Elevated tumor mutation burden and immunogenic activity in patients with hormone receptor-negative or human epidermal growth factor receptor 2-positive breast cancer

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Received February 27, 2018; Accepted April 5, 2019

DOI: 10.3892/ol.2019.10287

Abstract. Immunotherapy has been found to be efficient in a variety of cancers and, therefore, may be a promising strategy for breast cancer (BC), particularly due to the limited therapeutic options currently available for triple-negative BC (TNBC). However, heterogeneity of tumor mutation burden (TMB), immune gene expression and mismatch repair (MMR) gene activity across BC subtypes has not been well characterized. In the present study, a comprehensive analysis of TMB, expression levels of immune cell type marker genes, and expression levels of MMR-associated genes was performed. A total of 5 MMR-associated genes, including *MLH1*, *MLH3*, *MSH2*, *MSH6* and *PMS2*, were analyzed. Patients that harbored at least two MMR genes with expression levels in the lower 10% percentile across the cohort were considered as potentially aberrant (lost expression). Hormone receptor (HR)-negative BC is associated with a higher TMB and immune gene expression compared with HR-positive BC [TMB, estrogen receptor (ER)-negative vs. ER-negative, 55 vs. 32, respectively; $P=4.1x10^{-13}$; progesterone receptor (PR)-negative vs. PR-positive, 53 vs. 31, respectively; $P<2.2x10^{-16}$]. By contrast, human epidermal growth factor receptor 2 (HER2)-negative BC tended to have a lower TMB and decreased immune gene expression compared with HER2-positive BC (TMB, HER2-negative vs. HER2-positive, 36 vs. 48, respectively; P=0.02). Furthermore, aberrant expression of MMR genes was found to be more common in HR-negative compared with HR-positive BC (P<0.001). Significantly higher expression levels of each immune marker gene of four major immune cell types were found in patients who were HR-negative compared with patients who were HR-positive (P<0.001). The findings of the present study suggest that HR-negative or HER2-positive BC exhibits elevated TMB and immunogenic activity, and immunotherapeutic options are recommended.

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Key words: breast cancer, hormone receptor, tumor mutation burden, mismatch repair

Introduction

Breast cancer (BC) is the most common malignancy in women worldwide, and the majority of BC subtypes are hormone-associated (1). Estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER2) status, routinely available in BC specimens, are reliable and useful tools for therapeutic decision-making (2). Hormone receptor (HR)-positive tumors comprise the majority of the cases and have a relatively better outcome (3). By contrast, triple-negative BC (TNBC) is a clinically heterogeneous disease with an aggressive clinical course (4). The lack of targeted therapies and the relatively poor prognosis of patients with TNBC have created the need to evaluate novel treatment approaches, including immunotherapy (5,6). A number of studies have investigated the efficacy of checkpoint inhibitor immunotherapy against TNBC (5,7-9). For example, pembrolizumab and atezolizumab plus nab-paclitaxel have demonstrated encouraging clinical benefits in patients with advanced TNBC (5,9).

Cancer immunoediting is the process of eliminating highly immunogenic tumor cells by somatic evolution and protecting the host from tumor development through the host immune system (10). Increased burden of somatic mutations has been associated with an increased number of pathogenic germline mutations in high- and moderate-risk BC genes in patients with BC (11). The frequency of somatic mutations or tumor mutation burden (TMB) is associated with the immunogenicity of BC (10). Assessment of TMB is becoming increasingly important for immunotherapy decisions in patients with melanoma and lung cancer (12); however, TMB heterogeneity across BC subtypes has not been well characterized. The aim

of the present study was to examine whether the TMB of ER (or PR and HER2)-negative BC differs from that of the corresponding positive subtypes. In addition, the study aimed to examine what molecular cues are associated with the differences in TMB between the negative and positive subtypes and whether there is a difference in immunogenic activity between ER (or PR and HER2)-negative and -positive BC. The distribution of TMB, expression of mismatch repair (MMR) genes and immune-associated genes were comprehensively compared among BC subtypes. HR-negative BC was found to have a higher TMB and increased expression of major immune cell types [B cells, $CD4^+$ and $CD8^+$ T cells, and natural killer (NK) cells] compared with HR-positive BC. Of note, HER2-positive BC tended to have higher TMB and increased immune gene expression compared with HER2-negative BC.

