Molecular profile comparison of endometrial, renal and ovarian clear cell carcinoma: Is it the same disease at different sites?

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Abstract #5595

Background: Clear cell carcinomas (CCC) are histologically similar, however their clinical course varies widely based on the organ of origin. Clear cell uterine carcinoma (CCUC) accounts for approximately 5% of endometrial carcinomas and exhibit aggressive clinical behavior with poor outcomes. Clear cell ovarian cancers (CCOCs) are a subtype of epithelial ovarian cancers that are chemo-resistant with a poorer prognosis than other subtypes. 70% of renal cell carcinomas are clear cell (CCRCs), and respond to TKIs and mTOR inhibitors. It's unknown if these CCC rely on similar molecular pathways. Tumor profiling was used to identify subsets of CCC that may benefit from different therapies.
Methods: 136 CCUCs, 409 CCOCs and 94 CCRCs were tested using a commercial multiplatform profiling service (Caris Life Sciences, Phoenix, AZ). Specific tests performed included sequencing (Sanger, NGS), protein expression (IHC) and gene amplification (CISH or FISH).
Results: CCUCs had more TP53 mutations than CCOCs and CCRCs (40% vs 16% vs 14%). Compared to CCUCs and CCOCs, CCRCs had fewer mutations in the mTOR pathway (PIK3CA – 4% vs 25% vs 40%; PTEN - 1% vs 26% vs 3%) and the MAPK pathway (KRAS – 0% vs 14% vs 11%). VHL mutations were only seen in CCRCs (47% vs 0% vs 0%). ER and PR overexpression was more common in CCUCs than CCOCs and rare in CCRCs (ER – 35% vs 8% vs 0%; PR – 22% vs 13% vs 2%). AR overexpression was more common in CCRCs (26% vs 7% vs 5%). In contrast to CCUC and CCOC, no Her2 alterations measured by IHC, ISH or SEQ were seen in CCRCs.TOP2A, TS and RRM1 were expressed at a higher rate in CCUCs and CCOCs than CCRCs (TOP2A – 81% vs 63% vs 27%; TS - 46% vs 51% vs 16%; RRM1 – 22% vs 19% vs 2%). All CCC types had some immune-positivity for PD-1 (73%,47%, 68%) or PD-L1 (13%, 6%,29%).
Conclusions: While CCUCs and CCOCs share similarities, the molecular profiling shows significant differences compared with CCRCs. This data suggest blockade of the mTOR and/or MAPK pathways may be important in CCUCs and CCOCs. Further, anti-angiogenic agents are more likely to be of benefit in CCRCs. Immunotherapies warrant further investigation in selected CCC patients. Future studies are needed to correlate these markers with sensitivity to chemotherapy.

Background

- Clear cell renal cell cancer accounts for 70% of renal cell carcinomas. They are generally considered resistant to cytotoxic chemotherapies, but respond to TKI's and mTOR inhibitors.
- Clear cell ovarian cancer is a relatively uncommon histological subtype with poor prognosis in advanced stages and the tumors are typically chemo-resistant.
- Clear cell endometrial cancer accounts for only 1-6% of uterine malignancies and are considered estrogen-independent type II tumors with aggressive behavior and poor clinical outcome.
- We aim to compare the molecular characteristics of the three clear cell cancers to identify differences and similarities that can potentially provide a molecular rationale for treatment selection.

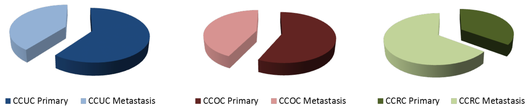
Methods

- Retrospective data analysis was done on uterine (CCUC), ovarian (CCOC) and renal (CCRC) clear cell carcinoma cases that were submitted to a commercial referral diagnostic laboratory (Caris Life Sciences, Phoenix, AZ) for molecular profiling aimed to provide therapeutic information based on tumor biomarkers.
- Specific testing was performed per physician request and included a combination of sequencing (Sanger, NGS), protein expression (IHC)and gene amplification (CISH or FISH).
- 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).
- 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.

Results

Table 1: Patient Characteristics

	CCUC		CCOC		CCRC	
Average Age	65.7		55.9		63	
Age Range	28-91		30-91		40-86	
Specimen site	Uterus	82	Ovary	233	Kidney	33
	Peritoneal tissue	15	peritoneal tissue	46	Lung	17
	Vagina	4	Pelvis, NOS	36	Connective tissue	10
	Cervix	5	Lymph nodes	18	Lymph nodes	4
	Ovary	6	Connective tissue	17	Bone	4
	Colon	4	Intestine	17	Skin	4
	Lung	3	Abdomen, NOS	14	Peritoneal tissue	3
	Lymph nodes	7	Liver	10	Liver	3
	Connective tissue	4	Uterus	4	Pancreas	2
	Pelvis	3	Vagina and Labia	4	Breast	2
	Liver	1	Lung	3	Adrenal gland	2
	Other	2	other	7	Other	10
Total N	136		409		94	



- CCOC patients are the youngest in age while CCUC and CCRC patients are older in age.
- The tumor samples are a mixture of primary tumors and various metastases.

