly thereafter. Overall survival (OS) was defined as time from diagnosis to either last follow-up or death. Progression-free survival (PFS) was defined as the time from surgery to either date of recurrence or death. Recurrences were diagnosed clinically or radiologically.

2.2. Sequencing

Out of 64 patients in the cohort with UPSC, 50 patients had a tumor sample in our tumor bank. Sections (8–12 mm) from fresh frozen surgical tumor samples were cut and stained with hematoxylin and eosin (H&E). Forty-two samples with a serous carcinoma content of over 90% were selected for subsequent analysis. Fig. 1A illustrates the study population for the genetic analysis. DNA was extracted from the cancer samples using the DNeasy Blood and Tissue Kit (Qiagen, Toronto, ON, Canada). DNA concentration and purity was assessed using the NanoDrop ND-100 spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA). Next Generation Sequencing was performed using the Illumina MiSeq platform (Illumina Inc., San Diego, CA). The list of targeted regions can be found in the supplementary files (Supplementary Table 1). 168 genes were targeted at 420 different mutational hotspots. The library was prepared using the Nimblegene TruSeqLT preparation kit (Illumina Inc., San Diego, CA). The Genome Reference Consortium Human Build 38 (hg38; RefSeq accession: GCF_000001405.26) was used for the reference alignment.

2.3. Mutation analysis

The resulting VCF files were annotated in silico using the Ensembl Variant Effect Predictor (Yates et al., 2016). Since carcinogenic genetic variants are thought to be sporadic in a healthy population, we selected for rare variants using their reported population allele frequency using the gnomAD database (Lek et al., 2016). Alleles with a population allele frequency below 1.5% were designated as rare and kept for further downstream analysis. Where needed, the raw BAM files were manually visualized using the Integrated Genome Viewer (Robinson et al., 2011) for possible reading mistakes by the variant caller. Synonymous or intronic mutations were also removed from our study, except if the mutation occurred within three base pairs of a coding exon, in which case the mutation was identified as a splice site mutation. Missense mutations were annotated using the following prediction tools: PolyPhen-2 (Adzhubei et al., 2010), Sift (Vaser et al., 2016), MCAP(Jagadeesh et al., 2016), MutationAssessor (Reva et al., 2011) and REVEL (Ioannidis et al., 2016).The same mutations were kept for further analysis if they were predicted as pathogenic by at least three out of the five tools. All data manipulations were done using the R program (www.cran.r-project.org).

2.4. Statistical analysis

Statistical analysis was performed using SPSS 24 (IBM Corp, College Station, TX). Statistical significance was calculated using the chi square or the Fisher's exact tests for differences in qualitative variables and the Wilcoxon rank sum test for differences in continuous variables.

Kaplan-Meier survival curves were used to calculate survival estimates (PFS and OS) and the log rank test was used in order to quantify survival differences according to different variables. A multivariate analysis using the Cox proportion hazards model was performed to assess the hazard ratio of the prognostic factors for PFS and OS.

3. Results

Out of the 83 patients with type II EC, 26 had LUSI (31.3%) and these were compared to 57 (68.7%) patients with upper uterine tumors. Patient and pathological characteristics and outcomes are summarized in Fig. 1B. Patients with LUSI, large tumors, and LVSI were more likely to be diagnosed with advanced FIGO (2009) stage disease (III-IV) ($P < .01$, $P = .03$, and $P < .01$, respectively).

