Assessment of Homologous Repair Deficiency status in Triple Negative Breast and Ovarian Carcinoma using Genetic and Epigenetic Next Generation Sequencing Assays

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Introduction

- Analyzing deficiencies in homologous recombination repair (HRR) machinery has become increasingly important to identify patients who respond to poly (adenosine diphosphate [ADP]-ribose) polymerase (PARP) inhibitors.
- It is well-documented that deleterious germline or somatic genetic variants in certain genes lead to the phenotype of homologous recombination deficiency (HRD).
- However, there is sparse information on the role of epigenetic silencing of the BRCA1, RAD51C, or XRCC3 genes by promoter hypermethylation in contributing to HRD.
- In this study, we have evaluated a quantitative NGS-based oncoReveal Methylation Assay for detection of methylation in promoter regions of BRCA1, RAD51C, and XRCC3.
- This assay was complemented with Pillar's oncoReveal HRDv2 assay to detect causal single nucleotide variants (SNVs) and indels for analyzing HRD.
- Based on identified genetic variants and a quantitative methylation value, we predicted an HRD status for the tumor specimens.
- The assays were performed on retrospective ovarian and triple negative breast cancer (TNBC) specimens at Temple University Hospital.

Methods

- A total of 71 formalin-fixed paraffin-embedded (FFPE) specimens (43 ovarian cancer and 28 TNBC) were evaluated for both methylation and mutation analysis.
- The ovarian cancer specimens were histologically classified as follows: 26 high-grade serous, 4 clear cells, 3 mucinous, 4 endometrioid, 1 poorly differentiated, and 1 mixed tumor specimen.
- FFPE specimens were microdissected, and DNA was extracted using standard laboratory protocols.
- The oncoReveal NGS assay was performed to detect mutations on the Illumina MiSeq platform.
- The methylation assay involved bisulfite conversion using the EpiTect Fast DNA Bisulfite kit, followed by NGS analysis using the methylation panel on the Illumina MiSeq.
- Bioinformatic analysis was conducted using PIVAT.

