Immunologic Research (2019) 67:469–477
https://doi.org/10.1007/s12026-019-09109-4

ORIGINAL ARTICLE

Retained or altered expression of major histocompatibility complex class I in patient-derived xenograft models in breast cancer

In Hye Song[1] · Young-Ae Kim[1,2] · Hyeonjin Lee[1,2] · Hye Seon Park[1,2] · In Ah Park[1] · Chan Kyu Sim[3] · Myeong Sup Lee[3] · Gyungyub Gong[1] · Hee Jin Lee[1]

Published online: 3 January 2020

Abstract

The expression of major histocompatibility complex class I (MHC I) in tumor cells is regulated by interferon signaling, and it is an important factor in the efficacy of cytotoxic T cell–dependent immunotherapy. To determine the impact of immune cells in MHC I expression on tumor cells, we compared the expression of MHC I in tumor cells derived from primary breast cancers and patient-derived xenograft (PDX) models. MHC I and myxovirus resistance gene A (MxA) expression were analyzed using immunohistochemistry in 23 cases of tumor tissue and corresponding primary and secondary PDXs. The median H score of MHC I was 210 (0–300) in patient tumor tissues, 197.5 (0–300) in primary PDX tumors, and 157.5 (5–300) in secondary PDX tumors. Cases were divided into four groups based on the difference in MHC I expression between the patient tumor tissues and secondary PDXs. Eleven cases constituted the high MHC I group, four constituted the low MHC I group, six comprised the decreased MHC I group, and two comprised the increased MHC I group. MHC I and MxA expressions in each tumor were weakly correlated within patients' tumors, while strongly correlated within PDX models. Retained or altered expression of MHC I in breast cancer PDXs reveals the presence of intrinsic and extrinsic interferon signaling pathways in tumor cells. Thus, considering MHC I expression in PDX is important when using PDX models to evaluate the efficacy of cancer immunotherapy in a preclinical setting.

Keywords Breast carcinoma · Tumor-infiltrating lymphocytes · Major histocompatibility complex I · Human leukocyte antigen

Introduction

Immunotherapy has recently become a mainstay of cancer treatment, particularly since checkpoint inhibitors and adoptive T cell therapies using chimeric antigen receptor T (CAR-T) cells have been reported to be effective in various tumor types. Immunotherapy has been approved by the US Food and Drug Administration for the treatment of malignant

In Hye Song and Young-Ae Kim contributed equally to this work.

Précis Tumor cells derived from PDX models showed retainment or alteration of MHC I expression. Thus, considering MHC I expression in PDX is important when using PDX models to evaluate the efficacy of cancer immunotherapy in a preclinical setting.

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s12026-019-09109-4) contains supplementary material, which is available to authorized users.

✉ Gyungyub Gong
gygong@amc.seoul.kr

✉ Hee Jin Lee
backlila@gmail.com

[1] Department of Pathology, University of Ulsan College of Medicine Asan Medical Center, 88 Olympic-ro 43-gil, Songpa-gu, Seoul 138-736, South Korea

[2] Asan Center for Cancer Genome Discovery Asan Institute for Life Sciences, University of Ulsan College of Medicine Asan Medical Center, Seoul, South Korea

[3] Lab of Molecular Immunology and Medicine Department of Biomedical Sciences, University of Ulsan College of Medicine, Seoul, South Korea

melanoma, non-small cell carcinoma, renal cell carcinoma, urothelial carcinoma, head and neck cancer, Hodgkin lymphoma, non-Hodgkin lymphoma, and acute lymphoblastic leukemia [1, 2]. However, as with other therapeutic anticancer treatments, cancer immunotherapy has its limitations, including intrinsic and acquired resistance [3]. Studies of cancer immunotherapy have begun to reveal the mechanisms of cancer immunotherapy resistance. Tumor-intrinsic factors include expression of immune checkpoints, low mutation rate, mutation in interferon signaling pathway molecules, and loss of major histocompatibility complex class I (MHC I) expression, while tumor-extrinsic factors include paucity of tumor-infiltrating lymphocytes (TILs), infiltration of immune suppressive cells, and alteration of immune checkpoints that are targets of specific inhibitors. Tumor-intrinsic and tumor-extrinsic factors both contribute to cancer immunotherapy resistance [3].

