Molecular Profiling of Mucinous Epithelial Ovarian Carcinomas (mEOC)

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Abstract

Background:
mEOCs are an uncommon subset of epithelial ovarian cancers. Most patients have early stage disease at presentation and a good prognosis. Patients with advanced stage disease at diagnosis are rare and can be difficult to distinguish from gastrointestinal metastases (GIM) to the ovary. They have a poor prognosis and a low response to standard chemotherapy. Molecular profiling of mEOCs may help differentiate primary mEOCs from GIMs and also identify patient subsets that could potentially benefit from targeted therapies and help design basket phase 2 trials.

Methods:
304 mEOCs referred to Caris Life Sciences (from 2009 - 2014) were evaluated. The diagnosis was based on reported pathology. 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.

Results:
Alterations in the MAP Kinase pathway were common in mEOCs with frequent mutations in KRAS (49%). BRAF had a lower mutation rate of 3.5%. Three cases had coexisting KRAS and BRAF mutations. Alterations in the mTOR pathway were also observed but at a less frequent rate (PIK3CA 12% and PTEN 6%). PD-1 positivity was observed in 43% of tumor infiltrating lymphocytes and PD-L1 was positive in 14% of mEOCs. cMET overexpression was seen in 33% of cases but no cMET amplification. HER2 amplification by FISH was observed in 11%. EGFR amplification was seen in 50% of cases and 57% had overexpression of EGFR by IHC. P53 mutated (n=68) and wildtype (n=37) mEOCs differed in ER, PR and HER2 expression and BRAF, PIK3CA and PTEN mutation prevalence.

% PREVALENCE	ER (IHC)	PR (IHC)	HER2 (IHC)	BRAF (NGS)	PIK3CA (NGS)	PTEN (NGS)
Mucinous (All) (n=304)	23	20	8	8	12	6
Mucinous (P53 wildtype) (n= 68)	35	28	3	12	19	9
Mucinous (P53 mutated) (n=37)	14	8	24	0	3	0

Conclusions:
Molecular profiling underscores the genomic heterogeneity in a large series of patients with mEOCS. It is likely that the P53 mutant mEOCs represent GIMs. There are a number of potential treatment targets in mEOCS identified in this study that could be addressed in clinical trials.

Background

- mEOC's are a histologic subgroup of epithelial ovarian cancer (EOC) that account for between 3 and 10% of EOC. It is difficult to reliably differentiate primary mEOC's from secondary mucinous carcinomas arising in other sites which are predominantly in the gastrointestinal tract (GIT) .
- Patients with stage 1 mEOC have an excellent prognosis in contrast to those with advanced stage disease who have a poor outcome and relative resistance to platinum and taxane based chemotehrapy. They are underrepresented in clinical trials in advanced EOC . There is uncertainty how to best treat these patients and in particular whether they should be treated with chemotherapy/targeted therapy regimens that are used in GIT cancers.
- Molecular profiling has shown that mEOCs are very different to the more common high grade serous cancers and develop along separate pathways. KRAS mutations are an early event in the development of mucinous tumors of the ovary but BRAF mutations have not been described (1, 2). In contrast to high grade serous cancers, P53 mutations appear to be uncommon. HER2 overexpression/amplification has been reported in 18-35% of mEOCs (3) suggesting a possible role for trastuzumab (4).
- We hypothesize that comprehensive multiplatform tumor profiling may identify potential therapeutic targets and possibly help distinguish primary mEOCs from mucinous cancers arising outside the ovary.

Methods

- 304 mEOCs were referred to Caris Life Sciences for profiling between 2013 and 2014 and considered for inclusion in this cohort. 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.
- No clinical data on disease stage, recurrence or prior treatment history was collected for these samples.
- 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.
- Chromogenic in-situ hybridization (ISH) 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). The case was considered amplified when the ration was ≥2.0.
- Direct sequence analysis was performed on genomic DNA isolated from formalin-fixed paraffin-embedded tumor samples using the Illumina MiSeq platform. Specific regions of 45 genes of the genome were amplified using the Illumina TruSeq Amplicon Cancer Hotspot panel.
- PD-1 staining is read from the tumor infiltrating lymphocytes (TILs) using the MRQ-22 clone of the PD-1 antibody. 1 TIL count per HPF with a 40X objective was considered as PD-1 positivity.
- PD-L1 testing was performed using the 130021 clone of the PD-L1 antibody. The staining is read from the cytoplasmic or membrane staining of the cancer cell and the result is considered positive if staining intensity is ≥2+ in ≥ 5% of tumor cells.

Results - Molecular Characterization of Mucinous Ovarian Cancer (n=304)

- ER and PR overexpression was present in 20% of tumors tested. In addition, 16 of 191 (8.4%) tumors tested had overexpression of Androgen receptors.
- 114 of 199 (57.3%) tumors tested had loss of ERCC1.
- BCRP (88.6%; 31 of 35 tumors tested) and MRP1 (83.1%; 30 of 36 tumors tested) were commonly overexpressed in this patient group.
- PD-1 and PD-L1 were expressed in 42.9% and 14.3% of tumors tested, respectively.