	TUMOR TYPE	Overall SNV/Indel Calls^			Methylation	
		P	VUS	Benign	BRCA1	RAD51C
1	ENDOMETRIOID	ARID1A D1850Tfs*33 16.2% PTEN R130Q 29.3%	ATM L612F 50.3%			
2	SEROUS	TP53 L289Pfs*56 4.7% BRCA1 Q1756Pfs*74 50.4%	ATR R1451W 47.7%			
3	SEROUS	TP53 P58Qfs*65 91.2% BRCA2 G1376Afs*11 89.4%	FANCA A746V 10.8% BARD1 R150G 5.8% MRE11 E600Q 41.4%			
4	SEROUS		ATM T2333I 63.2%			
5	CLEAR CELL	PIK3CA H1047R 34% ARID1A Y417Tfs*16 76.3%	CDK12 S987F 37.1%			
6	SEROUS	BRIP1 R798* 84.7% TP53 H179Y 65.9%	FANCD2 M782T 49.3% ATM R2580S 35.9%			
7	ENDOMETRIOID	KRAS G12V 43.4%	MRE11 V64G 47.9% BARD1 L239Q 48.5%			
8	SEROUS	TP53 Y220C 59.8%	BRCA2 E2599del 55%	BRCA2 Y42C 77.9%		
9	SEROUS	TP53 E271* 68.9%	PALB2 L323H 59.8%		29.44%	
10	SEROUS	TP53 N239Ifs*16 72.2%	FANCA R756C 14.7% ATR Y291D 16.4%		51.50%	
11	CLEAR CELL	ARID1A Q878* 38.8% PTEN T167Ifs*16 13.4% PTEN P248Tfs*5 14.2%	RAD50 R327H 53%			
12	SEROUS	TP53 c.920-1G>T 91.4%	ATM T2333I 8.1%			
13	SEROUS	TP53 C242F 4.2% TP53 C124* 39.9%				
14	MIXED	PIK3CA E542K 27.6% KRAS G12A 61.8%				
15	SEROUS	TP53 H193R 86.1%	FANCD2 P732L 45%			
16	SEROUS	CDK12 c.2964-1G>A 13.9% TP53 R280G 33.3%	FANCD2 R1452I 48.4%			
17	SEROUS	TP53 C242S 82.8%	BRCA1 E1456K 18.1% BRCA1 M1783T 81.9%			
18	SEROUS	BRCA2 N404Mfs*26 20.8% TP53 Y107* 37.3%	BRCA1 A1708V 60.7%			
19	SEROUS	TP53 R175H 31.2%				
20	ENDOMETRIOID	CTNNB1 S37C 26.7%	ATR R668W 48.3% FANCE R69Q 31.4%	FANCE G246del 46.5%		
21	SEROUS	TP53 P75Lfs*48 74.7% BRCA1 E23Vfs*17 90.2%	NBN P199S 94.2% ATR K764E 69.3%			
22	MUCINOUS	TP53 G245S 21.5% KRAS G12V 15%				
23	SEROUS	TP53 W146* 40.8%			34.90%	
24	SEROUS	TP53 L111R 20.5%	ARID1A A45V 18.3% ATR Y2132D 52.4%			
25	CLEAR CELL	TP53 C141Afs*29 63.9%	BRCA2 S1115P 14.7%			
26	MUCINOUS	KRAS G12V 40.5% TP53 L330Ifs*15 49.2%	RAD50 R726H 48.3% FANCD2 R779H 29.9%	FANCD2 F389V 30.3%		
27	SEROUS			TP53 P72R 99.9%	46.91%	
28	CLEAR CELL			BRCA1 P871L 47.3%		
29	MUCINOUS	TP53 G280fs*6 62.7 BRAF V600E 38.3				
30	SEROUS	TP53 R273H 45.8				
31	SEROUS			BRCA1 P871L 84.9%	24.05	
32	SEROUS	BRCA1 p.Asn1255LysfsTer12 69.4% TP53 p.Arg273His 40.8%		Undefined	Negative	Negative
33	ENDOMETRIOID	PTEN p.Arg233Ter 17.3%	BRCA1 p.Lys654SerfsTer47 18.2%		Negative	Negative
34	POORLY DIFFERENTIATED		MRE11 p.Gly600Gln 49.17% BRCA2 p.Asp980Tyr 43.4%		Negative	Negative
35	SEROUS			ATR p.Ile774TyrfsTer5 20.2%	Negative	Negative
36	POORLY DIFFERENTIATED	None	None		Negative	Negative
37	SEROUS		FANCE c.1316+19G>A 50.89%		Negative	Negative
38	SEROUS	None	None		Negative	Negative
39	SEROUS	TP53 p.Ser241Phe 15.4%	FANCE c.1316+19G>A 40.9%		Negative	Negative
40	SEROUS		FANCE p.Ser204Leu		Negative	38.5
41	ENDOMETRIOID	BARD1 p.Cys557Ser 54.2% TP53 p.Arg337Cys 3.3% CTNNB1 p.Asp32Tyr 33.17%	BARD1 p.Asp612Val 52.1% BRCA2 p.Pro465Ser 23.8%		Negative	Negative
42	ENDOMETRIOID	NBN p.Lys219Asnfs*Ter16 88.91%		RAD51B p.Pro365Arg 82.3%	Negative	Negative
43	CLEAR CELL	None	None		Negative	Negative

Table 1: DNA mutations and methylation detected by NGS in the ovarian study cases

