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Case series

Targeted sequencing of histologically defined serous endometrial cancer reflects prognosis and correlates with preoperative biopsy

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ABSTRACT

The aim of this study was to evaluate the impact of discordant endometrial sampling on the prognosis of patients finally diagnosed with uterine papillary serous carcinoma (UPSC) and to analyze UPSC mutational profile. Retrospective cohort study comparing outcomes of patients post-operatively diagnosed with UPSC and pre-operatively diagnosed with endometrioid endometrial cancer (EEC) or UPSC. Genes commonly implicated in carcinogenesis were analyzed in a subgroup of 40 patients post-operatively diagnosed with UPSC, using next generation sequencing. 61 patients with UPSC on post-surgical, final pathology were included in the study. Prior to surgery, 15 were diagnosed with EEC (discordant) and 46 were correctly diagnosed with UPSC (concordant). After a median follow-up of 41.6 months [5.4–106.7], a preoperative diagnosis of EEC was associated with better 3-year progression-free survival (100% vs. 60.9%, P = 0.003) and longer disease free interval (63.5 versus 15 months, P = 0.026) compared to patients with an initial diagnosis of UPSC. Patients with a concordant diagnosis of UPSC were 5 times more likely to progress or die compared to those with a discordant EEC diagnosis (P = 0.02, P = 0.03, respectively), and their tumors were associated with higher rates of TP53 (88.9% vs. 61.5%, P = 0.04), and a lower rate of PTEN (14.8% vs. 38.5%, P = 0.09) and ARID1A (3.7% vs. 23.1%, P = 0.05) mutations. A pre-surgical diagnosis of EEC is associated with improved prognosis in patients with UPSC. Some histologically defined UPSC tumors contain endometrioid-like molecular characteristics that may confer a survival advantage, suggesting a possible need for molecular approaches to better stratify patients into risk groups.

1. Introduction

Uterine papillary serous carcinoma (UPSC) tumors are generally associated with aggressive clinical behaviors. UPSC is frequently a component of mixed histology tumor with endometrioid components. Previous studies have suggested controversial conclusions concerning the survival of patients with mixed tumors compared to pure UPSC.

Gilks et al. (2013) suggested that in high grade endometrial cancer, agreement between two reviewers regarding the interpretation of pathological slides was reached in only 25–40% of cases, and final diagnostic consensus among three reviewers was reached in 62.5% of cases (Gilks et al., 2013). While tumor heterogeneity may be a contributing factor, this suggests that, even among experienced pathologists, histotype and grade assignment in EC, particularly in large high-grade tumors, is inaccurate, leading to inconsistent categorization of tumors (Gilks et al., 2013).

The aim of the current study was to evaluate the prognostic value of preoperative histology of endometrioid carcinoma on survival and to assess whether next-generation sequencing could be used to detect the UPSC or EC features of the post-surgical samples.

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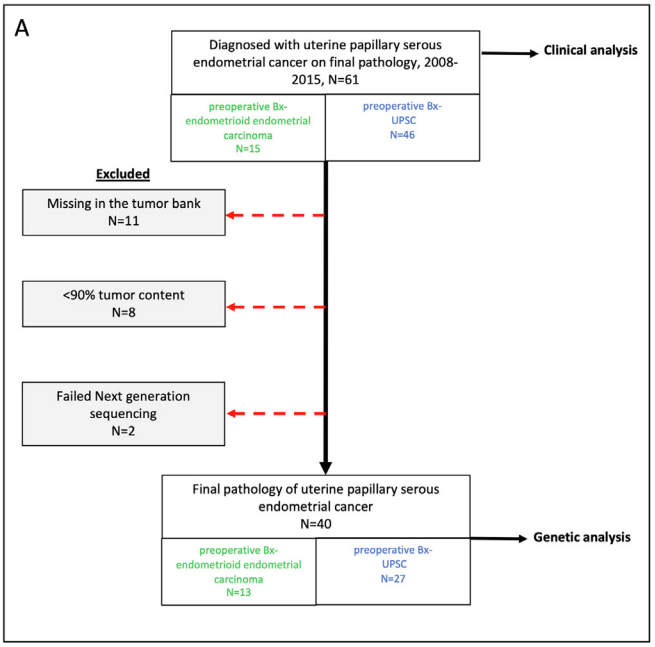