Materials and methods

The Cancer Genome Atlas (TCGA) whole-exome sequencing (WES) and RNA-seq data. Whole-exome somatic variants, gene expression and clinical data, including ER, PR and HER2 status, of 974 patients with BC were downloaded from Broad TCGA GDAC (v2016_01_28) (13). In the TCGA clinical dataset, ER or PR status is reported as negative or positive. HER2 status is reported as a score ranging between 0 and 3. A score of 0 or 1 is considered as HER2-negative, and a score of 3 as HER2-positive.

Calculation of TMB. TMB was defined as the total number of somatic missense substitutions of the exomes examined. Alternatively, TMB may be defined as the total number of somatic, coding, base substitution and indel mutations. In this case, all base substitutions and indels in the coding region, including silent alterations, are counted. Both methods were used for calculation of TMB, and the results were essentially identical. Only the results based on all base substitutions and indels are presented.

Expression analysis of MMR genes. The normalized expression levels (RNAseqV2, quantile normalized by RSEM) were downloaded from Broad TCGA GDAC (v2016_01_28) (13). Five MMR-associated genes (14), including *MLH1*, *MLH3*, *MSH2*, *MSH6* and *PMS2*, were analyzed. Patients that harbored at least two MMR genes with expression levels in the lower 10% percentile across the cohort were considered as potentially aberrant (lost expression).

Expression analysis of immune cell types. To investigate the difference in the expression levels of immune cell types among different BC subtypes, 4 major immune cell types were selected for examination, including B cells, $CD4^+$ T cells, $CD8^+$ T cells and NK cells. Cell type marker genes were identified based on the previous literature (15,16). B-cell marker genes include *CD79A*, *CD79B*, *BTLA*, *FCRL1*, *FCRL3*, *BANK1*, *BLK* and *RALGPS2*. $CD4^+$ T-cell marker genes include *CTLA4*, *IL32*, *FOXP3*, *GPR15* and *C15orf53*. *CD8A* is considered as a $CD8^+$ T-cell marker. NK-cell marker genes include *KLRF1* and *KLRC1*.

Statistical analysis. Analyses were performed using SPSS (v.20.0, IBM Corporation, Armonk, NY, USA). Data are presented as mean ± SD. The non-parametric Wilcoxon's rank-sum test was used to assess the differences in TMB between patients with negative and positive hormone receptor status (ER, PR or HER2). Differences in the proportion of samples with aberrant expression of MMR genes between ER-, PR- or HER2-negative and -positive status were assessed by the χ^2 test. Similar results were observed when the lower 5% percentile was considered as the cut-off to define aberrant expression. $P<0.05$ was considered to indicate a statistically significant difference.

Results

TMB differs significantly between HR-negative and HR-positive BC. To investigate the mutation burden heterogeneity across BC subtypes, TMB was compared between patients who were ER (or PR, HER2)-negative and patients who were ER (or PR, HER2)-positive. TMB was defined as the total number of coding SNVs6 and indels. Across the TCGA cohort of 974 BC samples, the number of exome mutations in individual cancers varies widely (range, 2-4,561; median, 38). The TMB and ER/PR/HER2 status were analyzed (data not shown). Patients who were ER-negative were found to have a significantly higher TMB ($P=4.1x10^{-13}$; Wilcoxon's rank-sum test) compared with patients who were ER-positive (Fig. 1A). The median TMB was 55 and 32 for patients ER-negative and ER-positive, respectively. PR-negative tumors also had a significantly higher TMB ($P<2.2x10^{-16}$; Wilcoxon's rank-sum test) compared with PR-positive tumors (Fig. 1B). The median TMB was 53 and 30.5 for patients who were PR-negative and PR-positive, respectively. By contrast, HER2-negative tumors had a significantly lower TMB ($P=0.02$; Wilcoxon's rank-sum test) compared with HER2-positive tumors (Fig. 1C). The median TMB was 38 and 46 for patients who were HER2-negative and HER2-positive, respectively. The increased TMB in the HER2-positive group was more evident in patients who were HR-positive, however was not observed in the HR-negative group (data not shown). Patients with TNBC also had a significantly higher TMB ($P=9.4x10^{-06}$; Wilcoxon's rank-sum test) compared with patients with non-TNBC (Fig. 1D). The median TMB was 55.5 and 38 for patients with TNBC and non-TNBC patients, respectively. These results suggest that TMB heterogeneity is common among different BC subtypes based on HR and HER2 status. In general, HR-negative BC has a higher TMB compared with HR-positive BC, and HER2-negative BC tends to have a lower TMB compared with HER2-positive BC.