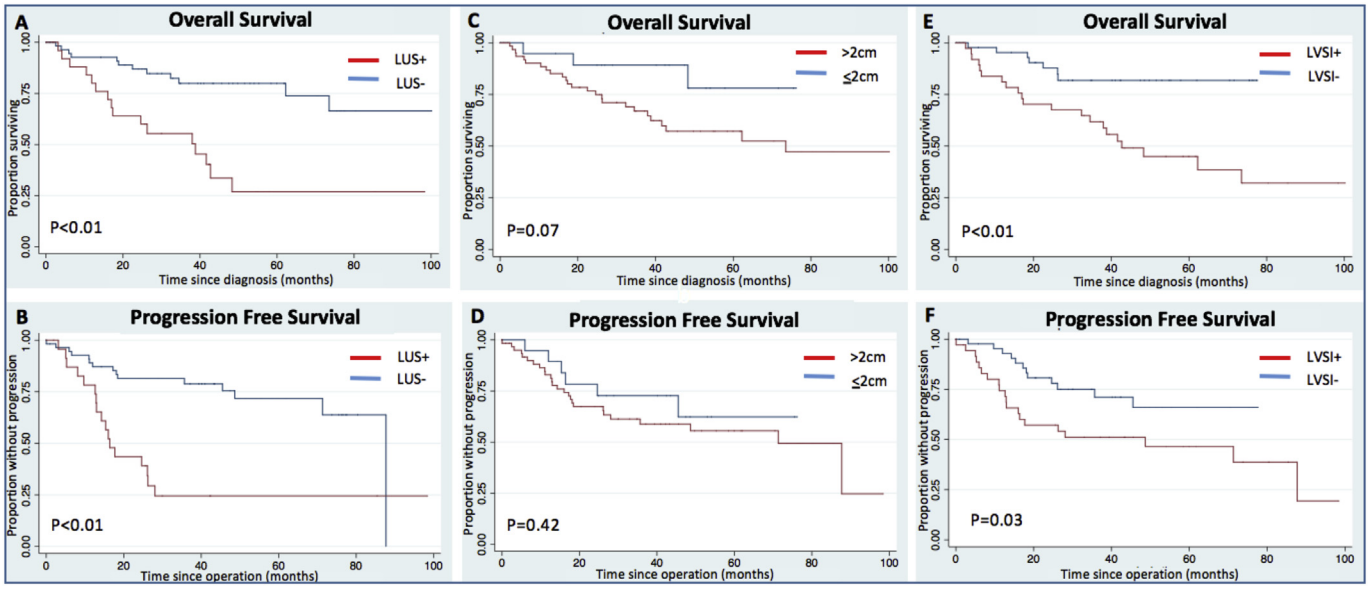


Fig. 2. Kaplan-Meier OS and PFS analyses by: (A,B) Tumor location (LUS vs. upper uterine segment) OS. (C,D) Tumor size (> 2 cm vs. < 2 cm). (E,F) LVSI+ vs LVSI−.

Table 1
Multivariate analysis - risk factors for recurrence.

	PFS 95% confidence interval				OS 95% confidence interval			
Risk factor	Hazard ratio	Lower	Upper	*P*-value	Hazard ratio	Lower	Upper	*P*-value
LUS+	2.9	1.3	6.2	0.007	2.6	1.1	5.9	0.025
LVSI+	1.5	0.6	3.4	0.38	2.1	0.8	5.5	0.14
Tumor size > 2 cm	0.9	0.3	2.6	0.83	1.2	0.3	4.7	0.78
Age (≥65 vs. < 65)	2.3	1.0	5.0	0.05	2.5	1.04	6.00	0.04
Stage (I/II vs III/IV)	7.8	3.3	18.3	< 0.001	6.9	2.6	17.9	< 0.001