44	TNBC		FANCE c.1316+19G>A 49	FANCD2 p.Gln934Hs 49.2%	Negative	Negative
45	TNBC	TP53 p.Arg110Pro 4.7%	FANCC p.Ser264Gly 51.6%		Negative	Negative
46	TNBC	PIK3CA p.His1047Arg 55.9% TP53 p.Arg110Pro 56.5%	FANCE p.Arg696Gln 50.9%	ERBB2 p.Ile655Val 59%	Negative	Negative
47	TNBC		TP53 p.Gln144Pro 44.4% FANCE p.Ser204Leu 63.9%		Negative	Negative
48	TNBC	BRCA2 p.Lys2950Asn 54.1%			Negative	Negative
49	TNBC		FANCE p.Ser204Leu 29.77% TP53 p.Phe109Ser 51.2%		Negative	Negative
50	TNBC		PTEN c.802-51_802-14del 25.6% FANCE p.Ser204Leu 51%		Negative	Negative
51	TNBC	TP53 p.His179Arg 39.58%			Negative	Negative
52	TNBC		TP53 p.Gly266Glu 28.1%		Negative	Negative
53	TNBC	TP53 p.Arg175His 51.6%		ERBB2 p.Ile655Val 78%	Negative	Negative
54	TNBC		BRCA1 p.Thr856Lys 61.66% TP53 p.Glu258Lys 25.2%		Negative	66%
55	TNBC	TP53 p.Ser303Alafs*Ter42 71.39%	BRCA1 p.Arg974His 15.5%		Negative	Negative
56	TNBC	PIK3CA p.His1047Arg 13.86% TP53 p.Glu224* 29.91%			Negative	Negative
57	TNBC	PIK3CA p.His1047Arg 16.5% TP53 p.Leu119ProfsTer40 6.38% TP53 p.Trp91Ter 23.56% ERBB2 5%	BRCA1 p.Gly834Arg 2.72% p.His1326Tyr 2.97% p.Pro897Ser 2.9% BRCA2 p.Tyr2726Cys 2.9% p.Arg2787His 3.6% p.Arg2842Cys 4.35% FANCA p.Met415Ile 8.6%		Negative	Negative
58	TNBC		BRCA2 p.Asn1279Asp 2.7% FANCE p.Ser204Leu 55.69% MRE11 p.Gly579Glu 42.44% ARID1Ap.Pro1206Ser 23.33%		Negative	Negative
59	TNBC	PTEN p.Lys183Argfs*Ter16 11.85%			Negative	Negative
60	TNBC	TP53 p.Glu221Ter 51.82%	FANCC p.Cys355Ser 36.5%		Negative	Negative
61	TNBC			ERBB2 p.Ile655Val 51.78%	Negative	Negative
62	TNBC	None	None		Negative	Negative
63	TNBC	BRCA1 p.Glu781Asnfs*Ter12 40.42% TP53 p.Arg248Gln 40%		FANCD2 p.Gln934Hs 71.3% CDK12 p.Leu1189Gln 71%	44.30%	Negative
64	TNBC	TP53 p.Gln167Ter 7.38%	ARID1A p.Pro1206Ser 40%		Negative	Negative
65	TNBC	TP53 p.Ser241Tyr 53.93%		CDK12 p.Thr1195Met 80%	Negative	Negative
66	TNBC		BARD1 p.Tyr597Cys 47.7% MRE11 p.Arg628Lys 34.9% FANCD2 p.Pro852Arg 45.7%		Negative	Negative
67	TNBC			CDK12 p.Leu1189Gln 35.62%	Negative	Negative
68	TNBC	TP53 c.994-1G>C 56.39%	RAD51D p.Glu307Lys 52.79% FANCE p.Ser204Leu 56.56%		Negative	Negative
69	TNBC	None	None		Negative	Negative
70	TNBC	NBN p.Arg43Ter 56.1%			Negative	Negative
71	TNBC	BRCA1 p.Gln1200Argfs*Ter18 16.3% TP53 p.Arg213Ter 6.6%	FANCE p.Arg696Gln 25.15%		Negative	Negative