Aberrant expression of MMR genes is more common in HR-negative BC. In order to investigate the molecular cues of increased TMB in ER- or PR-negative BC, statistical analysis was performed to test whether the presence of aberrant MMR expression was associated with BC subtypes. Five MMR-associated genes, including *MLH1*, *MLH3*, *MSH2*, *MSH6* and *PMS2*, were analyzed. Patients that harbored at least two MMR genes with expression levels in the lower 10% percentile across the TCGA cohort were considered as potentially aberrant (lost expression). The expression levels of each MMR gene and ER/PR/HER2 status were analyzed (data not shown). Aberrant expression of MMR genes was found to be more common ($P<0.001$; χ^2 test) in HR-negative BC compared

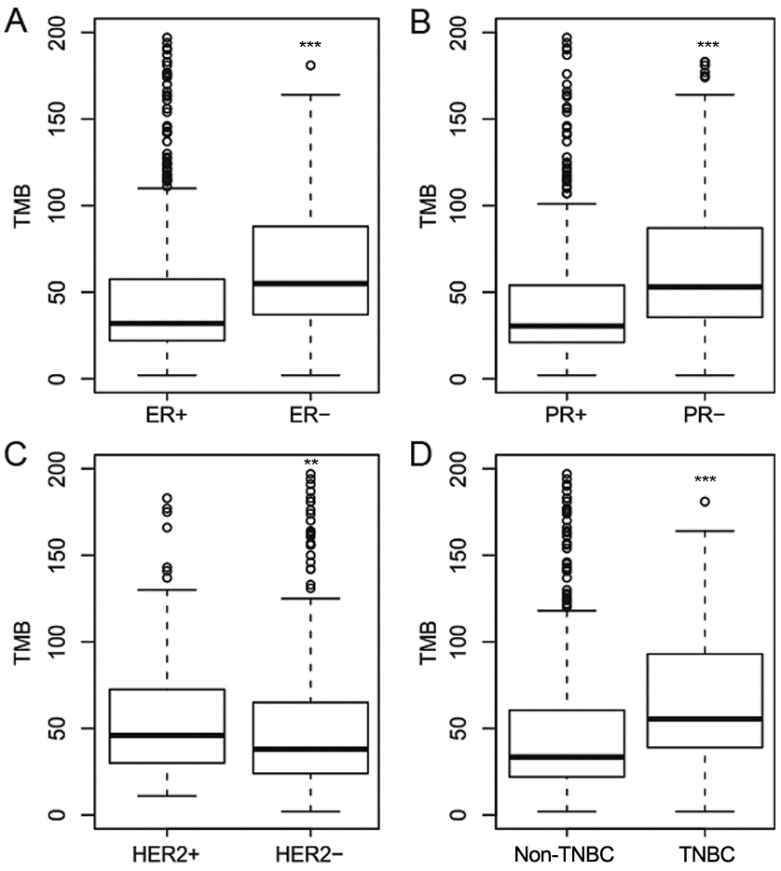

Figure 1. TMB distribution in patients with BC with different ER/PR/HER2 status. (A) Patients who were ER-negative had a higher TMB compared with patients who were ER-positive. (B) Patients who were PR-negative had a higher TMB compared with patients who were PR-positive. (C) Patients who were HER2-negative had a lower TMB compared with patients who were HER2-positive. (D) Patients with TNBC had a higher TMB compared with patients with non-TNBC. **P<0.05 vs. ER+ or HER2+; ***P<0.001 vs. PR+ or non-TNBC. TMB, tumor mutation burden, calculated as the sum of all base substitutions and indels in the coding region; BC, breast cancer; ER, estrogen receptor; PR, progesterone receptor; HER2, human epidermal growth factor receptor 2; TNBC, triple-negative breast cancer.

with HR-positive BC (Table I). However, the proportion of aberrant MMR gene expression did not differ significantly (P=0.1; χ^2 test) between patients who were HER2-positive and HER2-negative (Table I). This pattern is consistent with the pattern observed when comparing TMB among BC subtypes. The results suggest that aberrant expression of MMR genes is more common in HR-negative patients with BC.