Most immunotherapies are dependent upon the action of cytotoxic T cells. Cytotoxic T cells recognize cancer cells via the interaction between T cell receptors (TCR) and the peptide-MHC complex [4]. Therefore, the expression of MHC I and presentation of tumor cell antigens are critical for the effectiveness of most immunotherapies. MHC expression level varies widely between tissue types and is known to be stimulated by interferon signaling [4, 5]. The interferon signature can be evaluated with gene expression analyses or immunohistochemistry for biomarkers including myxovirus resistance gene A (MxA), which is one of the most prominent interferon-stimulated genes in vertebrate and is highly activated by type I and III interferons upon viral infection [6]. Although an in-depth understanding of tumor cell MHC expression could provide effective ways to overcome immunotherapy resistance, the mechanisms of MHC expression in breast cancer tissues are currently not well understood. Previous studies by the authors examined the relationship between the expression of estrogen receptors (ER), MHC I, and interferon signaling molecules and TILs in breast cancer [7, 8]. Tumors with high ER expression showed decreased expression of MHC I and interferon signaling molecules including MxA. These tumors also showed lower levels of TILs, suggesting that suppressed interferon signaling via the ER signaling pathway plays a role in the decreased expression of MHC I on tumor cells, resulting in reduced antigen presentation and infiltration of cytotoxic T cells. However, tumors with an intrinsically high level of interferon signaling may present more of their antigens, thus facilitating the infiltration of cytotoxic T cells, which produce and release interferon into the microenvironment. Therefore, MHC I expression on such tumor cells may be increased further. To evaluate the difference in MHC I expression between primary tumors and patient-derived xenograft (PDX) models, we performed MHC I immunohistochemical staining in primary and PDX tumors. In addition, we explored interferon signaling status via immunohistochemical staining of MxA. The relationship between the concentration of TILs in primary tumors and MHC I expression in primary tumors and PDX models was also analyzed.

Materials and methods

Origin of patient-derived xenograft (PDX) mouse model

Beginning in 2016, breast cancer tissues were collected during surgery from 23 patients with or without neoadjuvant systemic chemotherapy (after obtaining informed consent prior to surgery). All cancer tissues were derived from the breast except one, which was collected from a metastatic lesion in a lymph node. To derive the PDX mouse model implanted with tumor tissues, four tumor fragments (4 mm^3) from each tumor sample were implanted into the bilateral inguinal mammary fat pads of 4- to 6-week-old female immunodeficient nonobese diabetic CB17-*Prkdcs*$^{\text{severe combined immunodeficiency (scid)}}$ mice (NOD-SCID mice, Koatech Inc., Seoul, Korea) or NOD.*Cg-Prkdcscid Il2rgtm1Wjl/SzJ* mice (NSG mice, Jackson Laboratory, Bar Harbor, ME, USA). To stimulate the growth of breast cancer tissue in the PDX mouse model, an estradiol pellet (Innovative Research of America, Sarasota, FL, USA) was implanted under the skin of the upper back, as described previously [9]. Body weight and tumor growth were monitored once a week. When tumors reached 1 cm in diameter, they were excised and some fragments were cryopreserved for future analysis, while other fragments were retransplanted to NOD-SCID mice for functional assays.

Interferon treatment of PDX mouse model

Two tertiary PDX mice from one patient (BC16158) were treated with interferon–γ. When implanted tumors in the PDX mice reached 600–800 mm^3, the PDX mice were injected with interferon–γ (1.5 μg/mice, Peprotech, Rocky Hill, NJ, USA) intraperitoneally in one mouse and intratumorally in the other. The tumors were excised 3 days after injection and examined with immunohistochemistry.

Histological evaluation of breast cancer tissues

After collecting tissues for PDX, hematoxylin and eosin (H&E)-stained slides were prepared from surgical specimens from the remaining tumor tissues and were reviewed by two pathologists (HJL and IHS). Slides were histopathologically analyzed to determine the percentage of TILs (defined as mean percentage of the stroma from the invasive carcinoma infiltrated by lymphocytes and plasma cells in 10% increments; if less than 10% of stroma was infiltrated by TILs,

1%, 2%, or 5% criteria were used; all available full-sections were evaluated) [10]. Histological subtype and grade, tumor size, pT stage, pN stage, and lymphovascular invasion were also evaluated. For subtyping, cancer tissues from the patients and PDXs were immunohistochemically stained for detection of ER (1:200; NCL-L-ER-6F11, Novocastra, Newcastle-upon-Tyne, UK), progesterone receptors (PR, 1:200; NCL-L-PGR-312, Novocastra), and HER2 (1:8; 88-4422, Ventana Medical Systems, Tucson, AZ, USA). ER and PR levels were considered positive if the Allred score was higher than 2 [11]. Hormone receptor-positive (HR^+) tumors were defined as those with positive ER and/or PR staining. HER2-overexpressing tumors were defined as those with scores of 3+ by immunohistochemistry or gene amplification by silver in situ hybridization [12]. Triple-negative breast cancer (TNBC) was defined as tumors showing negative results for ER, PR, and HER2. Histological type was defined based on the 2012 World Health Organization (WHO) classification criteria, and histological grade was assessed using the modified Bloom-Richardson classification [13].