Hormone Receptors			
AR	IHC	8.4%	(16/191)
ER	IHC	22.5%	(67/298)
PR	IHC	20.1%	(60/299)
Growth Factor Receptors			
cMET	IHC	37.7%	(100/265)
EGFR	IHC	64.7%	(11/17)
HER2	IHC	7.6%	(21/276)
HER2	ISH	9.8%	(25/254)
IGF1R	IHC	53.9%	(55/102)
PDGFRA	IHC	32.4%	(11/34)
DNA Repair			
ERCC1 Loss	IHC	57.3%	(114/199)
MGMT Loss	IHC	27.6%	(81/293)
DNA Replication			
TOPO1	IHC	54.7%	(146/267)
TOPO2A	IHC	46.7%	(114/244)
TLE3	IHC	25.1%	(67/267)
TS Loss	IHC	72.2%	(122/169)
TUBB3 Loss	IHC	67.0%	(152/227)
Immunomodulatory Checkpoints			
PD-1	IHC	42.9%	(18/42)
PD-L1	IHC	14.3%	(6/42)
Drug Resistance Associated Proteins			
BCRP (ABCG2)	IHC	88.6%	(31/35)
MRP1	IHC	83.1%	(30/36)
PgP	IHC	54.4%	(141/259)
Other			
PTEN Loss	IHC	45.2%	(135/299)
RRM1 Loss	IHC	80.7%	(218/270)
SPARC	IHC	30.9%	(94/304)

Table 1: Findings of comprehensive tumor profiling in a cohort of 304 mEOCs

- The most common mutation was *KRAS* observed in 51% (54/106) of tumors tested.
- *P53* mutations were found in 35% of cases (37/105).
- Mutations in *PIK3CA* and *PTEN* were observed in 12% (13/105) and 6% (6/102) of tumors respectively.
- *BRAF* mutations were found in 8% of tumors tested (9/109).

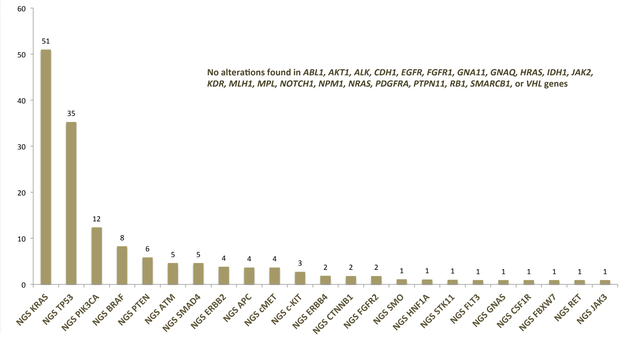


Figure 1: Mutation prevalence in mucinous ovarian cancer

Results - Comparison of P53 mutated (n=37) and P53 wildtype (n=68) Mucinous Ovarian Cancer

- Overexpression of ER (35.3% vs 13.5%) and PR (27.9% vs 8.1%) was greater in P53 wildtype mEOCs compared to those with P53-mutations (p=0.01 for both).
- HER2 overexpression (3.4% vs 24.2%) and gene amplification (3.0% vs 24.3%) was less common in patients with P53-wildtype tumors compared to P53 mutant tumors (p=0.0020 and p=0.0006 respectively).
- No significant differences in DNA repair, DNA replication or immuno-modulatory checkpoint proteins were observed between patient groups.

		P53 Wildtype (n=68)	*P53 Mutated (n=37)*	*p-Value*
Hormone Receptors				
AR	IHC	**11.8%** *(8/68)*	**8.1%** *(3/37)*	
ER	IHC	**35.3%** *(24/68)*	**13.5%** *(5/37)*	0.0169
PR	IHC	**27.9%** *(17/68)*	**8.1%** *(3/37)*	0.0169
Growth Factor Receptors				
cMET	IHC	**61.8%** *(42/68)*	**59.5%** *(22/37)*	
EGFR	IHC	**44.4%** *(4/9)*	**100%** *(4/4)*	
HER2	IHC	**3.4%** *(2/58)*	**24.2%** *(8/33)*	0.0020
HER2	ISH	**3.0%** *(2/66)*	**24.3%** *(9/37)*	0.0006
DNA Repair				
ERCC1 Loss	IHC	**72%** *(18/25)*	**77.8%** *(14/18)*	
MGMT Loss	IHC	**36.8%** *(25/68)*	**18.9%** *(7/37)*	0.0586
DNA Replication				
TOPO1	IHC	**43.9%** *(29/66)*	**52.8%** *(19/36)*	
TOPO2A	IHC	**52.3%** *(34/65)*	**72.7%** *(24/33)*	0.0527
TLE3	IHC	**22.1%** *(15/68)*	**10.8%** *(4/37)*	
TS Loss	IHC	**29.4%** *(20/68)*	**37.8%** *(14/37)*	
TUBB3 Loss	IHC	**69.1%** *(47/68)*	**70.3%** *(26/37)*	
Immunomodulatory Checkpoints				
PD-1	IHC	**38.1%** *(8/21)*	**58.3%** *(7/12)*	
PD-L1	IHC	**19.0%** *(4/21)*	**8.3%** *(1/12)*	
Drug Resistance Associated Proteins				
PgP	IHC	**45.6%** *(31/68)*	**54.1%** *(20/37)*	
Other				
PTEN Loss	IHC	**25.0%** *(17/68)*	**16.2%** *(6/37)*	
RRM1 Loss	IHC	**82.4%** *(56/68)*	**91.9%** *(34/37)*	
SPARC	IHC	**39.7%** *(27/68)*	**35.1%** *(13/37)*	