HR-negative BC exhibits increased expression levels of immune cell marker genes. Previous studies have used genomic data from TCGA to characterize cytolytic activity estimated using the expression of two genes (15,17,18). Recently, marker gene expression was used to analyze infiltration of various immune cell types (16,19-21). In the present study, 4 major immune cell types, including $CD4^+$ T cells, $CD8^+$ T cells, B cells and NK cells, were analyzed. The expression levels of the aforementioned marker gene sets for each cell type were compared between different BC subtypes. Significantly higher expression levels of each immune marker gene were found in patients who were HR-negative compared with patients who were HR-positive (P<0.001; Fig. 2 and Table II). By contrast, the HER2-positive group exhibited significantly increased expression of immune marker genes, such as CTLA4, FCRL1, C15orf53 and CD79A, compared with the HER2-negative group (P<0.001; Wilcoxon's rank-sum test). A similar trend was observed in gene expression between HER2-positive and HER2-negative groups only in patients who were HR-positive. The median expression levels for each marker gene in patients with different ER/PR/HER2 status are provided in Table II. These results suggest that HR-negative BC may exhibit increased immunogenic activity compared with HR-positive BC. Furthermore, HER2-positive BC may exhibit increased immunogenic activity compared with HER2-negative BC.

Table I. MMR gene expression in patients with BC with different ER/PR/HER2 status.

Status	Aberrant MMR expression	Normal MMR expression	P-value[a]
ER-	46	167	P<0.0001
ER+	75	641	
PR-	62	245	P<0.0001
PR+	59	560	
HER2-	59	427	P=0.091
HER2+	4	76	

[a]χ^2 test. BC, breast cancer; MMR, mismatch repair; HR, hormone receptor; HER2, human epidermal growth factor receptor 2; ER, estrogen receptor; PR, progesterone receptor.