Immunohistochemistry of MHC I and MxA

Whole sections of tumors were immunostained with antibodies for MHC I (HLA-ABC, 1:1600; ab70328, EMR8-5, Abcam) and MxA (1:1000; ab95926; Abcam, Cambridge, UK). The immunostaining intensity was evaluated as a four-value intensity score (0, 1, 2, and 3), and the percentage of membranous and/or cytoplasmic expression was also evaluated. An "H score" was generated by summing the products of the intensity and the percentage of positive cells (H score = (% of cells stained at intensity score 1 × 1) + (% of cells stained at intensity score 2 × 2) + (% of cells stained at intensity score 3 × 3)). If multiple tumors of the same generation from the same patient were stained, the median of the H scores was used to compare the changes in HLA-ABC and MxA expression according to PDX generation.

The Cancer Genome Atlas (TCGA) data

A total of 1100 breast-invasive carcinoma samples (TCGA, Provisional) were analyzed for the correlation between *IFNA1*, *IFNA2*, *IFNB1*, *IFNG*, *MX1*, *HLA-A*, *HLA-B*, and *HLA-C* mRNA expression (RNA Seq V2 RSEM) via cBioPortal (www.cbioportal.org) [14, 15].

Statistical analysis

SPSS version 20 (SPSS, Chicago, IL, USA) was used to carry out the statistical analyses. Mann-Whitney test and Spearman correlation were used to determine significance. All the tests were two-sided and were performed using a significance level of $\alpha = 0.05$.

Results

Clinicopathologic characteristics of the patients

Table 1 summarizes the clinicopathologic characteristics of the 23 patients. Median tumor size was 3.5 cm (1.5–11.5). Twenty patients were diagnosed with primary breast cancer, and surgical breast cancer tissues were used to generate PDX tumors. Among them, 16 patients (70%) had received neoadjuvant systemic therapy prior to surgery. One patient (4%) had undergone breast cancer surgery, and lymph node metastasis of the cancer was diagnosed 11 months after surgery. Metastatic lymph node tissue from this patient was used for the generation of PDX. When hormone receptor and HER2 expression status was classified, two (9%) were HR+/HER2− type, one (4%) was HR+/HER2+ type, and 20 (87%) were TNBC. The subtypes of the generated PDXs were the same as the patients' tumors in 20 of 23 cases. In three cases (BC16036, BC16110, and BC18050), primary and secondary PDXs were HR+ type while the patients' tumors were TNBC. The median TIL level was 10% (1–80%) in patient tumor tissues. Median number of primary and secondary PDX tumors was 2 (1–2) and 4 (1–10), respectively. In total, 37 primary PDX tumors and 98 secondary PDX tumors were analyzed.

One-third of PDX tumors showed abrupt changes in MHC I expression

The median H score of MHC I was 210 (0–300) in patient tumor tissues, 197.5 (0–300) in primary PDX tumors, and 157.5 (5–300) in secondary PDX tumors. For MxA, the median H score was 130 (5–280), 90 (10–285), and 81 (21–289) in patient tumors, primary PDX tumors, and secondary PDX tumors, respectively (Fig. 1). H scores of MHC I and MxA expression in different tumors of secondary PDX could be compared in 18 cases. Median of the difference in H scores was 25 (0–180) for MHC I and 60 (15–210) for MxA.

H scores of MHC I in patient tumor tissues showed a wide range of distribution (Fig. 2). Based on the changes in MHC I expression between patient tumor tissues and secondary PDXs, samples were divided into four groups: (1) high MHC I group, high expression of MHC I (H score ≥ 150) in patient tumors, primary PDXs, and secondary PDXs (median H score, 260; range of H score, 157.5–300; range of H score change, − 123–37); (2) low MHC I group, low expression of MHC I (H score ≤ 100) in patient tumors, primary PDXs, and secondary PDXs (median H score, 17.8; range of H score, 0–80; range of H score change, − 19–27); (3) increased MHC I group, low expression of MHC I in the patient tumors and high expression in primary and secondary PDXs (range of H score change; 160–250); and (4) decreased MHC I group,

Table 1 Clinicopathological characteristics of the patients according to tissue source

