Molecular Profiling Reveals Distinct Molecular Landscapes in 545 Cases of Triple Negative Endometrial Cancer

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Abstract

Objective: The term "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 poor prognosis, and have limited treatment options. It is unknown whether similar phenotypes found in other cancer types, such as endometrial cancer, harbor similar molecular alterations and prognosis. We sought to compare genetic and molecular features of triple negative endometrial cancers (TNEC) with non-TNEC to identify possible therapeutic targets.

Methods: A total of 3133 endometrial cancer samples were evaluated by Caris Life Sciences (Phoenix, AZ) from March 2011 to July 2014 by multiplatform profiling, which included sequencing (Sanger or NGS), protein expression (IHC), and /or gene amplification (CISH or FISH). The molecular profiles of 545 TNEC and 2162 non-TNEC were identified based on expression profiles and compared using Fisher exact tests.

Results: The frequency of TNEC in our cohort was 17%. Of 545 TNEC cases, 13% were endometrioid, 22% serous, 26% carcinosarcoma (MMMT), 7% clear cell, and 22% other. Table 1 compares molecular and genomic alterations between TNECs and non-TNECs. Compared to non-TNEC, TNECs had more frequent TP53 and BRCA1 mutations and more frequent alterations of the DNA synthesis pathway with higher TOPO1, TOPO2, TS, and RRM1 expression. Immune modulatory, FGFR and Wnt pathways were less often altered in TNECs as evidenced by lower PDL1 expression, fewer FGFR2 and fewer CTNNB1 mutations, respectively. PI3K/Akt/mTOR pathway aberrations were less common in TNEC with fewer PIK3CA, PTEN, and AKT mutations. Finally, expression of AR and TLE3 was less common in TNEC than in non-TNEC.

Conclusion: TNEC appears to have a distinct molecular background from non-TNEC. Differences were seen in pathways involved in DNA repair, DNA synthesis, immune modulatory function, and the PI3K/Akt/mTOR pathway. Further studies are warranted to validate the clinical applicability of these findings.

Background

- The term "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 poor prognosis
- We sought to compare genetic and molecular features of triple negative endometrial cancers (TNEC) with non-TNEC to identify possible therapeutic targets.

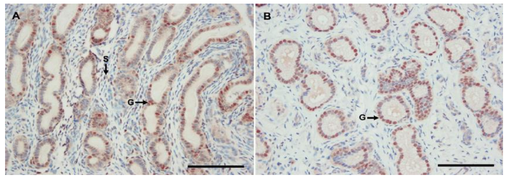

Fig. 10. A, B: Immunohistochemical localization of hormone receptors in glandular epithelial (G) and stoma cell (S) nuclei in the endometrium. (A) Intense nuclear staining of ER a during estrus. (B) High PR expression in the progestational endometrium. Scale bars 1⁄4 100 l m.

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.
- 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

Table 1. Comparison of molecular differences between TNEC and non-TENC

Marker	Triple Neg Endometrial Cancer	Non-Triple Neg Endometrial	p value
FISH-EGFR FISH	9%	5%	ns
FISH-TOP2A FISH	1%	2%	ns
IHC-Androgen Receptor	4%	28%	<0.0001
IHC-cMET	16%	14%	ns
IHC-ERCC1	10%	14%	ns
IHC-MGMT	41%	42%	ns
IHC-PD-1	74%	73%	ns
IHC-PD-L1	14%	28%	0.0069
IHC-PGP	9%	9%	ns
IHC-PTEN	50%	37%	<0.0001
IHC-RRM1	46%	37%	<0.0001
IHC-TLE3	18%	14%	0.0178
IHC-TOP2A	91%	79%	<0.0001
IHC-TOPO1	44%	38%	0.0107
IHC-TS	68%	48%	<0.0001
IHC-TUBB3	20%	20%	ns
ISH-cMET	1%	0%	ns