B

Variable	All patients N=61	Initially UPSC N=46	Initially endometrioid N=15	P -value
Patient factors				
Median age	71(43-85)	72(43-85)	68(50-77)	0.09
BMI (kg/m²)	26.8(17-53)	26.2(17-53)	31.3(18.4-45.4)	0.127
ASA score				0.806
1	8(13.1%)	7(15.2%)	1(6.7%)	
2	31(50.8%)	22(47.8%)	9(60%)	
3	20(32.8%)	15(32.6%)	5(33.3%)	
4	1(1.6%)	1(2.2%)	0	
missing	1(1.6%)	1(2.2%)	0	
Follow-up (months)*	41.6(5.4-106.7)	37.1(5.4-88.2)	56.9(9.6-106.7)	0.038
Tumor factors				
Final Histology				
Endometrioid comp ≥ 10%	29(47.5%)	18(38.1%)	11(73.3%)	0.036
Endometrioid comp ≥ 25%	23(37.7%)	12(26.1%)	11(73.3%)	0.002
Endometrioid comp ≥ 50%	18(29.5%)	8(17.4%)	10(66.7%)	0.001
FIGO 2009 Stage				0.806
I	29(47.5%)	22(47.8%)	7(46.7%)	
II	8(13.1%)	5(10.9%)	3(20%)	
III	18(29.5%)	14(30.4%)	4(26.7%)	
IV	6(9.8%)	5(10.9%)	1(6.7%)	
Myometrial invasion	54(88.5%)	41(89.1%)	13(86.7%)	1.0
LVSI	30(49.2%)	22(47.8%)	8(53.3%)	0.772
Lymph nodes dissection	60(98.4%)	45(97.8%)	15(100%)	1.0
Mean dissected LN	12.2(7.2)	11.4(6.6)	14.5(8.4)	0.151
Lymph nodes+	14(23%)	9(19.6%)	5(33.3%)	0.302
Peritoneal wash+	5(8.2%)	4(8.7%)	1(6.7%)	0.684
Mean tumor dia.(cm)	3.6(2.3)	3.5(2.2)	3.8(2.6)	0.834
Treatment factors				
Adjuvant rad	46(75.4%)	33(71.7%)	13(86.7%)	0.317
Adjuvant chem	53(86.9%)	38(82.6%)	15(100%)	0.182
Lab factors				
Preop CA125	26(5-2160)	29(5-2160)	16.5(7-93)	0.244

Data are median (range) or n (%). ASA = American Society of Anesthesiologists. UPSC= Uterine Papillary Serous Carcinoma. FIGO=International Federation of Gynecology and Obstetrics. LVSI=Lymph-Vascular Space Invasion.* From diagnosis.

Fig. 1. Study population: (A) Selection criteria. (B) Patient characteristics, final pathology and treatment by preoperative histology.

2. Materials and methods

2.1. Population and data collection

The study was conducted at the division of Gynecologic Oncology, Segal Cancer Center, Jewish General Hospital, a tertiary care hospital in Montreal, Canada. The study is in accordance with the declaration of Helsinki and was approved by the Institutional Review Board (protocol #2019-1547 and #15-070), with annual reviews. The study population consisted of 61 consecutive patients with UPSC, identified from our prospectively maintained computerized database composed of 544 consecutive patients who were diagnosed and surgically staged for EC between the years 2008–2015. All cases were originally pre- and post-operatively pathologically evaluated, and reviewed again, double blindly, as part of this study by two experienced gynecologic-pathologists. Both study pathologists agreed on the pre- and post-operative diagnoses 100% of the time, based on the microscopic definition of serous carcinoma, where the specimen consists of at least 10% serous component. Preoperative endometrial biopsies for all 61 cases post-operatively diagnosed with UPSC, were examined (Fig. 1A). Patients who had initially been diagnosed with UPSC (a concordant diagnosis between the biopsy and the final pathology) were compared to those who had initially been diagnosed with EEC (discordant diagnosis).

Overall survival (OS) was defined as time from diagnosis (date of endometrial biopsy) to either last follow-up or death. Progression-free survival (PFS) was defined as the time from surgery to either diagnosed date of recurrence or death. Recurrences were diagnosed clinically or radiologically.

2.2. Sequencing

8–12 mm sections from fresh frozen surgical blocks of 50 patients were stained with hematoxylin and eosin (H&E) and reviewed by a pathologist. Forty-two samples with carcinoma content of over 90% were selected for subsequent analysis. Fig. 1A illustrates the selected study population. DNA was extracted with the DNeasy Blood and Tissue Kit (Qiagen, Toronto, ON, Canada). DNA concentration and purity was measured with 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). 420 hotspots representing 168 cancer-related genes were targeted (genomic regions targeted listed in supplementary Table 1). Nimblegene TruSeqLT preparation kit (Illumina Inc., San Diego, CA) was used to prepare the library. The Genome Reference Consortium Human Build 38 (hg38; GCF_000001405.26) was used for the reference alignment.

