Identifying Potential Therapeutics by Molecular Profiling of 136 Cases of Uterine Clear Cell Carcinoma

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Abstract

Background: Clear cell carcinoma of the endometrium (CCE) is a rare subtype of endometrial cancer (EC) associated with worse prognosis when compared to other high grade EC subtypes. We aim to evaluate molecular, genomic and protein expression patterns in a large cohort of CCEs in order to direct patients to rational therapeutic strategies and clinical trials.
Methods: Out of 3133 EC submitted to Caris Life Sciences from March 2011 to July 2014, 136 CCEs were identified based on reported pathology. Testing was ordered per physician request and included a combination of sequencing (Sanger or next generation sequencing), protein expression (immunohistochemistry), and /or gene amplification (FISH/CISH).
Results: Of the samples evaluated by sequencing, the most common genetic mutations were P53 (40%) and BRCA2 (33%). Hormone receptor expression was low: ERα (35%), PR (22%) and AR (7%). ERBB2 was mutated in 8%, while its protein product Her2, a biomarker for HER2-directed therapies, was amplified in 12% and expressed in 5% of patients. Aberrations of the PI3K pathway, including 26% PTEN and 25% PIK3CA mutation rate, with 69% loss of PTEN expression, highlight potential utility with inhibitors of this pathway. DNA repair pathway was altered in our CCE cohort as well: low ERCC1 (6%) and MGMT (34%) expression, and high BRCA2 mutation rate, suggesting sensitivity to alkylating agents. Evaluation of the cMET pathway showed 40% IHC expression. TUBB3, a class III β-tubulin, was infrequently expressed (15%), and TLE3 expression was high (29%) compared to other EC subtypes, implicating sensitivity to microtubule-stabilizing agents. Increased TOPO2A expression, associated with anthracycline efficacy, was seen in over 80% of cases. Loss of RRM1, a DNA synthesis protein known to determine efficacy of gemcitabine, was seen in 78% of cases.
Conclusions: Our findings highlight the genetic heterogeneity of CCE, and identified altered cellular pathways with potential diagnostic and predictive values for therapeutic intervention. Drugs targeting the pathways for DNA repair, PI3K, and receptor tyrosine kinases, as well as gemcitabine and taxanes, may warrant consideration in selected patients with CCE.

Background

- Uterine clear cell carcinoma is a rare subtype of endometrial cancer that accounts for only 1-6% of all uterine malignancies.[1-2]
- Uterine clear cell carcinomas are considered to be Type-II estrogen-independent and exhibit aggressive behavior with worse clinical outcomes compared Type I tumors.[3]
- Uterine clear cell carcinomas most often occur in post menopausal women with a median age of 66-68 years.[4]
- Similar to uterine serous cancers, clear cell carcinoma is more prevalent in African American women.[5]
- While the pathogenesis of many subtypes of uterine cancer have been well studied, the molecular pathways involved in uterine clear cell carcinoma development are poorly understood.
- Broader understanding of the molecular and genomic characteristics of uterine clear cell carcinomas could contribute to the growing body of literature this disease and potentially provide treatment options with targeted therapies.

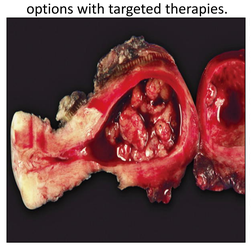


Figure 1a. Gross example of uterine clear cell carcinoma showing exophytic polypoid mass occupying the endometrial cavity[5]

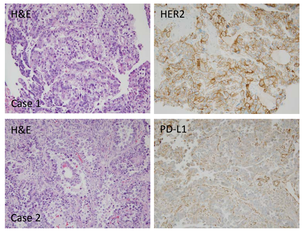


FIGURE 1b. (Case 1) Clear cell carcinoma, H&E and Her2 positive IHC staining, (Case 2) Clear cell carcinoma, H&E and PD-L1 positive IHC staining.

Methods

- 136 out of 3133 (4.3%) of endometrial cancers submitted to Caris Life Sciences from March 2011 to July 2014 were identified as clear cell carcinoma.
- Specific testing was performed per physician request and included a combination of sequencing (Sanger, NGS or pyrosequencing), protein expression (IHC), gene amplification (CISH or FISH), and/or RNA fragment analysis.
- IHC analysis was performed on formalin-fixed paraffin-embedded tumor samples using commercially available detection kits, automated staining techniques (Benchmark XT, Ventana, and AutostainerLink 48, Dako), and commercially available antibodies.
- Fluorescent in-situ hybridization (FISH) was used for evaluation of the HER-2/neu [HER-2/CEP17 probe], EGFR [EGFR/CEP7 probe], and cMET [cMET/CEP7 probe] (Abbott Molecular/Vysis). HER-2/neu and cMET status were also evaluated by chromogenic in-situ hybridization (INFORM HER-2 Dual ISH DNA Probe Cocktail; commercially available cMET and chromosome 7 DIG probe; Ventana). The same scoring system was applied as for FISH.
- Direct sequence analysis was performed on genomic DNA isolated from formalin-fixed paraffin-embedded tumor samples using the Illumina MiSeq platform. Specific regions of 47 genes of the genome were amplified using the Illumina TruSeq Amplicon Cancer Hotspot panel.
- Mutation analysis by Sanger sequencing included selected regions of BRAF, KRAS, NRAS, c-KIT, EGFR, and PIK3CA genes and was performed by using M13-linked PCR primers designed to amplify targeted sequences.
- Retrospective data analysis; Statistical analysis (unpaired t-tests used to compare biomarker expression across histologic subtypes) performed using Prism™ v6. Biomarker associations were calculated by two-tailed Fisher Exact tests.