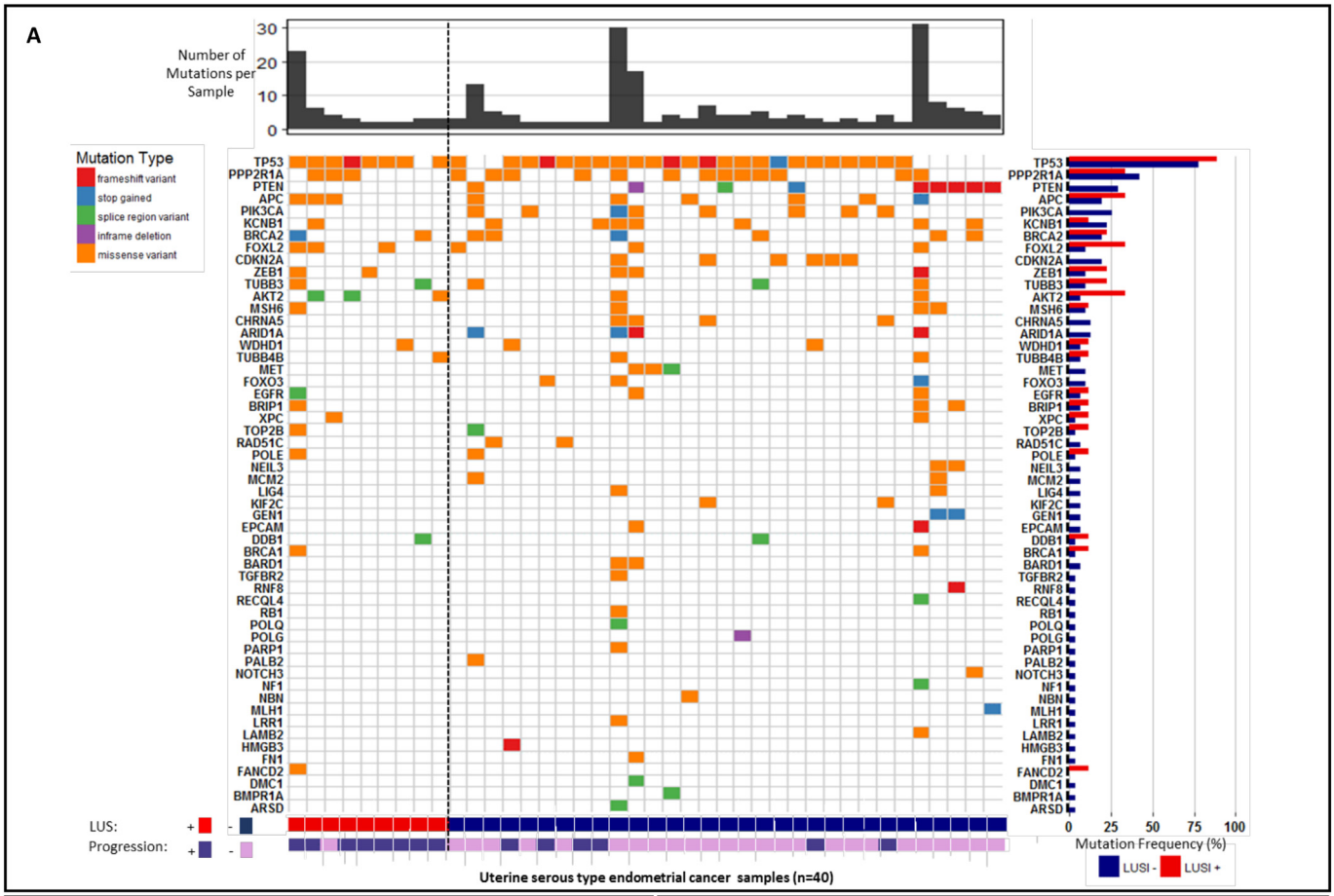
Data for time-to-event analyses were updated up to September 27, 2016. The median follow-up time for all patients was 45.5 months (range, 1.7–103.6 months). During the follow-up period, 26 women (31.3%) had recurrent disease and 28 died (33.7%). Of the 3 uterine factors studied, LUSI was identified as the only factor associated with disease recurrence. Patients with LUSI had more distant recurrences (50%, 13 out of 26, versus 17.5%, 10 out of 57, $P = .004$) and local recurrences (19.2%, 5 out of 26 versus 3.5%, 2 out of 57, $P = .03$). LUSI was associated with a greater risk of recurrence in stages I-II only (44.4% vs 7.9%, $P = .018$). Presence of LUSI was inversely associated with survival (38.5% versus 78.9%, $P = .01$). Fig. 2 presents the Kaplan-Meier curves for overall survival (OS) and Progression-Free Survival (PFS) in patients with LUSI, tumor diameter larger than 2 cm, and LVSI. On multivariate analysis, LUSI remained the only significant uterine risk factor (Table 1), in addition to age and stage.

