Profiling of Triple Negative Endometrial Cancers (TNEC) and Triple Negative Breast Cancers (TNBC) Reveals Unique Expression Profiles

Nathaniel L Jones[1], Joanne Xiu[2], S. Chatterjee[1], A. Buckley de Meritens[1], 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, Phoenix, AZ

COLUMBIA UNIVERSITY College of Physicians and Surgeons

NewYork-Presbyterian The University Hospital of Columbia

Abstract

Objective: Triple negative" has been used to characterize a subtype of breast cancer that lacks estrogen, progesterone, and HER2 receptor expression. They are aggressive cancers with limited treatment options. It's unknown if similar phenotype found in other cancer types, like endometrial cancer, harbor similar molecular alterations and prognosis. We aim to compare molecular features between TNEC and TNBC.

Methods: A total of 3133 endometrial cancer samples were evaluated by Caris Life Sciences (Phoenix, AZ) from Mar, 2011 to Jul, 2014 by multiplatform profiling, which included a combination of sequencing (Sanger or NGS), protein expression (IHC), and/or gene amplification (CISH or FISH). 545 TNEC and 2049 TNBC were identified based on reported pathology and compared using Fisher exact tests.

Results: Compared to an incidence of 15-20% TNBC in breast cancer, 17% (545/3133) TNEC was seen in our cohort, of which 13% were endometrioid, 22% serous, 26% carcinosarcoma, 7% clear cell, and 22% other. Compared with TNBC, TNEC showed 1.9 exonic mutations per case while TNBC showed 1.2 mutations per case. As shown in the table, AR expression is lower in TNEC than TNBC. TP53 mutation was common in both but more frequent in TNBC. While BRCA1/2 mutation rates were similar, low MGMT and ERCC1 were more common in TNEC, suggesting increased aberrant DNA repair. DNA synthesis protein expression was higher in TNEC including TS, RRM1, and TOPO2A, although TNBC had higher TOPO1. PD-1 expression was more common in TNEC suggesting immune pathway involvement. PI3K/AKT/mTor, MAPK and Wnt pathways were more involved in TNEC with greater PTEN, PIK3CA, FBXW7, KRAS and CTNNB1 mutations.

Conclusion: Our study reveals significantly higher overall mutation rates in TNEC than in TNBC, and specifically higher activations of multiple molecular pathways including PI3K/Akt/mTor, MAPK and Wnt. Further studies are warranted to validate these findings in clinical trials.

Background

- Triple negative" has traditionally been used to characterize a subtype of breast cancer that lacks estrogen, progesterone, and Her2 receptor expression
- Triple negative breast cancers behave aggressively, are associated with a poor prognosis, and have limited treatment options
- Less than 15% of breast cancers have the triple negative phenotype
- It is unknown whether similar phenotypes found in other cancer types, such as endometrial cancer, harbor similar molecular alterations and prognosis
- We aim to compare genetic and molecular features of triple negative endometrial cancers (TNEC) and triple negative breast cancers (TNBC).

Fig. 10. A, B: Immunohistochemical localization of hormone receptors in glandular epithelial (G) and stroma cell (S) nuclei in the endometrium. (A) Intense nuclear staining of ER during estrus. (B) High PR expression in the progestational endometrium. Scale bars 1/4 100 l m.

Methods

- 3133 cases of endometrial cancers were submitted to Caris Life Sciences from March 2011 to July 2014 and 545 of which were determined as TNEC based negative IHCs of ER, PR, Her2 and lack of Her2 amplification by ISH. Similarly, 2049 TNBC tumors were identified and analyzed.
- Specific testing was performed per physician request and included sequencing (Sanger, NGS or pyrosequencing), protein expression (IHC), 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], TOP2A [TOPO2A/CEP17 probe], cMET [cMET/CEP7 probe] (Abbott Molecular/Vysis, Ventana). 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 1. Comparison of molecular differences between TNBC and TNEC