Results

Figure 2. Pie chart representing the histology of all 3133 cases of endometrial cancer reviewed.

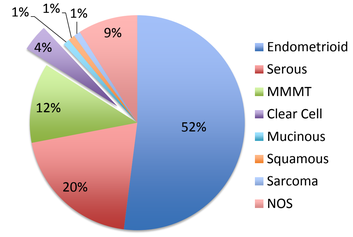
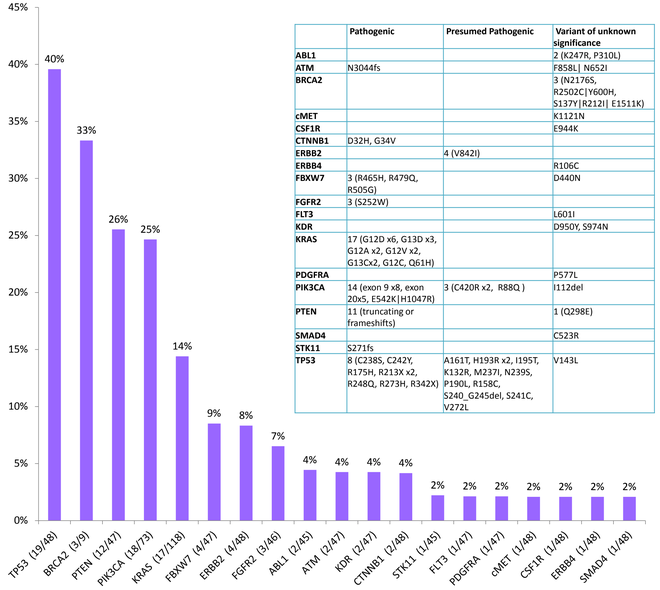


Figure 3. Clear Cell Carcinoma patient and tumor characteristics

Patient Age	N
20-40	4
41-60	35
61-80	85
81-91	12
Average	65.7 yrs

Specimen Site	N
Uterus|endometrium	82
Peritoneal tissue	15
Vagina	4
Cervix	5
Ovary	6
Colon	4
Lung	3
Lymph nodes	7
Others	10

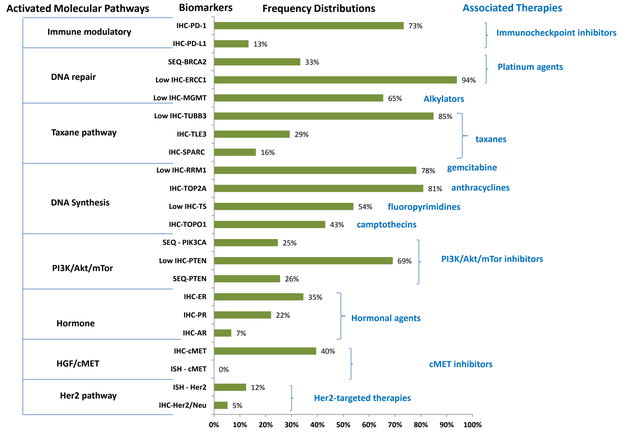
Figure 4. Mutation Prevalence in Clear Cell Carcinoma



	Pathogenic	Presumed Pathogenic	Variant of unknown significance
ABL1			2 (K247R, P310L)
ATM	N3044fs		F858L| N652I
BRCA2			3 (N2176S, R2502C|Y600H, S137Y|R212I| E1511K)
cMET			K1121N
CSF1R			E944K
CTNNB1	D32H, G34V		
ERBB2		4 (V842I)	
ERBB4			R106C
FBXW7	3 (R465H, R479Q, R505G)		D440N
FGFR2	3 (S252W)		
FLT3			L601I
KDR			D950Y, S974N
KRAS	17 (G12D x6, G13D x3, G12A x2, G12V x2, G13Cx2, G12C, Q61H)		
PDGFRA			P577L
PIK3CA	14 (exon 9 x8, exon 20x5, E542K|H1047R)	3 (C420R x2, R88Q)	I112del
PTEN	11 (truncating or frameshifts)		1 (Q298E)
SMAD4			C523R
STK11	S271fs		
TP53	8 (C238S, C242Y, R175H, R213X x2, R248Q, R273H, R342X)	A161T, H193R x2, I195T, K132R, M237I, N239S, P190L, R158C, S240_G245del, S241C, V272L	V143L

Results (cont)

Figure 5. Molecular and genomic aberrations of endometrial clear cell carcinomas and potential pathway directed therapeutics



Conclusions

- Our findings contribute to a growing fund of knowledge in this rare and poorly-understood endometrial cancer.
- The data eludes to the molecular heterogeneity of this disease , confirms many of the known pathway aberrations, and offers novel pathways for therapeutic exploration.
- We identified PI3K pathway aberrations suggesting potential utility in targeting this pathway.
- DNA repair pathways were altered as well, suggesting sensitivity to alkylating agents in a subset of patients with uterine clear cell carcinoma.
- TUBB3 was infrequently expressed and TLE3 expression was high in a subset of our patients, suggesting sensitivity to microtubule-stabilizing agents
- Increased TOPO2A expression, know to be associated with anthracycline efficacy, was seen in over 80% of cases.
- More than a third of tumors expressed ER suggesting a role for hormonal therapy in a subset of patients.
- Prospective studies and additional clinical data are needed to better understand the significance of these findings and offer prognostic and therapeutic guidance.

References

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