When analyzing a subgroup of forty-two UPSC samples, using next generation sequencing, 54 out of 168 sequenced genes harbored mutations in forty samples following mutation filtering, as sequencing failed for two samples (Fig. 1A). Nine of the sequenced tumors originated in the lower uterine segment. Fig. 3A shows a landscape overview of the frequency of mutated genes, the number of mutations per sample and the type of mutation in function of tumor location in the uterus. Six samples exhibited a hyper-mutated phenotype ($N > 8$ mutations) due to their increased mutational frequency compared to all other samples. One out of these six samples originated from the LUS. *TP53* was found to be the most commonly mutated gene in the UPSC cohort (Fig. 3A, 80%), without a statistically significant difference between lower and upper uterine segment tumors (88.9% vs. 77.4%, respectively, $P = .449$, Fig. 3B). *PPP2R1A* was found to be mutated in 40% of the patients (41.9% and 33.3% of the lower and upper segment tumors, respectively, $P = .643$). *PTEN*, *PIK3CA*, *CDKN2A* and *ARID1A* were found to be mutated only in upper uterine segment tumors (29%, 12.9%, 19.4% and 12.9%, respectively). Fig. 3B shows the most recurrently mutated genes in our patient cohort (≥10% of the samples). *AKT2* mutation frequencies were found to be significantly different between LUS positive and negative groups (33.3% vs 6.5%, respectively, $P = .03$). A large frequency of mutations within the PTEN-PI3K-AKT signaling axis were found in the tumors from the upper segment only (45%, $P = .04$, Fig. 3C). No other pathways were found to be differently mutated between the two groups.

4. Discussion

The association between tumors located in the lower uterine segment and worse prognosis has been previously described for type I cancers (Masuda et al., 2011; Gemer et al., 2009). LUSI was associated with pelvic and para-aortic nodal disease, but not with recurrence in a study of 208 patients with high grade EC that included a small subset of 35 serous and 12 clear cell cancers (Doll et al., 2014).To understand the difference between our results on type II cancers and this study containing a mix of high grade type I cancers and type II cancers, we evaluated a subgroup of high grade, type I cancers and found that using a multivariate analysis, LUSI in high grade type I tumors did not predict adverse outcome (data not shown) in contrast to type II cancers. This suggests that type I and type II high grade cancers are clinically different. In our cohort, with only type II cancers, LUSI was significantly associated with worse outcome.

The frequency of three of four recurrently mutated genes (*TP53*,



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Gene	Frequency LUS + (%)	LUS - (%)	Total %	P value
TP53	88.9	77.4	80	0.449
PPP2R1A	33.3	41.9	40	0.643
PTEN	0	29	22.5	0.066
APC	33.3	19.4	22.5	0.377
KCNB1	11.1	22.6	20	0.449
BRCA2	22.2	19.4	20	0.85
FOXL2	33.3	9.7	15	0.08
CDKN2A	0	19.4	15	0.152
ZEB1	22.2	9.7	12.5	0.316
TUBB3	22.2	9.7	12.5	0.316
AKT2	33.3	6.5	12.5	0.032
PIK3CA	0	12.9	10	0.256
MSH6	11.1	9.7	10	0.9

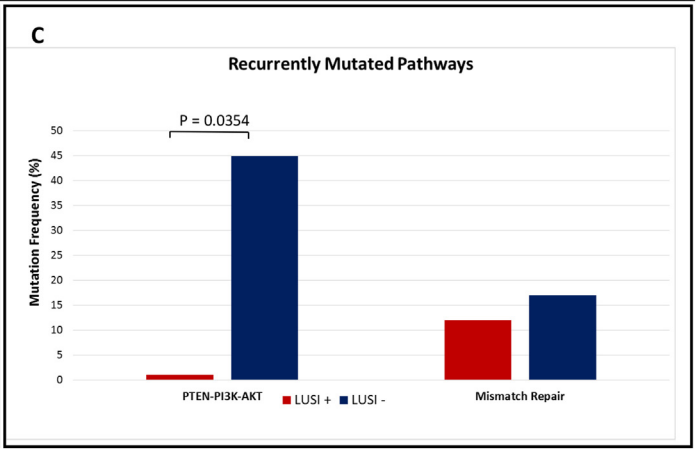

Fig. 3. Somatic mutations profiles of lower uterine segment UPSC versus upper uterine tumors: A. A landscape overview of the frequency of mutated genes, the number of mutations per sample and the type of mutation in function of tumor location in the uterus. B. Frequency of the most mutated genes (≥10%) in lower and upper UPSC tumors. C. Frequency of mutations within the PTEN-PI3K-AKT signaling axis and the mismatch repair pathway in function of tumor location.