Table 2: Comparison of potentially actionable biomarker alterations based on predictive associations in P53 wildtype and P53 mutated mEOCs

- 32 concomitant mutations were found in the 37 patients with *P53*-mutated tumors (an average of 0.85 mutations per patient), compared to 86 (1.25 mutations per patient) in 68 tumors wildtype for *P53*.
- No *BRAF* or *PTEN* mutations were found in the *P53* mutated tumors, compared to 12% and 9% of *P53* wildtype tumors (p=0.0301 for *BRAF*, p=0.0626 for *PTEN*). *PIK3CA* mutations also occurred significantly less frequently in *P53* mutated tumors (3% vs 18%; p=0.0216).
- Mutations in *ERBB2* (Her2) gene trended to occur more frequently in *P53* mutated tumors compared to those that are *P53* wildtype (9% vs 1%; p=0.0818).

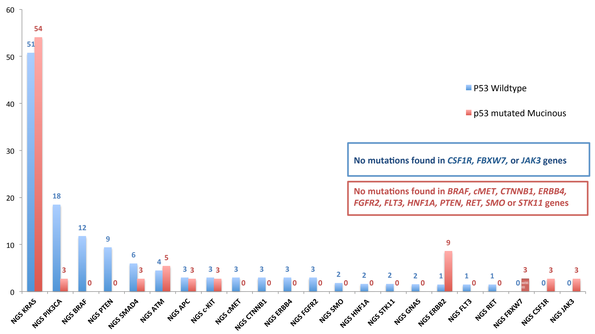


Figure 2: Mutation prevalence in P53 wildtype and P53 mutated mEOCs

Results – Potential treatment strategies

- Comprehensive genomic profiling demonstrates a number of potential therapeutic targets with differences in P53 mutated and P53 wildtype tumors.
- Overexpression of hormone receptors and HER2 overexpression was mutually exclusive in both P53-mutated and wildtype tumors.
- Simultaneous overexpression of AR, ER and PR was observed in 6 P53 wildtype tumors and 1 P53 mutated mEOC.
- The proportion of patients with no mutations in receptor tyrosine kinases (RTK2), the MAP Kinase pathway (MAPK) and PI3K pathway (PI3K) was similar in both groups.
- Treatment strategies directed against mTOR may be useful in selected P53 wildtype tumors based on PIK3CA mutations.

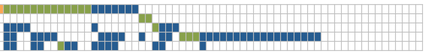
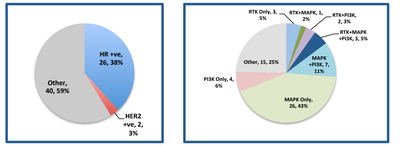


Figure 3: Overlap of hormone receptor, HER2 and pathway alterations in P53 wildtype mEOCs

(orange indicates 3 concomitant alterations, green indicates the presence of 2 and navy indicates a single alteration per tumor)



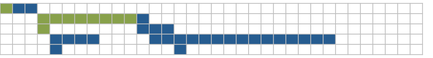
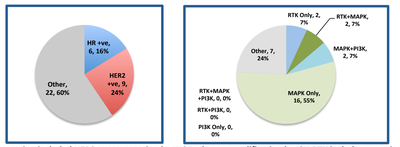


Figure 4: Overlap of hormone receptor, HER2 and pathway alterations in P53 mutated mEOCs



Hormone receptors included AR, ER and PR. HER2 expression included HER2 overexpression by IHC and gene amplification by ISH.RTK includes mutations in either cKIT, cMET, CSF1R, EGFR, ERBB4, FGFR1, FGFR2, FLT3, HER2 and PDGFRA. MAPK includes mutations in KRAS, NRAS, HRAS or BRAF. PI3K includes alterations in PIK3CA, PTEN, FBXW7, AKT1 or STK11.

Conclusions

- This study demonstrates the genomic heterogeneity of MEOCs and confirms the findings of other groups with respect to the frequency of KRAS mutations, HER2 overexpression/amplification, hormone receptor expression and P53 mutations.
- Clear differences were observed between P53 wildtype and P53 mutant mEOCs suggesting that they are distinct entities with a different biology .
- The absence of hormone receptor expression and PI3K alterations, along with the higher HER2 expression/amplification in the P53 mutated group suggest that they may be of a gastrointestinal origin(5).
- There may be a role for PD-L1 checkpoint inhibitors in a subset of patients with mEOCs.
- Comprehensive multiplatform tumor profiling of advanced stage mEOCs identifies a number of potential therapeutic targets. The clinical utility of this approach could be investigated in small proof of concept basket trials given how uncommon these tumors are.

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