Table 1. Molecular differences between TNBC and TNEC
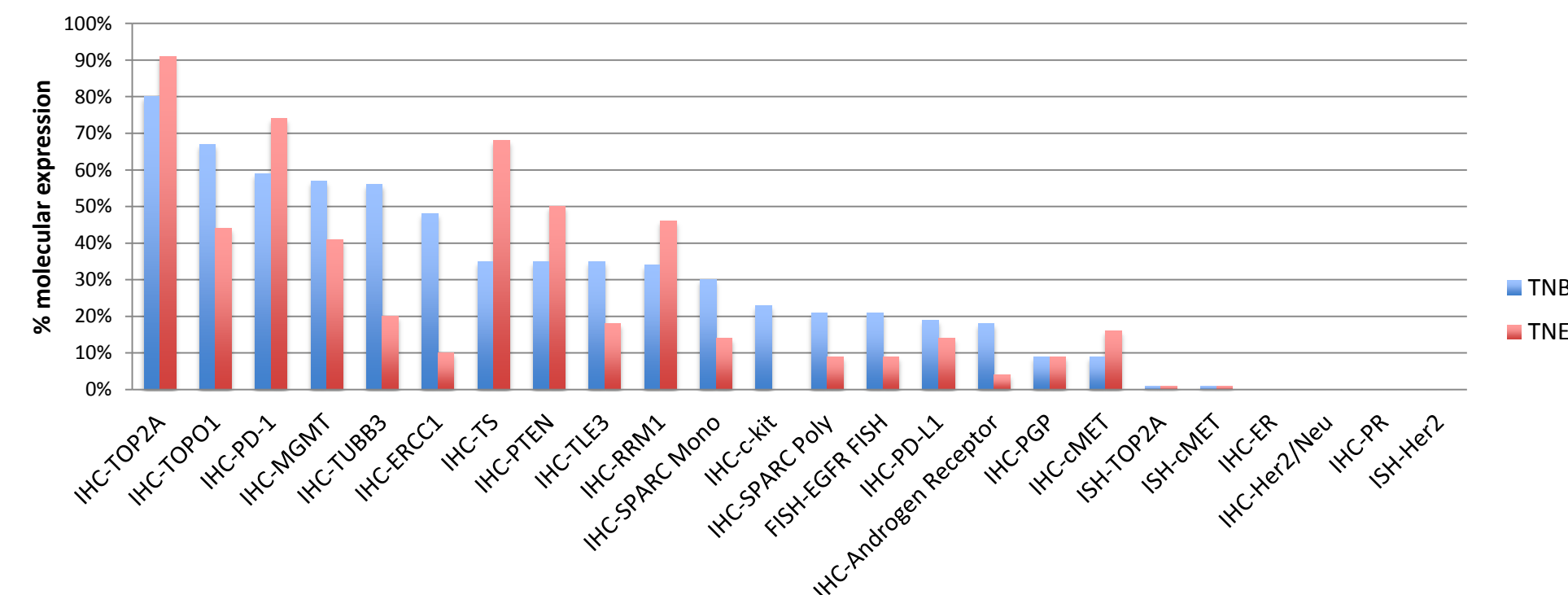
	Triple Neg Breast Cancer	Triple Neg Endometrial Cancer	p value
IHC-TOP2A	80%	91%	<0.01
IHC-TOPO1	67%	44%	<0.01
IHC-PD-1	59%	74%	<0.01
IHC-MGMT	57%	41%	<0.01
IHC-TUBB3	56%	20%	<0.01
IHC-ERCC1	48%	10%	<0.01
IHC-TS	35%	68%	<0.01
IHC-PTEN	35%	50%	<0.01
IHC-TLE3	35%	18%	<0.01
IHC-RRM1	34%	46%	<0.01
IHC-SPARC Monoclonal	30%	14%	<0.01
IHC-c-kit	23%	0%	<0.01
IHC-SPARC Polyclonal	21%	9%	<0.01
FISH-EGFR FISH	21%	9%	
IHC-PD-L1	19%	14%	
IHC-Androgen Receptor	18%	4%	<0.01
IHC-PGP	9%	9%	
IHC-cMET	9%	16%	<0.01
ISH-TOP2A	1%	1%	
ISH-cMET	1%	1%	
IHC-ER	0%	0%	
IHC-Her2/Neu	0%	0%	
IHC-PR	0%	0%	
ISH-Her2	0%	0%	