3. Mutation analysis

Missense variants were annotated *in silico* with the Ensembl Variant Effect Predictor (Yates et al., 2016). We prioritized rare variants with a reported population allele frequency below 1.5% in the gnomAD database (Lek et al., 2016). Synonymous or intronic mutations were removed from the analysis, unless the mutation occurred within three base pairs of a coding exon. The potential pathogenicity of each missense variant was assessed 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). Variants were kept if they were predicted as pathogenic by at least three out of five tools. All data manipulations were within the R environment (www.cran.r-project.org).

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 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.

5. Results

5.1. Patient characteristics and oncologic outcomes

Sixty-one patients met the study inclusion criteria diagnosed post-operatively with UPSC on surgical pathology. Fifteen subjects (24.6%)

that had been preoperatively diagnosed with EEC were compared to 46 patients (75.4%) with a concordant diagnosis of UPSC (Fig. 1A).

Patient characteristics are summarized in Fig. 1B. The median age of the women in the cohort was 71 [range, 43–85]. Other clinical characteristics such as BMI and ASA scores were not significantly different between the groups, although patients previously diagnosed with EEC had a trend toward higher BMI compared to patients, originally diagnosed with UPSC (median BMI of 31.3 [18.4–45.4] versus 26.2 [17–53], p = 0.127). Almost half of patients also had an endometrioid component of at least 10% (mixed tumor) on final pathology. Patients who were biopsied with EEC were more likely to be finally diagnosed with mixed tumors compared to patients with UPSC in their preoperative endometrial sampling (73.3% versus 38.1%, P = 0.036). This association was found to be stronger with higher endometrioid component cut-offs of 25% and 50% (p = 0.002 and p = 0.001, respectively). There were no statistically significant differences in the rate and number of dissected nodes, FIGO stage, myometrial invasion, positive peritoneal cytology, and maximal tumor diameter.

Forty-six (75.4%) and fifty-three (86.9%) women received adjuvant radiation treatment and chemotherapy, respectively, without significant statistical differences between the two groups (p = 0.317 and p = 0.182, respectively).

Data for time-to-event analyses were updated up to Sep 27, 2016. The median follow-up time for all patients was 41.6 months (range, 5.4–106.7 months) with significantly longer follow-up in patients preoperatively diagnosed with EEC (56.9 [9.6–106.7] versus 37.1 [5.4–88.2], P = 0.038). Table 1A summarizes the survival outcomes of the patients in each cohort. During the follow-up period, seventeen women (27.9%) had recurrent disease and 21 (34.4%) died. In a univariate analysis, a preoperative diagnosis of EEC was associated with significantly better 3-year PFS (100% vs. 60.9%, p = 0.003) and OS (100% vs. 69.6%, p = 0.014), longer disease free interval (63.5 vs. 15 months, P = 0.026) and better survival (66.5 versus 20 months, p = 0.011) compared to women initially diagnosed with UPSC.

Fig. 2 and Table 1B present the Kaplan-Meier OS and PFS in patients initially diagnosed with EEC compared to patients with a concordant diagnosis of UPSC, adjusted by stage and age in a cox-regression model. Age 70 or greater (hazard ratio 2.4 (95% CI 1.0–6.2), P = 0.055), advanced FIGO stage (16.4 (5.6–48), P < 0.001), and preoperative diagnosis of EEC (0.2 (0.1–0.8), P = 0.02) significantly impacted PFS. Preoperative diagnosis of EEC also significantly impacted OS 0.2 (0.1–0.9), P = 0.037).

