Molecular and immune landscape of metaplastic triple negative breast cancer compared with invasive ductal triple negative breast cancer

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BACKGROUND

- Metaplastic Breast Cancer (MBC) is rare and aggressive form of BC with majority having triple-negative receptor status.
- There are no standard therapeutic approaches for MBC and majority are treated similar to invasive ductal triple negative breast cancer (ID-TNBC) but with worse outcomes in comparison to other BC subtypes.
- There is an urgent need for new drug targets and therapies for MBC. Here, we characterize the molecular and immune signature of metaplastic TNBC (M-TNBC).

METHODS

- 455 BC samples (M-TNBC, n=91; ID-TNBC, n=364) were analyzed by next-generation sequencing (592, NextSeq; WES, NovaSeq), Whole Transcriptome Sequencing (WTS; NovaSeq) (Caris Life Sciences, Phoenix, AZ).
- Tumor mutational burden (TMB) totaled somatic mutations per tumor (high>10 mt/MB).
- Microsatellite-instability (MSI) was tested by IHC and NGS.
- Immune cell fractions were calculated by deconvolution of WTS: Quantiseq.
- Pathway enrichment was determined by Gene Set Enrichment Analysis (GSEA, Broad Institute).
- Statistical significance was determined using chi-square and Mann-Whitney U test and p-value <0.05 was considered significant.

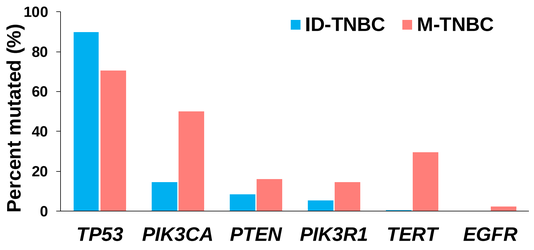
Table 1: Sample demographic information

		Invasive ductal TNBC (ID-TNBC)	Metaplastic TNBC (M-TNBC)
Count (N)		364	91
Median Age [range]		59 [24 - >89]	64 [22 - >89]
Sex	Female	99.7% (363/364)	100% (91/91)
	Male	0.3% (1/364)	0.0% (0/91)
Race	White	64.8% (175/270)	66.7% (48/72)
	Black/AA	28.5% (77/270)	19.4% (14/72)
	Asian/Pacific Islander	1.9% (5/270)	4.2% (3/72)
	Other	4.8% (13/270)	9.7% (7/72)
Ethnicity	Not Hispanic or Latino	83.1% (217/261)	81.4% (57/70)
	Hispanic or Latino	16.9% (44/261)	18.6% (13/70)

Race/ethnicity data is self-reported

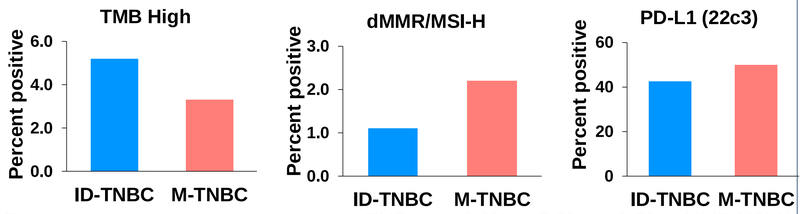
RESULTS

Figure 1. Mutation analysis of ID-TNBC and M-TNBC



M-TNBC had a higher frequency of *PIK3CA* (50.0% vs 14.5%), *PTEN* (16.1% vs. 8.4%), *EGFR*, (2.2% vs 0%), *PIK3R1 (14.4% vs. 5.5%)*, *TERT* (29.7% vs. 0.5%), but lower frequency of *TP53* (70.6% vs. 89.7%) compared to ID-TNBC (all p<0.05).

Figure 2. TMB-high, dMMR/MSI-H and PD-L1 positivity



There was no differences in frequency of TMB-high (3.3% vs 5.2%, p=0.58), dMMR/MSI-H (2.2% vs 1.1%, p=0.34) and PD-L1 positivity (22c3) (50.0% vs 42.6%, p=0.38) between M-TNBC and ID-TNBC.

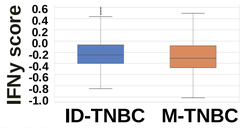
Figure 3. Immune cell infiltration

	ID-TNBC	M-TNBC
B cells	4.46	3.99 *
Mφ M1	3.45	3.46
Mφ M2	2.94	3.38 *
DC	3.21	1.73 *
Neutrophils	2.65	5.97 *
NK cells	3.05	2.82
T cells CD8	0.58	0.00 *
Tregs	1.99	1.02 *

Low Median% High Median%

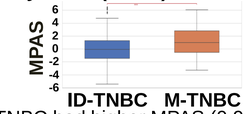
M-TNBC had increased infiltration of M2 Mφ (3.3% vs. 2.9%) and neutrophils (5.9% vs. 2.6%) but decreased infiltration of B cells (3.9% vs. 4.5%), Treg (1.0% vs. 1.9%), DC (1.7% vs. 3.2%) and CD8 T cells (0% vs. 0.5%). For CD4 T cells median was 0 in both groups. *p<0.05.

