Distinct molecular landscape between endometrioid and non-endometrioid uterine carcinoma

Nathaniel L Jones[1], Joanne Xiu[2] , Sandeep K. Reddy[2], Ana I. Tergas[1], William M. Burke[1], Jason D. Wright[1], June Y. Hou[1]
[1]Columbia University College of Physicians and Surgeons and New York Presbyterian Hospital [2]Caris Life Sciences

Abstract

Background: Endometrial carcinoma (EC) is typically divided into endometrioid (Type I) and non-endometrioid (Type II) subtypes, despite considerable heterogeneity within each phenotype. We examined molecular alterations between Type I and Type II EC to better understand differences and identify optimal treatment strategies.
Methods: Out of a total of 3133 ECs submitted to Caris Life Sciences between Mar 2011 and Jul 2014, 1634 cases were Type I and 1226 cases were Type II EC based on reported pathology. Multiplatform molecular analysis included gene sequencing (Sanger or next generation sequencing), immunohistochemistry (IHC) of protein expression, and /or gene amplification (FISH/CISH).
Results: 3133 cases were analyzed and included 628 cases of uterine serous cancer (USC), 136 cases of clear cell adenocarcinoma (CC), 361 cases of carcinosarcoma (CS), 38 cases of mucinous, and 36 cases of squamous cell cancer. Overall there was a high frequency of ER/PR hormone receptor expression: USC (60%/32%), CC (35%/22%), CS (25%/21%), mucinous (74%/66%), and squamous (49%/40%). USCs express high AR compared with other non-endometrioid EC: USC (27%), CC (7%), CS (12%), mucinous (16%), and squamous (6%). PD1/PDL1 protein expression was high in USC (68%/11%), CC (73%/13%) and CS (84%/25%), suggesting potential benefit with PD-1/PD-L1 inhibitors. c-met overexpression was notably high in CC (40%) and mucinous (43%) tumors, suggesting promise with anti-eGFR therapy. TP53 was mutated most frequently in USC (76%) and CS (69%), followed by PIK3CA and KRAS, with highest mutation rates in mucinous tumors, 33% and 41%, respectively. DNA repair pathway was altered with low ERCC1 expression, notably in CC (6%) and squamous (8%) suggesting benefit with platinum therapy. BRCA proteins were frequently mutated in CS: 18% in BRCA1, 27% in BRCA2. CS also had high frequency of FBXW7 mutation (12%). Her2 overexpression in USC was 10% via IHC, amplification was 17% via CISH/FISH and mutation was 2% via NGS. Squamous cell showed 44% CTNNB1 mutation rate.
Conclusion: Overall we were able to identify differential molecular profiles within the high-risk uterine cancer subtypes that could guide future therapy. Correlating molecular profiles with clinical outcomes will assist in developing rational guidelines for therapy in individuals with EC.

Background

- Endometrial cancer has traditionally been divided into Type I and Type II disease based on unique histologic and pathologic characteristics.[1]
- Type I disease typically arises in the setting of unopposed estrogen stimulation and has a well-defined precursor lesion (complex atypical hyperplasia or CAH). Patients usually present with early stage disease and have an overall good prognosis.[1-2]
- Type II disease on the other hand, typically arises in the setting of an atrophic endometrium without a hormonally driven pathogenesis. It is associated with higher grade, higher stage, aggressive behavior, and worse prognosis.[1-2]
- Characteristic mutations, amplifications, and overexpression profiles are seen more in association with each type although some overlap exists. Therefore, it may be more helpful from a therapeutic standpoint to understand molecular alterations within specific histologic subtypes.[3]
- Because the non-endometrioid subtypes are uncommon, using a large tumor database with molecular and genetic information helps to identify unique tumor profiles and identify thematic pathways for therapeutic exploration.

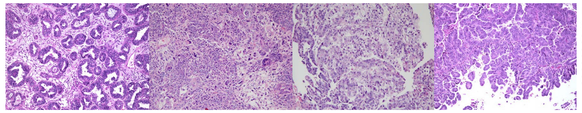


FIGURE1. H&E Stains.
(a) endometrioid
(b) carcinosarcoma
(c) clear cell
(d) serous

Methods

- 3133 cases of endometrial cancers were submitted to Caris Life Sciences from March 2011 to July 2014.
- 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.