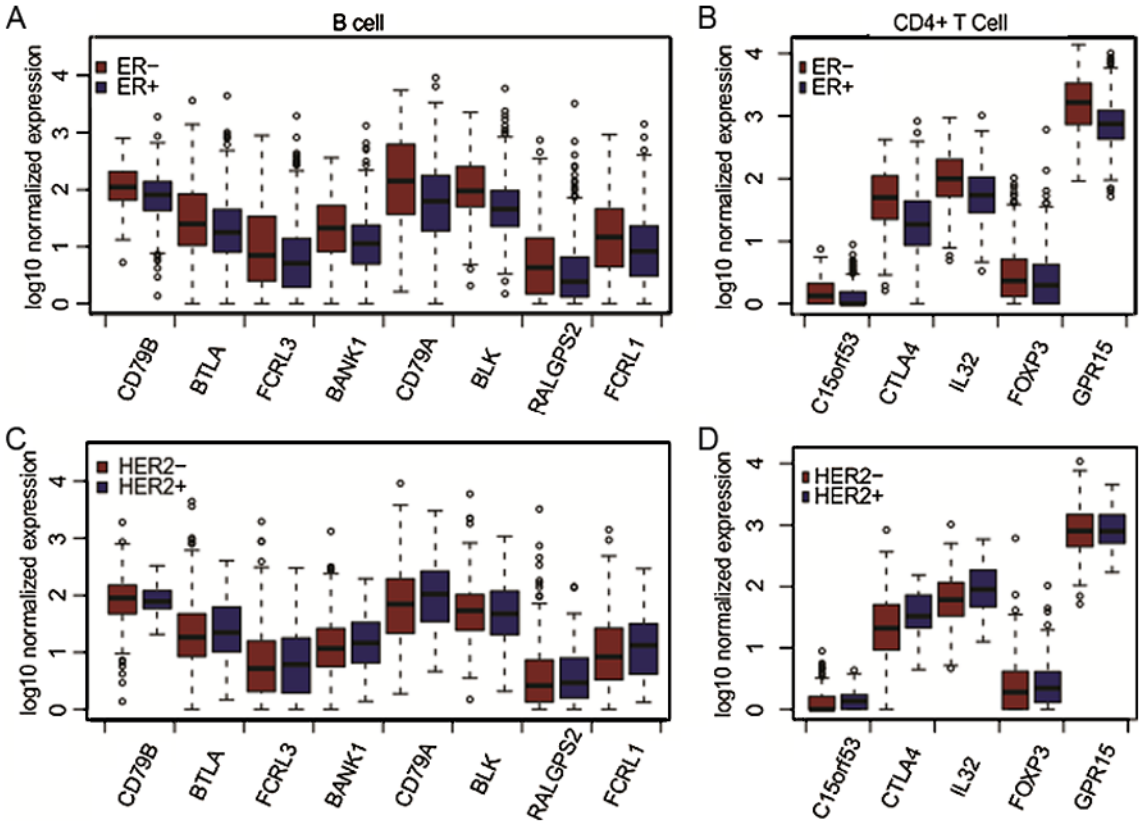

Figure 2. Expression levels of immune cell types in patients with BC with different HR/HER2 status. (A) Patients who were ER-negative consistently exhibited higher expression levels of B-cell marker genes compared with patients who were ER-positive. (B) Patients who were ER-negative consistently exhibited higher expression levels of $CD4^+$ T-cell marker genes compared with patients who were ER-positive. (C) Patients who were HER2-negative exhibited lower expression levels of B-cell marker genes compared with patients who were HER2-positive. (D) Patients who were HER2-negative exhibited lower expression levels of $CD4^+$ T-cell marker genes compared with patients who were HER2-positive. BC, breast cancer; HR, hormone receptor; HER2, human epidermal growth factor receptor 2; ER, estrogen receptor.

Discussion

Cancer immunoediting is the process of eliminating highly immunogenic tumor cells by somatic evolution, and protecting the host from tumor development through the host immune system (7). Molecular studies have reported that mutational heterogeneity in BC was associated with novel cancer-associated genes, such as breast cancer genes 1 and 2 (BRCA1/2) and ataxia telangiectasia mutated (ATM) serine/threonine kinase, and antigens (CD8+, PD-L1+) that were produced by mutated genes, aberrantly expressed normal genes, or genes encoding viral proteins (7-9,22). The frequency of somatic mutations or TMB was associated with the immunogenicity of breast cancer. The present study characterized and provided extensive data describing TMB differences between ER (or PR, HER2)-negative and ER (or PR, HER2)-positive patients with BC. It was observed that the HR-negative group had a higher TMB compared with the HR-positive group. Shaw *et al* (23) demonstrated that the level of TMB calculated by total circulating free DNA and the circulating tumor cell count (≥5) were both significantly associated with overall survival in patients with metastatic BC, unlike the cancer-associated biomarkers, including cancer antigen 15-3 and alkaline phosphatase (23,24). The similar trend of estrogen receptor 1 and KRAS proto-oncogene, GTPase gene mutations was absent from primary tumor tissue, and appeared to be acquired with disease progression (23,25). This TMB marker may reflect the degree of metastatic burden and may serve as a favorable predictor for clinical decision-making. The total mutation burden was correlated with response to chemotherapy and poly (ADP-ribose) polymerase inhibitors in patients with ovarian cancer with *BRCA1/2* mutations (26). Low TMB predicted resistance to chemotherapy, whereas high TMB predicted a remarkably favorable clinical outcome in *mBRCA*-associated ovarian cancer in the TCGA cohort (26). Our previous study revealed that the TMB value in patients with breast cancer can be predicted based on the expression levels of ER, PR, HER-2 and Ki-67 (27). These observations suggest that TMB coupled with HR negativity in BC is a genomic marker of prognosis and a predictor of response to immunotherapy.

These results revealed that the aberrant expression of MMR genes may contribute to the increased TMB in HR-negative patients. BC is a relatively heterogeneous disease, and deficiency of major BC-susceptibility genes in DNA repair pathways, including MMR, may be involved in familial BC and implicated in higher TMB (28,29). An increasing number of studies suggest that triple-negative, luminal B-like or HER2-positive tumors harbor a high mutational burden, and these molecular types are considered as immunogenic (7,27). An interesting finding of the present study is that patients who were HER2-positive (particular in the HR-positive group) indicated to have higher TMB

Table II. Gene expression from B Cell/T Cell/NK Cell in patients with BC with different ER/PR/HER2 status.

A, B cell

Gene	ER_NEG	ER_POS	PR_NEG	PR_POS	HER2_NEG	HER2_POS	TRIPLE_NEG	NON_TRIPLE
CD79B	108.6660	80.5172	94.0301	81.7910	80.8910	75.8138	113.5951	72.3639
BTLA	24.1280	16.8827	21.9850	16.9827	16.3043	20.5051	31.5309	16.2235
FCRL3	6.0477	4.1124	4.9558	4.1163	3.8578	4.9408	6.54325	4.1272
BANK1	20.2347	10.3978	15.2576	10.5348	10.5432	13.4445	18.9792	11.8900
CD79A	141.0011	61.8596	106.4768	62.0842	63.7342	102.0126	151.2536	73.3673
BLK	94.1826	44.5720	78.2432	44.7591	49.9627	48.1587	108.8669	35.4945
RALGPS2	3.3266	1.4465	2.2954	1.4508	1.4550	1.8520	3.3051	1.7174
FCRL1	13.7678	7.3211	10.6117	7.3071	7.2333	12.0030	12.9948	11.8360

