Gynecologic Oncology Reports 24 (2018) 43–47

Contents lists available at ScienceDirect

Gynecologic Oncology Reports

journal homepage: www.elsevier.com/locate/gynor

Case series

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

Liron Kogan[a,c,1], David Octeau[b,c,1], Zainab Amajoud[a], Jeremie Abitbol[a,b], Ido Laskov[a,d], Alex Ferenczy[e], Manuela Pelmus[e], Neta Eisenberg[f], Roy Kessous[a], Susie Lau[a], Amber Yasmeen[c], Walter H. Gotlieb[a,c,*], Shannon Salvador[a]

[a] Division of Gynecologic Oncology, Segal Cancer Center, Jewish General Hospital, McGill University, Montreal, Quebec H3T 1E2, Canada.
[b] Department of Experimental Medicine, Faculty of Medicine, McGill University, Montreal, Quebec, Canada
[c] Segal Cancer Center, Lady Davis Institute of Medical Research, McGill University, Montreal, Quebec, Canada
[d] Department of Obstetrics and Gynecology, Lis Maternity Hospital, Tel Aviv Sourasky Medical Center, Tel Aviv University, Tel Aviv, Israel
[e] Department of Pathology, Segal Cancer Center, Jewish General Hospital, McGill University, Montreal, Quebec H2T 1E2, Canada
[f] Department of Obstetrics and Gynecology, Rabin Medical Center, Tel-Aviv University, Tel Aviv, Israel

ARTICLE INFO

Keywords:
Lower uterine segment
Uterine papillary serous carcinoma
Type II endometrial cancer
Sequencing
Gene analysis
PTEN mutation

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

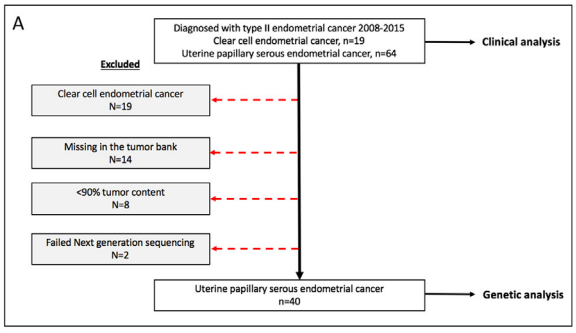
2.1. Study population

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

* Corresponding author at: Division of Gynecologic Oncology, McGill University, SMBD Jewish General Hospital, 3755 Chemin de la Cote-Ste-Catherine, Montreal, Quebec H3T 1E2, Canada.
E-mail address: walter.gotlieb@mcgill.ca (W.H. Gotlieb).
[1] Both authors contributed equally.

https://doi.org/10.1016/j.gore.2018.03.004
Received 2 January 2018; Received in revised form 4 March 2018; Accepted 14 March 2018
Available online 19 March 2018



B

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).