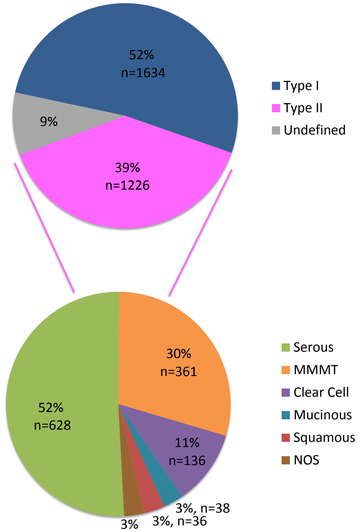
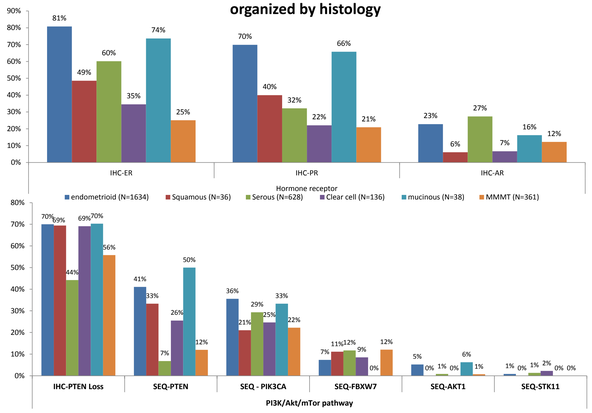


Figure 3. Pie chart representing the histology of Type II endometrial cancers

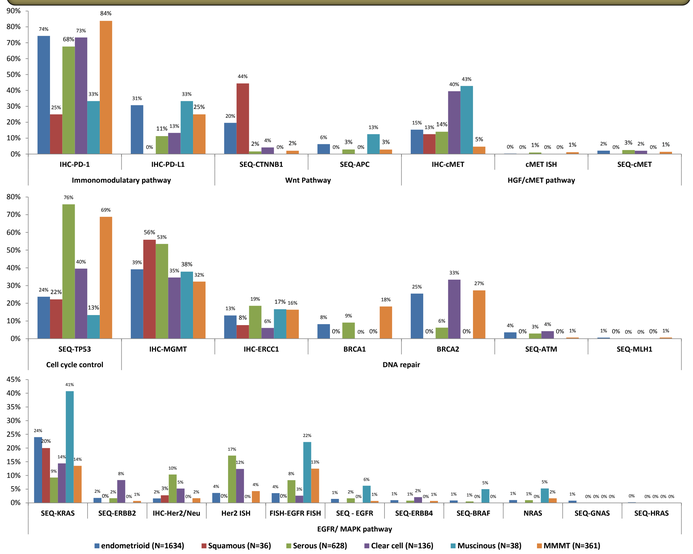
Figure 4. Patient age based on histology

Patient Age	Endometrioid	Mucinous	USC	CC	Squamous	CS
20-40	N=43	N=0	N=0	N=4	N=1	N=4
41-60	N=588	N=9	N=123	N=35	N=14	N=75
61-80	N=916	N=26	N=459	N=85	N=19	N=254
81-94	N=87	N=3	N=46	N=12	N=2	N=28
Average	63.3yrs	65.8yrs	67.5 yrs	65.7yrs	64.1 yrs	66.9 yrs

Figure 5. Pathway alterations in endometrial cancer subtypes organized by histology



Results (continued)



Conclusions

- We identified several pathways that warrant further exploration in the histologic subtypes of a large cohort (n=3133) of uterine carcinomas
- There is a high frequency of ER/PR receptor expression suggesting potential benefit with hormone therapy
- USCs express high AR compared with other EC and may benefit from anti-androgen therapy
- PD1/PDL1 protein expression was high in USC, CC, and CS, suggesting benefit with PDL/PDL1 inhibitors
- C-met overexpression was high in CC and mucinous tumors, suggesting promise with anti-cMET therapy
- PIK3CA and KRAS mutation rates were high in mucinous tumors suggesting benefit from targeted therapy.
- DNA repair pathways were altered in CC and squamous suggesting benefit with platinum therapy.
- BRCA proteins and FBWX7 proteins were frequently mutated in CS.
- Overall, we identified differential molecular profiles within the high-risk uterine cancer subtypes that could guide future therapy.
- Correlating molecular profiles with clinical outcomes will assist in developing rational guidelines for therapy in individuals with EC.

References

1. Lax, S. F. & Kurman, R. J. A dualistic model for endometrial carcinogenesis based on immunohistochemical and molecular genetic analyses. Verh. Dtsch. Ges. Pathol. 81, 228–232 (1997)
2. J.V. Bokhman. Two pathogenetic types of endometrial carcinoma. Gynecol Oncol, 15 (1983), pp. 10–17
3. Kandoth C, Schultz N, Cherniack AD, et al. Integrated genomic characterization of endometrial carcinoma. Nature 497 (7447): 67-73, 2013