B, CD4+ T cell

Gene	ER_NEG	ER_POS	PR_NEG	PR_POS	HER2_NEG	HER2_POS	TRIPLE_NEG	NON_TRIPLE
C15orf53	0.3477	0.0000	0.3127	0.0000	0.0000	0.3605	0.2955	0.3269
CTLA4	49.0202	17.7471	39.7454	17.4976	20.1526	32.0127	56.9741	32.1545
IL32	98.8562	54.1037	86.7804	52.8931	56.4775	88.3388	99.1651	79.2368
FOXP3	1.3298	0.9843	1.2318	0.9814	0.8780	1.2063	0.9259	0.7234
GPR15	1657.1686	751.7339	1184.3091	755.4254	803.0842	786.9443	1861.1596	848.1356

C, CD8+ T cell

Gene	ER_NEG	ER_POS	PR_NEG	PR_POS	HER2_NEG	HER2_POS	TRIPLE_NEG	NON_TRIPLE
CD8A	188.2387	148.8562	163.3699	151.1880	151.2229	152.5424	194.0695	168.3510

D, NK cell

Gene	ER_NEG	ER_POS	PR_NEG	PR_POS	HER2_NEG	HER2_POS	TRIPLE_NEG	NON_TRIPLE
KLRF1	5.4054	2.9576	4.39585	3.01835	3.1873	3.6188	5.0695	3.3683
KLRC1	3.6041	3.0883	2.9054	3.22965	2.9617	3.2038	3.1361	2.5931

BC, breast cancer; MMR, mismatch repair; HR, hormone receptor; HER2, human epidermal growth factor receptor 2; ER, estrogen receptor; PR, progesterone receptor; NK, natural killer.

and increased expression levels of immune cell marker genes compared with patients who were HER2-negative. Immunotherapeutic strategies may increase the quality or quantity of immune effector cells, reveal additional protective tumor antigens, and/or eliminate cancer-induced immunosuppressive mechanisms (7). Large clinical trials of multiple immunotherapy approaches in patients with BC are ongoing, including therapeutic administration of monoclonal antibodies to target and relieve cancer-induced immunosuppression, including CTLA-4, PD-1 or Treg cells (7,9). Previous studies have been focused on immunotherapy for TNBC instead of other subtypes of BC. In triple-negative breast cancer, Atezolizumab plus nab-paclitaxel prolonged progression-free survival among patients with metastatic triple-negative breast cancer in both the intention-to-treat population and the PD-L1-positive subgroup; among patients with PD-L1-positive tumors, the median overall survival was prolonged by ~10 months following Atezolizumab plus nab-paclitaxel treatment (9). In HER-2 positive breast cancer, six (15%) of 40 PD-L1-positive patients achieved an objective response ratio (ORR) with pembrolizumab, a PD-1 inhibitor in the PANACEA study (30). Similarly, an ORR of only 12.0% and CBR of 20% with monotherapy of pembrolizumab were observed in ER-positive/HER2-negative metastatic breast cancer (31). Based on gene markers in $CD4^+$ T cells, $CD8^+$ T cells, B cells and NK cells in the present study, the findings suggested that HER2 status was correlated to a certain extent with immunogenic activity and, therefore, HER2 status may also be considered for immune checkpoint inhibition, particularly in patients who are HR-positive.

In conclusion, in the present study, HR-negative or HER2-positive BC were found to exhibit increased TMB and immunogenic activity. The present study presents immunotherapeutic options recommended for such patients.

Acknowledgements

Not applicable.

Funding

The present study was supported by the Liaoning Province Doctor Startup Fund Program (grant no. 201501108), National Nature Science Foundation (grant no. 81502188), National Natural Science Foundation of Liaoning (grant no. 2015020251), Central Guidance for Special Funds (grant no. 2016007011) and the Clinical Capability Construction Project for Liaoning Provincial Hospitals (grant no. LNCCC-C05-2015).

Availability of data and materials

The datasets used and/or analyzed during the present study are available from the corresponding author on reasonable request.