Figure 1. Comparison of molecular differences between TNEC and non-TENC

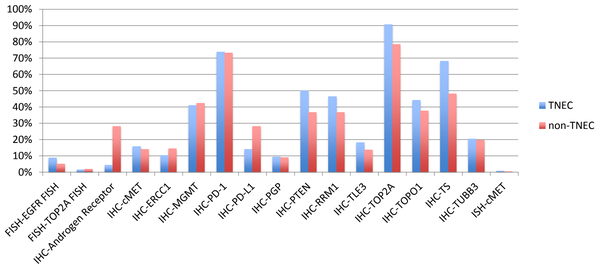

Table 2. Comparison of genomic differences between TNEC and non-TENC

Marker	TNEC expression	Non-TNEC expression	p value
SEQ-TP53	56%	37%	<0.0001
SEQ-PIK3CA	25%	35%	<0.0001
SEQ-KRAS	20%	17%	ns
SEQ-BRCA2	19%	23%	ns
SEQ-PTEN	18%	33%	<0.0001
SEQ-BRCA1	17%	5%	0.0141
SEQ-FBXW7	13%	7%	0.0005
SEQ-CTNNB1	8%	15%	0.0006
SEQ-FGFR2	4%	8%	0.011
SEQ-HNF1A	4%	5%	ns
SEQ-ATM	4%	3%	ns
SEQ-APC	4%	5%	ns
SEQ-cMET	3%	2%	ns
SEQ-NRAS	2%	1%	ns
SEQ-ERBB2	2%	1%	ns
SEQ-SMO	2%	1%	ns
SEQ-ABL1	2%	1%	ns
SEQ-RB1	1%	2%	ns
SEQ-SMAD4	1%	1%	ns
SEQ-ERBB4	1%	1%	ns
SEQ-FLT3	1%	1%	ns
SEQ-EGFR	1%	1%	ns
SEQ-KDR	1%	1%	ns
SEQ-MLH1	1%	0%	ns
SEQ-GNAS	1%	0%	ns
SEQ-STK11	1%	1%	ns
SEQ-cKIT	1%	1%	ns
SEQ-RET	1%	0%	ns
SEQ-VHL	1%	0%	ns
SEQ-BRAF	1%	1%	ns
SEQ-PDGFRA	1%	1%	ns
SEQ-AKT1	1%	4%	0.0006
SEQ-CSF1R	1%	1%	ns
SEQ-JAK3	1%	2%	0.0762
SEQ-GNAQ	1%	0%	ns
SEQ-GNA11	0	1%	ns
SEQ-CDH1	0	1%	ns
SEQ-SMARCB1	0	0%	ns
SEQ-FGFR1	0	0%	ns
SEQ-IDH1	0	0%	ns
SEQ-ALK	0	0	ns
SEQ-HRAS	0	0	ns
SEQ-JAK2	0	0	ns

Results (continued)

Figure 2. Comparison of genetic differences between TNEC and non-TNEC

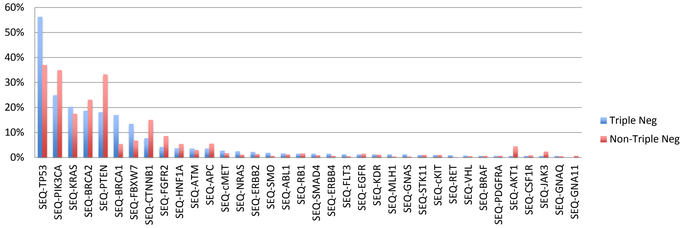

Table 3. Summary of TNEC and non-TENC molecular signatures

	Marker	TNEC	non-TNEC	p-value
IHC	AR	4%	28%	P<0.05
	PD-L1	14%	28%	
	PTEN	50%	37%	
	RRM1	46%	37%	
	TLE3	18%	14%	
	TOP2A	91%	79%	
	TOPO1	44%	38%	
	TS	68%	48%	
SEQ	TP53	56%	37%	
	PIK3CA	25%	35%	
	PTEN	18%	33%	
	BRCA1	17%	5%	
	FBXW7	13%	7%	
	CTNNB1	8%	15%	
	FGFR2	4%	8%	

Conclusions

- We identified several pathways that warrant further exploration in a large cohort (n=545) of triple negative endometrial cancers.
- There is a significantly higher frequency of TP53 mutations in TNEC, suggesting a more aggressive subtype and inferior outcomes.
- Greater involvement in the DNA synthesis pathway was noted in TNECs with higher TOPO1, TOPO2, TS, and RRM1 expression
- Immune modulatory, FGFR and Wnt pathways were less often altered in TNECs with lower PDL1 expression and fewer FGFR2 and CTNNB1 mutations, respectively
- PI3K/Akt/mTOR pathway aberrations were less common in TNEC with fewer PIK3CA, PTEN, and AKT mutations.
- Expression of AR and TLE3 was less common in TNEC than in nonTNEC.
- Overall we identified differential molecular profiles within triple negative endometrial cancers that could guide future therapy.
- Correlating molecular profiles with clinical outcomes will help to determine if the triple negative phenotype has therapeutic or prognostic significance in endometrial cancer

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.