5.2. Mutational profiles

In forty patients who were post-operatively diagnosed with UPSC and also had their tumors sequenced (Fig. 1A), 54 out of 168 sequenced genes harbored mutations predicted to be pathogenic. Thirteen out of 40 sequenced tumors were misdiagnosed preoperatively as EEC. Fig. 3A shows a landscape overview of the frequency of mutated genes, the number of mutations per sample and the type of mutation (on final pathology) in function of preoperative pathology. Six samples exhibited a hyper-mutated phenotype (N > 8 mutations) due to their increased mutational frequency compared to all other samples. Three out of those six samples were preoperatively diagnosed with EEC. *TP53* was found to be the most commonly mutated gene in patients post-operatively diagnosed with UPSC, overall (Fig. 3A, 80%), but that frequency differed significantly between patients preoperatively diagnosed with EEC and UPSC tumors (61.5% vs. 88.9%, respectively, P = 0.04, Fig. 3B). *PPP2R1A* was found to be mutated in 40% of patients (23.1% and 48.1% of preoperatively EEC and UPSC, respectively, p = 0.135). *PTEN, PIK3CA* and *ARID1A* were found to be mutated in 22.5%, 20% and 10% of the samples (*PTEN*- 38.5% and 14.8%; *PIK3CA*- 30.8% and 14.8%; *ARID1A*-23.1% and 3.7% of preoperatively EEC and UPSC tumors, respectively, p = 0.09, p = 0.242, p = 0.06).

FOXO3 and *TUBB4B* were only mutated on final pathology of preoperative EEC tumors. (23.1%, p = 0.01). Interestingly, all four patients in the preoperative EEC group without *TP53* mutations harbor a *PTEN* mutation and did not recur. Fig. 3B illustrates the most frequently mutated genes in our patient cohort.

6. Discussion

Our findings suggest that women preoperatively diagnosed with UPSC had a 5-fold greater risk for progression and death compared with those initially diagnosed with EEC, even after adjusting for confounding variables. Endometrioid-like mutational features were overrepresented in the final pathology of preoperatively EEC group, which may account for the differences in pathogenesis and prognosis.

Roelfsen et al. compared 58 patients with mixed UPSC to 50 patients with pure UPSC and showed that the major prognostic factors for PFS and OS were stage and pure UPSC pathology (Roelofsen et al., 2012). In this multi-center study, women with pure UPSC histology had a 2.9 and 2.6-fold greater risk for recurrence and death respectively, compared with those with mixed tumors (Roelofsen et al., 2012). Other studies failed to show a significant difference in survival between patients with pure UPSC and those with mixed tumors (Elit et al., 2004). Preoperative biopsy might serve as a fingerprint of the endometrioid component of the tumor, as endometrial sampling results of EEC were found to be associated with a final pathology of mixed tumors in almost three-quarters of patients in this group.

Inter- and intraobserver variability of grade and histotype assignment in relatively large tumors might lead to a discrepancy between preoperative biopsies and surgical specimen (Batista et al., 2016). However, it has recently been shown that molecular classification can be achieved preoperatively and accurately predict the molecular features in the final hysterectomy specimens, demonstrating concordance superior to grade and histotype (Talhouk et al., 2016). Thus, the endometrioid component of the tumor, sampled before the surgery, may be reflected at the molecular profile of the final pathology specimen.

Patients for whom the initial diagnosis was changed after surgery had significantly less frequent *TP53* mutations in their tumors compared to patients with a concordant diagnosis. *TP53* gene mutations are markers of adverse outcomes in endometrial cancer mostly due to their

Table 1A
Prognosis by initial pathology.

Variable	All patients N = 61	Initially UPCS N = 46	Initially endometrioid EC N = 15	P-value
Overall mortality	21(34.4%)	18(39.1%)	3(20%)	0.222
3-years survival	47(77%)	32(69.6%)	15(100%)	0.014
Median survival time* (months)	24.4(5.4–76.6)	20(5.4–50)	66.5(42.1–76.6)	0.011
Recurrence	17(27.9%)	14(30.4%)	3(20%)	0.524
Local	5(8.2%)	5(10.9%)	0	0.321
Distant	16(26.2%)	14(30.4%)	2(13.3%)	0.312
3-years progression free survival	43(70.5%)	28(60.9%)	15(100%)	0.003
Median time to recurrence* (months)	17.0(5.1–74.4)	15(5.1–48.5)	63.5(52.7–74.4)	0.026