Authors' contributions

JX, HB and XW performed the literature search, data extraction and statistical analysis, and drafted the manuscript. TS, XNW and YS designed and supervised the study. All authors have read and approved the final manuscript.

Ethics approval and consent to participate

The study was approved by the Ethics Committee of Liaoning Cancer Hospital & Institute (Shenyang, China).

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

1. DeSantis CE, Ma J, Goding Sauer A, Newman LA and Jemal A: Breast cancer statistics, 2017, racial disparity in mortality by state. CA Cancer J Clin 67: 439-448, 2017.
2. Effi AB, Aman NA, Koui BS, Koffi KD, Traore ZC and Kouyate M: Breast cancer molecular subtypes defined by ER/PR and HER2 status: Association with clinicopathologic parameters in ivorian patients. Asian Pac J Cancer Prev 17: 1973-1978, 2016.
3. Dunnwald LK, Rossing MA and Li CI: Hormone receptor status, tumor characteristics, and prognosis: A prospective cohort of breast cancer patients. Breast Cancer Res 9: R6, 2007.
4. Davis SL, Eckhardt SG, Tentler JJ and Diamond JR: Triple-negative breast cancer: Bridging the gap from cancer genomics to predictive biomarkers. Ther Adv Med Oncol 6: 88-100, 2014.
5. Nanda R, Chow LQ, Dees EC, Berger R, Gupta S, Geva R, Pusztai L, Pathiraja K, Aktan G, Cheng JD, *et al*: Pembrolizumab in patients with advanced triple-negative breast cancer: Phase Ib KEYNOTE-012 study. J Clin Oncol 34: 2460-2467, 2016.
6. Hartman ZC, Poage GM, den Hollander P, Tsimelzon A, Hill J, Panupinthu N, Zhang Y, Mazumdar A, Hilsenbeck SG, Mills GB and Brown PH: Growth of triple-negative breast cancer cells relies upon coordinate autocrine expression of the proinflammatory cytokines IL-6 and IL-8. Cancer Res 73: 3470-3480, 2013.
7. Adams S, Schmid P, Rugo HS, Winer EP, Loirat D, Awada A, Cescon DW, Iwata H, Campone M, Nanda R, *et al*: Pembrolizumab monotherapy for previously treated metastatic triple-negative breast cancer: Cohort A of the phase II KEYNOTE-086 study. Ann Oncol 30: 397-404, 2019.
8. Adams S, Loi S, Toppmeyer D, Cescon DW, De Laurentiis M, Nanda R, Winer EP, Mukai H, Tamura K, Armstrong A, *et al*: Pembrolizumab monotherapy for previously untreated, PD-L1-positive, metastatic triple-negative breast cancer: Cohort B of the phase II KEYNOTE-086 study. Ann Oncol 30: 405-411, 2019.
9. Schmid P, Adams S, Rugo HS, Schneeweiss A, Barrios CH, Iwata H, Diéras V, Hegg R, Im SA, Shaw Wright G, *et al*: Atezolizumab and nab-paclitaxel in advanced triple-negative breast cancer. N Engl J Med 379: 2108-2121, 2018.
10. Criscitiello C and Curigliano G: Immunotherapy of breast cancer. Prog Tumor Res 42: 30-43, 2015.
11. Rummel SK, Lovejoy L, Shriver CD and Ellsworth RE: Contribution of germline mutations in cancer predisposition genes to tumor etiology in young women diagnosed with invasive breast cancer. Breast Cancer Res Treat 164: 593-601, 2017.
12. Chalmers ZR, Connelly CF, Fabrizio D, Gay L, Ali SM, Ennis R, Schrock A, Campbell B, Shlien A, Chmielecki J, *et al*: Analysis of 100,000 human cancer genomes reveals the landscape of tumor mutational burden. Genome Med 9: 34, 2017.
13. Tomczak K, Czerwińska P and Wiznerowicz M: The cancer genome atlas (TCGA): An immeasurable source of knowledge. Contemp Oncol (Pozn) 19: A68-A77, 2015.
14. Boland CR and Goel A: Microsatellite instability in colorectal cancer. Gastroenterology 138: 2073-2087 e2073, 2010.
15. Rooney MS, Shukla SA, Wu CJ, Getz G and Hacohen N: Molecular and genetic properties of tumors associated with local immune cytolytic activity. Cell 160: 48-61, 2015.
16. Danaher P, Warren S, Dennis L, D'Amico L, White A, Disis ML, Geller MA, Odunsi K, Beechem J and Fling SP: Gene expression markers of Tumor Infiltrating Leukocytes. J Immunother Cancer 5: 18, 2017.
17. Low SK, Zembutsu H and Nakamura Y: Breast cancer: The translation of big genomic data to cancer precision medicine. Cancer Sci 109: 497-506, 2018.
18. Kraya AA, Maxwell KN, Wubbenhorst B, Wenz BM, Pluta J, Rech AJ, Dorfman LM, Lunceford N, Barrett A, Mitra N, *et al*: Genomic signatures predict the immunogenicity of BRCA-deficient breast cancer. Clin Cancer Res pii: clincanres.0468.2018, 2019.
19. Stovgaard ES, Nielsen D, Hogdall E and Balslev E: Triple negative breast cancer-prognostic role of immune-related factors: A systematic review. Acta Oncol 57: 74-82, 2018.
20. Yang J, Xu J, E Y and Sun T: Predictive and prognostic value of circulating blood lymphocyte subsets in metastatic breast cancer. Cancer Med 8: 492-500, 2019.
21. Xu J, Jiang L, Cao H, Jia Y, Wu S, Jiang C and Sun T: Predictive value of $CD4^+/CD8^+$ ratio in patients with breast cancer receiving recombinant human thrombopoietin. J Interferon Cytokine Res 38: 213-220, 2018.
22. McGrail DJ, Federico L, Li Y, Dai H, Lu Y, Mills GB, Yi S, Lin SY and Sahni N: Multi-omics analysis reveals neoantigen-independent immune cell infiltration in copy-number driven cancers. Nat Commun 9: 1317, 2018.
23. Shaw JA, Guttery DS, Hills A, Fernandez-Garcia D, Page K, Rosales BM, Goddard KS, Hastings RK, Luo J, Ogle O, *et al*: Mutation analysis of cell-free DNA and single circulating tumor cells in metastatic breast cancer patients with high circulating tumor cell counts. Clin Cancer Res 23: 88-96, 2017.
24. Bidard FC, Hajage D, Bachelot T, Delaloge S, Brain E, Campone M, Cottu P, Beuzeboc P, Rolland E, Mathiot C and Pierga JY: Assessment of circulating tumor cells and serum markers for progression-free survival prediction in metastatic breast cancer: A prospective observational study. Breast Cancer Res 14: R29, 2012.
25. Xu J, Sun T, Guo X, Wang Y and Jing M: Estrogen receptor-α promoter methylation is a biomarker for outcome prediction of cisplatin resistance in triple-negative breast cancer. Oncol Lett 15: 2855-2862, 2018.