Table 2: DNA mutations and methylation detected by NGS in the Triple Negative Breast Cancer (TNBC) study cases

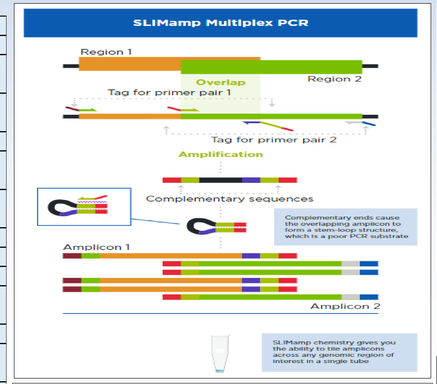

Fig 1:Workflow process for SLIMamp tumor panel

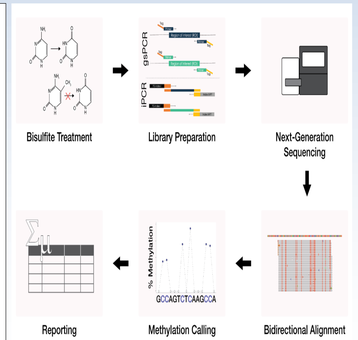

Fig 2: Flowchart for the SLIMamp NGS methylation panel

SAMPLE NO	TUMOR TYPE	PATHOGENIC MUTATIONS	METHYLATION	
			BRCA	RAD 51
2	SEROUS	TP53 L289Pfs*56 4.7% BRCA1 Q1756Pfs*74 50.4%	Neg	Neg
3	SEROUS	TP53 P58Qfs*65 91.2% BRCA2 G1376Afs*11 89.4%	Neg	Neg
6	SEROUS	BRIP1 R798* 84.7% TP53 H179Y 65.9%	Neg	Neg
18	SEROUS	BRCA2 N404Mfs*26 20.8% TP53 Y107* 37.3%	Neg	Neg
21	SEROUS	TP53 P75Lfs*48 74.7% BRCA1 E23Vfs*17 90.2%	Neg	Neg
71	TNBC	BRCA1 p.Gln1200Argfs*Ter18 16.3% TP53 p.Arg213Ter 6.6%	Neg	Neg
32	SEROUS	BRCA1 p.Asn1255LysfsTer12 69.4% TP53 p.Arg273His 40.8%		
9	SEROUS		29.44	
10	SEROUS		51.5	
23	SEROUS		34.9	
27	SEROUS		46.91	
31	SEROUS		24.05	
40	SEROUS			38.5
48	TNBC	BRCA2 p.Lys2950Asn 54.1%		
54	TNBC			66%
63	TNBC	BRCA1 p.Glu781Asnfs*Ter12 40.42% TP53 p.Arg248Gln 40%	44.30%	

Table 3: Specimens detected by NGS that exhibit HRD and benefit from PARP inhibitors

Results

- The NGS results were analyzed by the Pillar PIVAT pipeline.
- Table 1 and 2 shows pathogenic HRD mutations were detected in 56/71 (79%) specimens.
- The most common mutations detected were in TP53 (52%) and BRCA1/2 (11%), followed by mutations in genes such as ARID1A, KRAS, PTEN, CTNNB1, BRIP1, PIK3CA, and CDK12
- Methylation analysis showed that five high-grade serous carcinoma specimens harbored promoter methylation in BRCA1, and one in RAD51C.
- In the TNBC cohort, one specimen each was methylated for BRCA1 and RAD51C.
- The level of promoter methylation varied across the methylated specimens.
- Table 3 is a compilation of patient specimens that will show HRD using the Pillar HRD assay.
- These specimens either harbor a HRD related mutation or show promoter methylation in HRD genes.

Conclusions

This study provides encouraging preliminary evidence for the possibility of implementing in house based HRD testing in routine clinical practice. The oncoReveal methylation assay also provides a unique approach to investigate HRD in ovarian carcinoma and triple negative breast cancers.