Figure 4. IFNy score analysis



M-TNBC had decreased IFNy score (-0.31 vs -0.24, p=0.05)

Figure 5. MAP kinase pathway activity score (MPAS)



M-TNBC had higher MPAS (0.9 vs 0.08, p<0.05).

Figure 6. Immune-related gene expression in ID-TNBC and M-TNBC

Checkpoint gene	ID-TNBC	M-TNBC
CD274	4.01	5.60
PDCD1	0.52	0.45
CTLA4	1.61	1.52
PDCD1LG2	1.67	1.63
FOXP3	2.79	2.07
HAVCR2	19.70	18.71 *
LAG3	1.05	0.95
IDO1	2.90	1.94 *

MHC Class-I	ID-TNBC	M-TNBC
TAP2	41.62	43.03
TAP1	14.82	12.62
HLA-B	172.61	179.35
HLA-A	167.55	181.83
HLA-C	156.69	171.97
B2M	1464.34	1505.04

MHC Class-I	ID-TNBC	M-TNBC
HLA-DQB2	1.30	1.23
HLA-DRB1	100.58	102.67
HLA-DPA1	169.86	169.80
HLA-DPB2	0.06	0.08
HLA-DPB1	81.20	90.35
HLA-DQB1	22.24	20.09

ID-TNBC had higher expression of immune checkpoint genes (*FOXP3, IDO1*; FC: 1.3-1.5). *p<0.05.

Low TPM (Median) High TPM (Median)

Figure 7. Cancer progression-related gene expression in ID-TNBC and M-TNBC

Stem cell genes	ID-TNBC	M-TNBC
CD44	454.61	572.78*
ALDH1A2	1.28	1.65 *
ALDH1A3	9.90	7.81
POU5F1	2.70	2.88
KLF4	5.38	7.27 *
SOX2	0.16	0.38 *
NANOG	0.28	0.29

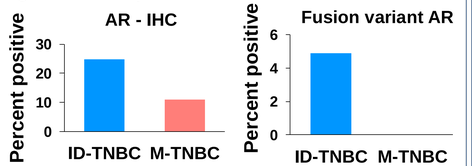
Cell cycle genes	ID-TNBC	M-TNBC
CDKN1A	12.72	19.95
CDKN1B	22.38	18.16 *
CCND2	6.74	7.71
E2F1	3.33	2.32 *
CCNE1	5.29	3.83 *
CCND1	330.37	402.02

Apoptosis-related	ID-TNBC	M-TNBC
NAIP	12.11	15.22
XIAP	40.79	44.97
BCL2	2.24	1.77
BIRC3	7.60	5.60 *
BCL2L1	18.41	21.03
BIRC6	93.79	86.25 *
BIRC2	31.63	34.65 *
BIRC5	2.61	2.69

M-TNBC had higher expression of stem cell-related genes (*CD44, ALDH1A2, KLF4, SOX2*; FC: 1.2-2.3), but lower expression of cell cycle genes (*CDKN1B, E2F1, CCNE1*; FC: 1.2-1.4), inhibition of apoptosis genes (*BIRC3, BIRC6, BCL2*; FC: 1.1-1.3). *p<0.05.

Low TPM (Median) High TPM (Median)

Figure 8. AR expression and fusion variant-AR



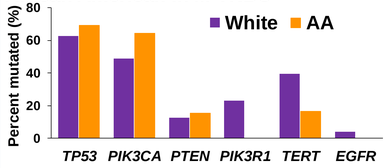
M-TNBC had lower AR protein expression (11.0% vs 24.8%) and lower frequency of fusion variant-AR (0% vs 4.9%) compared to ID-TNBC (all p < 0.05).

Figure 9. Gene set enrichment analysis

Term	NES	FDR
Epithelial Mesenchymal Transition (EMT)	1.52	0.00

M-TNBC had gene set enrichment of EMT pathway (NES: 1.5, FDR<0.25).

Figure 10. Mutation analysis of White and African American in M-TNBC



There was no difference in mutation frequency between White and African American M-TNBC

CONCLUSIONS

Our data indicate that M-TNBC is associated with an aggressive disease biology with higher frequency of *PIK3CA, PTEN, PIK3R1, TERT, EGFR* and gene set enrichment of EMT pathway. Higher expression of stem cell-related gene expression in M-TNBC indicates their association with therapy resistant phenotype. Also, M-TNBC had increased infiltration of M2 Mφ and neutrophils, lower IFNy score suggesting differential TME. However, these findings warrant further validation by larger studies.