26. Birkbak NJ, Kochupurakkal B, Izarzugaza JM, Eklund AC, Li Y, Liu J, Szallasi Z, Matulonis UA, Richardson AL, Iglehart JD and Wang ZC: Tumor mutation burden forecasts outcome in ovarian cancer with BRCA1 or BRCA2 mutations. PLoS One 8: e80023, 2013.
27. Xu J, Guo X, Jing M and Sun T: Prediction of tumor mutation burden in breast cancer based on the expression of ER, PR, HER-2, and Ki-67. Onco Targets Ther 11: 2269-2275, 2018.
28. Win AK, Lindor NM and Jenkins MA: Risk of breast cancer in Lynch syndrome: A systematic review. Breast Cancer Res 15: R27, 2013.
29. Kappil M, Terry MB, Delgado-Cruzata L, Liao Y and Santella RM: Mismatch repair polymorphisms as markers of breast cancer prevalence in the breast cancer family registry. Anticancer Res 36: 4437-4441, 2016.
30. Loi S, Giobbie-Hurder A, Gombos A, Bachelot T, Hui R, Curigliano G, Campone M, Biganzoli L, Bonnefoi H, Jerusalem G, *et al*: Pembrolizumab plus trastuzumab in trastuzumab-resistant, advanced, HER2-positive breast cancer (PANACEA): A single-arm, multicentre, phase 1b-2 trial. Lancet Oncol 20: 371-382, 2019.
31. Rugo HS, Delord JP, Im SA, Ott PA, Piha-Paul SA, Bedard PL, Sachdev J, Tourneau CL, van Brummelen EMJ, Varga A, *et al*: Safety and antitumor activity of pembrolizumab in patients with estrogen receptor-positive/human epidermal growth factor receptor 2-negative advanced breast cancer. Clin Cancer Res 24: 2804-2811, 2018.