PPP2R1A, *PIK3CA*) concurred with previously described frequencies (Cancer Genome Atlas Research N et al., 2013), confirming the serous histology of our samples (Supplementary Table 3). *PTEN* was mutated at a higher frequency in our cohort (22%) than in other studies. A significant difference in the mutation frequency of *AKT2* was observed between the two groups in this study, but three of the five variants were classified as likely benign in the Reference Sequence database (Supplementary Table 2). *FOXL2* was mutated in 33% of all LUSI tumors, but only in 9% of upper uterine segment tumors. The specific mutations have not previously been reported in the COSMIC or RefSeq databases (Supplementary Table 2). The PTEN-PI3K-AKT signaling axis is a known major pathway of carcinogenesis, especially in high-grade Type I endometrial cancer (Cancer Genome Atlas Research N et al., 2013). A pathway-driven analysis of UPSC of the upper uterine segment revealed multiple alterations in the PTEN-PI3K-AKT signaling axis (45%). Only one patient with a mutation in this pathway experienced a progression event, and no tumors situated in the lower uterine segment had a mutation in the same pathway. While the LUSI positive group is small (n = 9), our post hoc analysis estimated that we could detect a mutation in this pathway in that group, assuming a mutation rate equal to that of the LUSI negative group, with α = 5% and power = 77%. All nine *PTEN* mutations, including the missense mutations, are loss-of-function mutations. Conversely, all four *PIK3CA* mutations, have been described before and annotated as pathogenic in the ClinVar database

(Supplementary Table 2) and three of them were mutually exclusive from *PTEN* mutations. Moreover, they are all putative activating mutations due to their location with the C2 functional domain of the protein (Gymnopoulos et al., 2007). *TP53* mutations were found in six of the twelve patients mutated in this pathway and the tumors were confirmed to be histologically serous by two different gynecologic pathologists. Together, our results suggest that a significant subset of histologically serous tumors of the upper uterine segment may be driven by endometrioid-like, PTEN-PI3K-AKT oncogenic defects, despite mutations in *TP53*. This may partially explain the difference in outcome between the groups, since patients PTEN-driven serous tumors have better PFS and OS than patients with TP53-driven tumors (Supplementary Fig. 1A–B). However, both groups still fared better than patients with lower uterine segment tumors.

Our results suggest that tumor location in the lower uterine segment is an independent prognostic factor for local and distant recurrence, and survival in type II EC. This study has several limitations: other genetic events may play a significant role in UPSC carcinogenesis, the number of LUS tumors that were analyzed was limited, and somatic copy number alterations and epigenetic events were not considered in this study. The main strengths of this study lie in the fact that this data was collected in a single tertiary center where all the patients were fully staged, treated, and followed up. Finally, the two study cohorts were well balanced with regards to adjuvant treatment received and other clinical factors such as age, ASA, and BMI. All tumor samples underwent pathology review prior to molecular analysis and only tumors with > 90% UPSC were included. Moreover, our stringent mutation filtering pipeline allowed us to preferentially consider genomic mutations most likely to have a deleterious effect in the regression analyses.

In conclusion, our data suggests that LUSI is a significant adverse survival factor, associated with a unique mutational profile. Other well established risk factors in endometrioid type EC such as LVSI and tumor size may not be as relevant in patients with UPSC/clear cell carcinomas. Alternative prognostic markers based on the molecular biology of these tumors should be further investigated in the future in order to offer individualized treatments driven by molecular pathways.

Disclosure

The authors report no conflict of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.gore.2018.03.004.

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