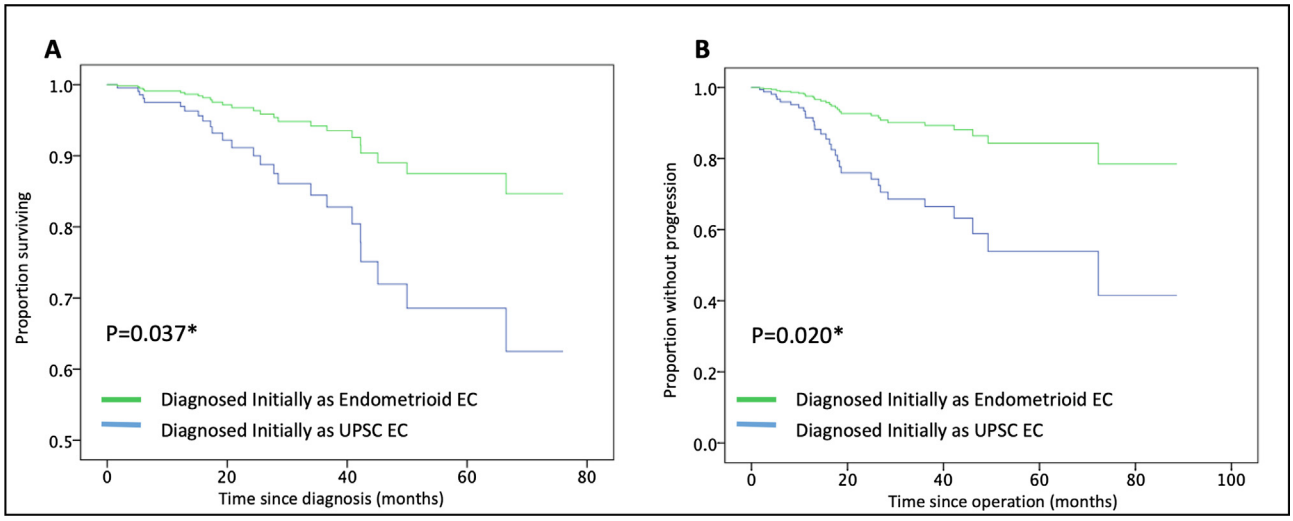

Fig. 2. Kaplan-Meier survival analyses by initial diagnosis: (A) Overall survival. (B) Progression free survival.

strong association with UPSC (Cancer Genome Atlas Research et al., 2013; Jones et al., 2015; Kuhn et al., 2012). Interestingly, in the subset of patients without a *TP53* mutations and preoperative diagnosis of EEC, loss-of-function *PTEN* mutations were found. The TCGA study previously observed this mutual exclusivity between the two genes that suggest distinct oncogenic mechanisms between EEC and UPSC. While histology does not seem to differentiate the two in post-operative surgical samples, next-generation sequencing was able to detect the endometrioid characteristics of a significant proportions of UPSCs. In addition to *PTEN* mutations, *PIK3CA* mutations potentially disrupting the PI3K/AKT pathway were also found more often in preoperative EEC samples. Disruptions in this pathway are a hallmark of endometrioid tumors. Preoperative EEC tumors seem to have strong endometrioid-like characteristics even though they were diagnosed as UPSC after surgery. These molecular differences may account for the survival differences observed in our cohort.

The relatively small number of UPSC tumors and genes analyzed clinically and genetically limits the ability to derive deeper conclusions from our clinical and molecular findings, despite our statistically significant results. Furthermore, our results rely on single tumor biopsy samples (snapshots) to portray tumor mutational profile and should therefore be interpreted carefully, taking into account intratumor heterogeneity. Thus, the genomic landscape of our tumor samples might not reflect all the changes responsible for the significantly different clinical behavior observed in serous tumors, namely those diagnosed initially as endometrioid compared to serous type. However, since EC is a molecularly heterogeneous disease and that UPSCs are often composed of mixed cancer cell populations, our results suggest that next-generation sequencing may be a powerful tool to detect and perhaps quantify the endometrioid and serous components of a particular tumor.

The main strength of this study lies in the fact that this data was collected in a single tertiary center where all the patients were fully staged, including lymphadenectomy, treated, and followed up. The two cohorts that were compared were well balanced with regards to clinical factors as age, ASA, and BMI. Finally, the data for the study was based on prospectively collected and computerized data as reported by gynecologic oncologists directly after the operation and in every follow-up visit thereafter. While misclassification of histology cases is possible, all tumor samples underwent pathology review prior to molecular analysis. Preoperative samples with different histological results than on final pathology were reviewed and examined in our hospital. Moreover, our stringent mutation filtering pipeline allowed us to preferentially consider mutations with putative deleterious effects.

In conclusion, this single-institution study evaluating surgically staged EC patients with UPSC suggests that preoperative EEC fingerprint is significantly associated with better outcomes and a unique mutational profile on final pathology, possibly reflecting a prominent endometrioid component at final pathology and potentially implicating different oncogenic pathways than other UPSC.

Author Contribution section

David Octeau – Bioinformatics, writing manuscript (methods, results and discussion).

Jeremie Abitbol – Statistics analysis, writing manuscript (methods, discussion).