Results

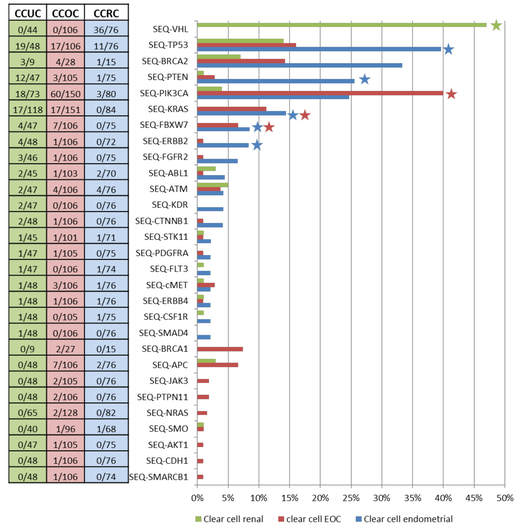
Figure 2: Biomarker frequency distribution, corresponding cancer pathways and associated therapies in CCUC, CCOC and CCRC.

Cancer pathways	Biomarkers	CCUC Percent|N		CCOC Percent|N		CCRC Percent|N		Associated therapies
DNA synthesis	IHC-TOP2A	81%	85/105	63%	207/331	27%	24/89	anthracycline
	IHC-TOPO1	43%	49/114	41%	148/359	53%	49/92	irinotecan, topotecan
	IHC-RRM1 (low)	78%	90/115	81%	294/361	98%	91/93	gemcitabine
	IHC-TS (low)	54%	62/115	49%	92/188	84%	76/91	fluorouracil, pemetrexed, capecitabine
Immune-Modulation	IHC-PD-1	73%	11/15	47%	15/32	68%	23/34	pembrolizumab, nivolumab
	IHC-PD-L1	13%	2/15	6%	2/32	29%	10/35	
HGF|cMET pathway	IHC-cMET	40%	32/81	24%	87/360	52%	48/92	cMET-targeted therapies
	ISH-cMET	0%	0/63	3%	5/152	0%	0/61	
DNA repair	IHC-MGMT (low)	65%	89/136	58%	221/380	48%	44/92	temozolomide
	IHC-ERCC1 (low)	94%	62/66	80%	215/268	67%	4/6	platinum agents
	SEQ-BRCA1	0%	0/9	7%	2/27	0%	0/15	PARP inhibitors, Platinum agents, mitomycin C
	SEQ-BRCA2	33%	3/9	14%	4/28	7%	1/15	
	SEQ-ATM	4%	2/47	4%	4/106	5%	4/76	
Hormone Receptors	IHC-ER	35%	47/136	8%	33/400	0%	0/90	Hormone therapies
	IHC-PR	22%	30/136	13%	51/397	2%	2/90	
	IHC-AR	7%	9/135	5%	10/207	26%	23/90	
PI3K/Akt/mTOR pathway	IHC-PTEN (low)	69%	94/136	45%	180/399	52%	48/93	PI3K/Akt/mTOR inhibitors
	SEQ-PTEN	26%	12/47	3%	3/105	1%	1/75	
	SEQ-PIK3CA	25%	18/73	40%	60/150	4%	3/80	
	SEQ-FBXW7	9%	4/47	7%	7/106	0%	0/75	
	SEQ-AKT1	0%	0/47	1%	1/105	0%	0/75	
	SEQ-STK11	2%	1/45	1%	1/101	1%	1/71	
Taxane pathway	IHC-TLE3	29%	24/82	47%	170/362	11%	10/90	paclitaxel, docetaxel
	IHC-TUBB3 (low)	85%	45/53	90%	279/311	79%	70/89	
	IHC-SPARCm	14%	19/136	14%	53/382	36%	34/94	nab-paclitaxel
Her2 pathway	ISH-Her2	12%	13/105	9%	32/346	0%	0/83	Her2-targeted therapies
	SEQ-ERBB2	8%	4/48	1%	1/106	0%	0/72	
	IHC-Her2/Neu	5%	7/134	2%	9/398	0%	0/93	
Multi-drug resistance	IHC-PGP	9%	10/107	16%	57/354	20%	18/88	

0
1-10
11-20
21-30
31-45
45-70
71-99

- Based on expression of PD1 and PDL1, immune modulatory agents are promising and warrant further investigation in clear cell cancers, especially in CCRC.
- BRCA1/2 mutations seen in clear cell cancers suggest the use of PARP inhibitors.
- VHL mutations are seen exclusively in CCRC, suggesting an activated angiogenesis pathway in CCRC.
- ER/PR expression is the highest in CCUC and CCOC and is low in CCRC; AR expression, on the other hand, is the highest in CCRC.
- PTEN loss is prevalent in all subtypes considered, while the mutation of genes including PIK3CA and PTEN is more frequent in CCUC and CCOC.
- Her2 aberration is seen in CCUC and CCOC, but not in CCRC.
- In spite of a favorable biomarker profile for some chemotherapies for CCRC, the inherent physiology may underlie the toxicity observed.

Figure 3: Gene mutation frequencies in CCUC, CCOC and CCRC; stars indicate that the difference has reached statistical significance by Fisher Exact test.



Conclusions

- Distinct molecular features detected by IHC, ISH and sequencing are seen when CCUC, CCOC and CCRC are compared, prompting consideration of differential treatment strategies in these cancers presenting a similar histology.
- Regarding PI3K/Akt/mTOR pathway activation, while PTEN protein loss is prevalent in all three subtypes, mutations of PIK3CA and PTEN are more prevalent in CCOC and CCUC than in CCRC.
- Her2 aberration by gene amplification, protein expression and gene mutation are seen in CCUC and CCOC, but are absent in CCRC, warranting investigation of Her2-targeted agents in CCUC and CCOC in clinical trials.
- While hormonal therapies targeting ER, PR can be considered in CCUC and CCOC based on hormone receptor expression, androgen receptor may be a therapeutic target in CCRC.
- Based on expression of PD1 and PDL1, immune modulatory agents are promising and warrant further investigation in clear cell cancers especially CCRC.