Table 2. Mutation differences between TNBC and TNEC

	Triple Neg Breast Cancer	Triple Neg Endometrial Cancer	p value
SEQ-TP53	66%	56%	<0.01
SEQ-PIK3CA	14%	25%	<0.01
SEQ-BRCA2	14%	19%	
SEQ-BRCA1	10%	17%	
SEQ-PTEN	5%	18%	<0.01
SEQ-APC	4%	4%	
SEQ-AKT1	3%	1%	<0.01
SEQ-cMET	3%	3%	
SEQ-ATM	2%	4%	
SEQ-RB1	2%	1%	
SEQ-JAK3	2%	1%	
SEQ-STK11	2%	1%	
SEQ-ERBB2	2%	2%	
SEQ-KRAS	1%	20%	<0.01
SEQ-HRAS	1%	0%	
SEQ-FBXW7	1%	13%	<0.01
SEQ-ABL1	1%	2%	
SEQ-MLH1	1%	1%	
SEQ-RET	1%	1%	
SEQ-BRAF	1%	1%	
SEQ-KDR	1%	1%	
SEQ-cKIT	0%	1%	
SEQ-VHL	0%	1%	
SEQ-ERBB4	0%	1%	
SEQ-SMAD4	0%	1%	
SEQ-CTNNB1	0%	8%	<0.01
SEQ-EGFR	0%	1%	
SEQ-SMO	0%	2%	
SEQ-CSF1R	0%	1%	
SEQ-CDH1	0%	0%	
SEQ-IDH1	0%	0%	
SEQ - NRAS	0%	2%	<0.01
SEQ-HNF1A	0%	4%	<0.01
SEQ-NOTCH1	0%	0%	
SEQ-FLT3	0%	1%	
SEQ-PDGFRA	0%	1%	
SEQ-FGFR2	0%	4%	<0.01
SEQ-PTPN11	0%	0%	
SEQ-SMARCB1	0%	0%	
SEQ-FGFR1	0%	0%	
SEQ-ALK	0%	0%	
SEQ-GNA11	0%	0%	
SEQ-JAK2	0%	0%	

Results (continued)

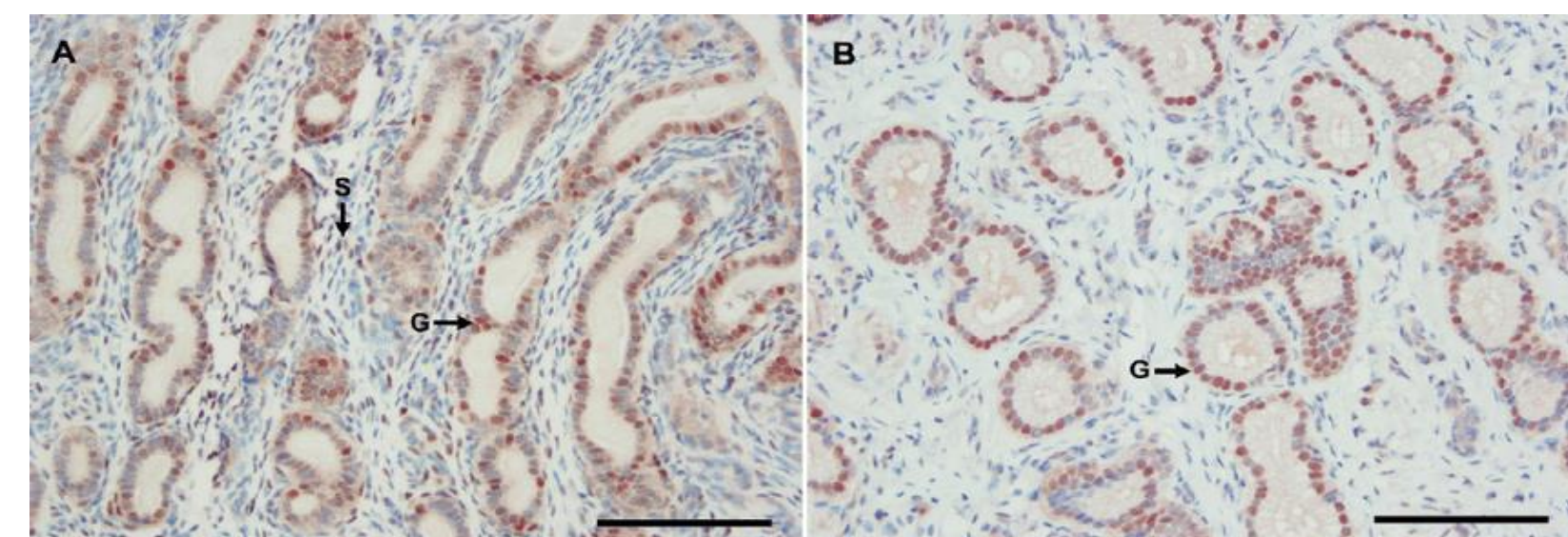**Figure 2. Comparison of molecular differences between TNBC and TNEC**
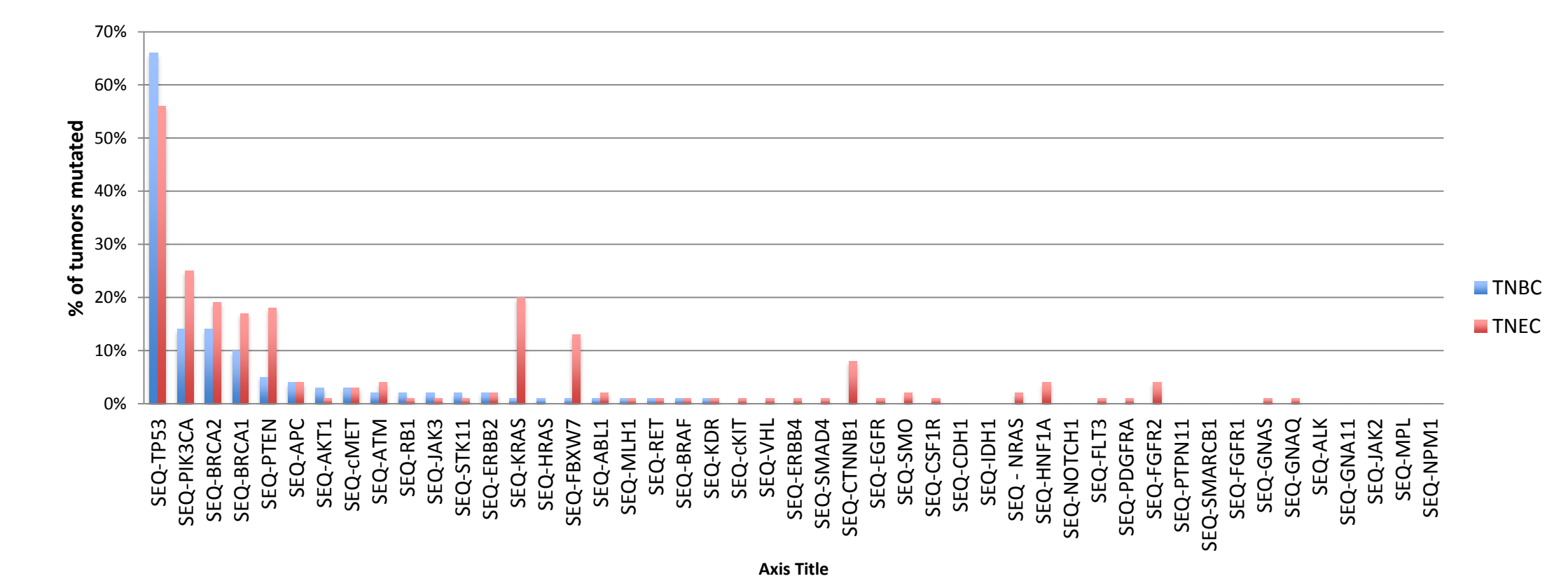
Table 3. Summary of TNBC and TNEC molecular signatures