Zainab Amajoud – Collecting data, editing manuscript.

Ido Laskov - Collecting data, editing manuscript.

Alex Ferenczy – Pathology review of all the cases.

Manuela Pelmus – pathology review of all the cases.

Neta Eisenberg - editing manuscript, designing research.

Roy Kessous - Collecting data, editing manuscript.

Emad Matanes - editing manuscript.

Susie Lau – Editing manuscript, designing research.

Amber Yasmeen – DNA extraction, editing manuscript, designing research.

Vanessa Lopez-Ozuna – Bioinformatics, editing manuscript.

Table 1B
A multivariate analyses of prognostic factors for OS and PFS in patients with UPSC (n = 61).

	Overall survival 95% confidence interval				Progression free survival 95% confidence interval			
Risk factor	Hazard ratio	Lower	Upper	P-value	Hazard ratio	Lower	Upper	P-value
Age ≥ 70	5.0	1.7	15.0	0.004	2.4	1.0	6.2	0.055
Initial diagnosis (Endometrioid vs. Serous)	0.2	0.1	0.9	0.037	0.2	0.1	0.8	0.020
Stage (III/IV)	13.4	4.4	40.6	< 0.001	16.4	5.6	48.0	< 0.001

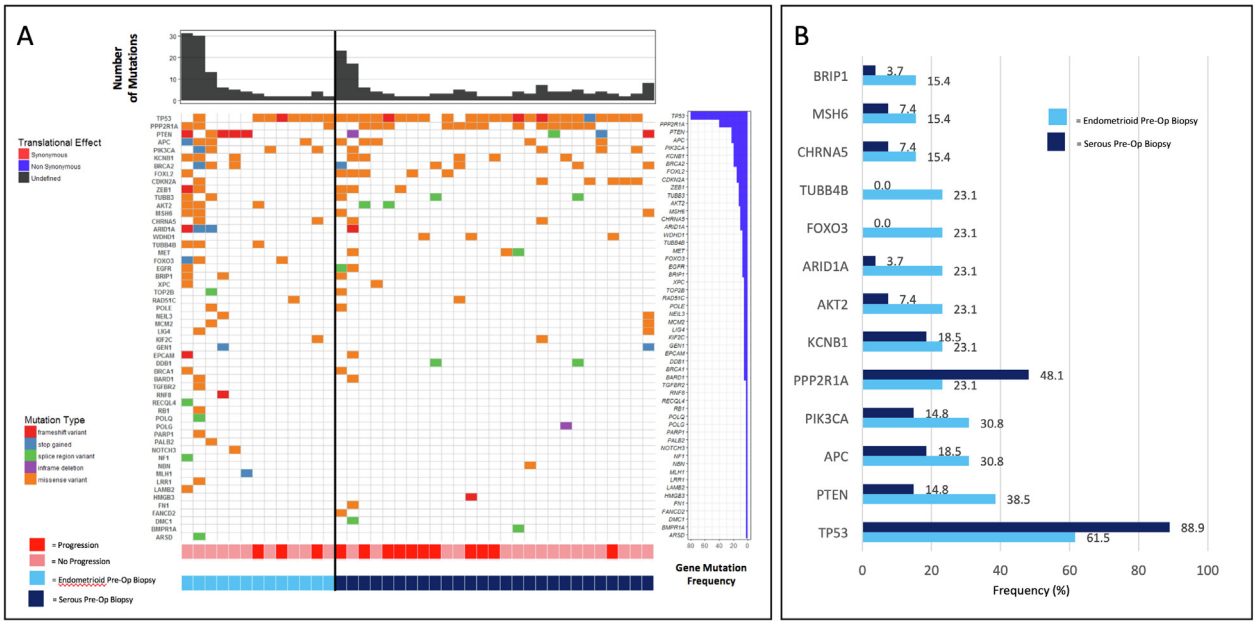

Fig. 3. Somatic mutations profiles of patients diagnosed finally with UPSC by their preoperative histology: (A) A landscape overview of the frequency of mutated genes, the number of mutations per sample and the type of mutation. (B) Frequency of the most mutated genes (≥10%).

Shannon Salvador- Editing manuscript, designing research.

Walter H. Gotlieb - Editing manuscript and writing discussion, designing research.

Liron Kogan – Collecting data, DNA extraction, statistics analysis, writing manuscript (intro, methods, results and discussion).

Declaration of Competing Interest

The authors report no conflict of interest.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.gore.2019.100521.

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