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Comprehensive Profiling of Metaplastic Breast Carcinoma Reveals Frequent Over-Expression of PD-L1

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Abstract (updated)

Background: Metaplastic breast carcinoma (MBC) is a rare subtype of breast carcinoma less responsive to conventional chemotherapy relative to usual breast carcinomas such as ductal and lobular subtype. In molecular terms, MBC usually clusters with triple negative breast cancers (TNBC), but MBCs portray a worse prognosis in comparison with TNBC. Published studies investigating MBCs for specific biomarkers of therapy response are rare and limited by the methodological approaches.

Methods: 297 samples [MBC (n=75), triple-negative breast cancer of no-special-type (TNBC-NOS, n=106), HER2-positive breast cancers (n=32) and luminal breast cancers (n=84)] were profiled using direct sequencing analysis [Illumina MiSeq Next Generation Sequencing (NGS)]. Immunohistochemistry for PD-L1 (SP142, Spring Bioscience) and PD-1 (NAT105, Ventana) was performed using automated procedures.

Results: 89% MBCs exhibited triple-negative immunophenotype (ER-/PR-/HER2-). The most common mutations in MBCs included *TP53* (67%) and *PIK3CA* mutations (23%). Other mutations were rare including *HRAS* mutations (7%), *STK11* (5%), *FBXW7, PTEN, c-MET* and *JAK3* (4%, respectively). PD-L1 expression on cancer cells was detected in significantly higher proportion of MBCs (46%) than in other molecular subtypes (6% in luminal and HER2+ breast cancers, respectively and 9% in TNBC-NOS, p<0.001). PD-1 positive tumors infiltrating lymphocytes (TILs) varied greatly in MBCs (0 to >50/mm2).

Conclusion: Comprehensive profiling of a large cohort of this rare carcinoma highlighted predominance of *TP53* mutations, wild type *EGFR* gene expression, a distinct increase in proportion of PD-L1 expression in carcinoma cells, and PD-1 expression in TILs. The latter properties can be exploited in clinical trials utilizing immune check point inhibitors.

Background

Metaplastic breast carcinoma (MBC) encompasses a group of mammary neoplasms characterized by differentiation of the neoplastic epithelium into squamous or mesenchymal cells (1). It is less responsive to conventional chemotherapy compared to the usual ductal and lobular breast carcinomas (2). In molecular terms MBC usually clusters with triple negative (ER-/PR-/ Her2-) breast cancer (TNBC), but MBCs portray a worse prognosis in comparison with TNBC. Published studies investigating MBCs for specific biomarkers of therapy response are rare and limited by the methodological approaches (3, 4).

Methods

The study included 297 samples [MBC (n=75), triple-negative breast cancer of no-special-type (TNBC-NOS, n=106), HER2-positive breast cancers (n=32) and luminal (ER+/Her2-) breast cancers (n=84)]. The samples were profiled using direct sequencing analysis [**Illumina MiSeq Next Generation Sequencing (NGS)**] using formalin-fixed paraffin embedded tissue blocks. **Immunohistochemistry** for PD-L1 (SP142, Spring Bioscience) and PD-1 (EH12.1, Pharmingen) was performed using automated procedures.

Results (updated)

Mean age of patients: 57 years (range, 35-93 years).

Histology: MBCs exhibited various morphologic features including squamous, myoepithelial, spindle, and rhabdoid morphology with heterologous elements: bone (n=3) and cartilage (n=22 cases).

ER, PR and **HER2** status were available for 71 patients; 63 cases (89%) of MBCs exhibited triple-negative phenotype; the remaining 8 cases (11%) were ER/or PR positive (7 cases) and HER2 positive (1 case).

PD-1 and PD-L1 expression in metaplastic carcinoma

Group	PDL-1 status		Total
	negative	positive	
Metaplastic carcinoma	39 (54%)	**33 (46%)**	72
TNBC NOS	93 (91%)	**9 (9%)**	102
HER2+	30 (94%)	**2 (6%)**	32
Luminal carcinoma	79 (94%)	**5 (6%)**	84
Total	241 (83%)	**49 (17%)**	290

Table 1. PD-L1 expression was significantly higher in metaplastic carcinomas in comparison with other breast cancer subtypes (46% vs. 6-9%, p<0.001).

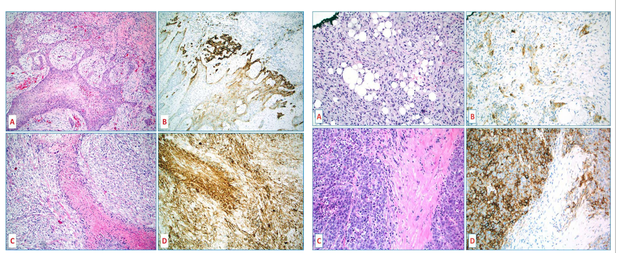
TME (PD-L1/TIL)		Frequency (%)
Type	Type 1 (PD-L1+, TILs+)	29 (40.8%)
	Type 2 (PD-L1-, TIL-)	14 (19.7%)
	Type 3 (PD-L1+, TIL-)	4 (5.6%)
	Type 4 (PD-L1-, TIL+)	24 (33.8%)
Total		71 (100%)

Table 2. The stratification of MBCs into 4 types (5) on the basis of PD-L1 and TILs status; The proposed stratification is a framework to tailored immunotherapy against tumor microenvironment (TME);

PD-1 positive tumors infiltrating lymphocytes (TILs) varied greatly in MBCs (0 to 400/mm^2, mean: 67.3). No significant association was found between the number of TILs and PD-L1 status (p=0.209).

Overall PD-L1 positivity among 75 metaplastic breast carcinomas was 46%; highest of all types.

Figure 1 Figure 2



Figures 1A-D, 2A-D. Despite various morphologic appearances (A, C) of metaplastic carcinomas, strong PD-L1 overexpression (B, D) was observed in nearly 50% of the cases.

Mutational profiling of metaplastic carcinoma

72 MBCs were tested by NGS of which 57 cases had interpretable results. Mutations were detected in 16 out of 45 tested genes affecting 48 out 57 metaplastic carcinomas (84%). ***TP53* mutation** was the most frequent mutation (32/57, 56%). In 20 cases, ***TP53*** was the sole mutation detected in the tumor tissue while in the remaining 12 cases other mutations co-occurred. ***PIK3CA*** mutation was the second most common mutation (13/72, 23%) while ***HRAS*** mutations was detected in 4 cases (7%), ***STK11*** in 3 cases (5%), ***FBXW7, PTEN, c-MET*** and ***JAK3*** in two cases, respectively (4%). Single cases harbored mutations of ***AKT1, APC, BRAF, RET, c-KIT, MLH1, RET***, and ***SMAD4*** genes. None of the cases harbored *EGFR* mutation.

Conclusions

Metaplastic carcinomas are characterized by increased PD-L1 expression in carcinoma cells, and PD-1 expression in TILs, which can be exploited in clinical trials utilizing immune check point inhibitors in this hard-to-treat subtype of breast cancer.

Comprehensive mutational profiling of MBC highlighted predominance of *TP53* and *PIK3CA* mutations and a wild type *EGFR* gene expression.

References

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