	Marker	TNEC	TNBC	p-value
IHC	AR	4%	18%	
	ERCC1	10%	48%	
	MGMT	41%	57%	
	PD-1	74%	59%	
	RRM1	46%	34%	
	TOP2A	91%	80%	
	TOPO1	44%	67%	
	TS	68%	35%	<0.01
SEQ	TP53	56%	66%	
	PIK3CA	25%	14%	
	KRAS	20%	1%	
	PTEN	18%	5%	
	FBXW7	13%	1%	
	AKT	3%	1%	
	CTNNB1	8%	0%	
	BRCA2	14%	19%	p>0.05
	BRCA1	10%	17%	

Conclusions

- We identified unique molecular and genomic differences between a large cohort of triple negative endometrial and triple negative breast cancers.
- The incidence of triple negative endometrial cancer (17%) in our cohort was similar to reported incidence of triple negative breast cancer (15-20%) in the literature.
- Compared with TNBC, TNEC showed 1.9 exonic mutations per case while TNBC showed 1.2 mutations per case.
- AR expression is lower in TNEC than TNBC
- TP53 mutation was common in both but more frequent in TNBC
- Greater involvement in the DNA synthesis pathway was noted in TNEC with higher TOPO2, TS, and RRM1 expression, although TNBC has higher TOPO1 expression
- While BRCA1/2 mutation rates were similar, low MGMT and ERCC1 were more common in TNEC, suggesting increased aberrant DNA repair
- PI3K/AKT/mTor, MAPK and Wnt pathways were more involved in TNEC with greater PTEN, PIK3CA, FBXW7, KRAS and CTNNB1 mutations

References

1. Colegrove, K.M., et al., *The normal genital tract of the female California sea lion (Zalophus californianus): cyclic changes in histomorphology and hormone receptor distribution.* Anat Rec (Hoboken), 2009. **292**(11): p. 1801-17.