Clinicopathologic variables	Primary tumor, did not receive neoadjuvant therapy ($n = 6$)	Primary tumor, received neoadjuvant therapy ($n = 16$)	Metastasis, lymph node ($n = 1$)
Age (years old)			
Median (range)	46 (33–51)	41 (31–69)	62
Tumor size (cm)	2.85 (1.7–5)	3.7 (1.5–11.5)	2.5
Subtype			
HR+/HER2-	1 (16.7%)	1 (6.25%)	0 (0%)
HR+/HER2+	0 (0%)	1 (6.25%)	0 (0%)
HR-/HER2+	0 (0%)	0 (0%)	0 (0%)
TNBC	5 (83.3%)	14 (87.5%)	1 (100%)
Histologic type			
IDC, NOS	5 (83.3%)	16 (100.0%)	1 (100%)
Metaplastic carcinoma	1 (16.7%)	0 (0%)	0 (0%)
Histologic grade			
2	0 (0%)	1 (6.25%)	1 (100%)
3	6 (100%)	15 (93.75%)	0 (0%)
LVI			
Absent	4 (66.7%)	7 (43.75%)	NA
Present	2 (33.3%)	9 (56.25%)	
TIL (%)	20 (0–80)	10 (0–60)	10
TNM stage			
I	1 (16.7%)	2 (12.5%)	0 (0%)
II	5 (83.3%)	6 (37.5%)	0 (0%)
III	0 (0%)	8 (50%)	1 (100%)
IV	0 (0%)	0 (0%)	0 (0%)
Miller-Payne grade			
1		8 (50%)	
2		4 (25%)	
3		4 (25%)	
RCB class			
II		6 (37.5%)	
III		10 (62.5%)	

HR, hormone receptor; *IDC*, invasive ductal carcinoma; *LVI*, lymphovascular invasion; *NA*, not available; *NOS*, not otherwise specified; *PDX*, patient-derived xenograft; *RCB*, residual cancer burden; *TIL*, tumor-infiltrating lymphocyte; *TNBC*, triple-negative breast cancer

lower expression of MHC I in PDXs than patient tumors (range of H score change, −85 to −210). Eleven out of twenty-three cases were in the high MHC I group, four were in the low MHC I group, six were in the decreased MHC I group, and two cases were in the increased MHC I group. One case with a PDX generated from a metastatic lymph node was in the decreased MHC I group (Table 2). Three cases (BC16036, BC16110, and BC18050) were obtained from TNBC patient tumors, but showed HR+ type in primary and secondary PDXs. They were classified in the high MHC I group, the decreased MHC I group, and the high MHC I group, respectively.

Tumors in the high MHC I group showed significantly higher MHC I expression H scores in patient tumor tissues (Mann-Whitney U test, $P = 0.005$) and primary ($P = 0.009$) and secondary PDXs ($P = 0.009$), and higher MxA expression in primary ($P = 0.009$) and secondary PDXs ($P = 0.036$) than those in the low MHC I group. Tumors in the high MHC I group also showed significantly higher MHC I expression H scores in patient tumor tissues ($P = 0.007$) and primary ($P = 0.001$) and secondary PDXs ($P = 0.001$), and higher MxA expression in secondary PDXs ($P = 0.014$) than those in the decreased MHC I group. Tumors in the decreased MHC I group showed significantly higher MHC I expression H scores in patient tumor tissues ($P = 0.036$) and primary PDXs ($P = 0.036$), and marginally or significantly higher MxA expression in patient tumor tissues ($P = 0.054$) and primary PDXs ($P = 0.036$) than those in the low MHC I group. The high MHC I group showed higher Ki-67 labeling index than the decreased MHC I group, which was statistically significant, but the difference was small (median percentage, 80% versus 75%; $P = 0.043$). The percentage of TIL was higher in the high MHC I group than in the other groups, but it was not statistically significant.

MHC I expression was strongly correlated with expression of MxA in PDX

We analyzed the correlation between MHC I and MxA expression in each tumor. In general, expression of both proteins was strongly correlated (Spearman's $\rho = 0.628$, $P < 0.001$). However, the correlation between these proteins in patient tumor tissues was weak and not statistically significant (Fig. 3, $\rho = 0.276$, $P = 0.202$). In contrast, their correlation in PDXs was strong and significant (primary, $\rho = 0.692$, $P < 0.001$; secondary, $\rho = 0.699$, $P < 0.001$).

MHC I expression in PDX models showed marked change after interferon–γ treatment

We compared the MHC I expression in the tumors from PDXs treated with interferon–γ with that in control PDX by immunohistochemistry. Both of the two tumors showed markedly higher MHC I expression (H scores, 170 and 270) compared with that of control PDX (H score, 90).

HLA-A, *HLA-B*, and *HLA-C* expression was correlated with *IFNB1*, *IFNG*, and *MX1* expression in TCGA data

We investigated the correlation in 1100 breast-invasive carcinoma samples of TCGA [14, 15] between *IFNB1*, *IFNG* and *MX1* expression and *HLA-A*, *HLA-B*, and *HLA-C* expression. *MX1* expression showed significant, positive correlation with

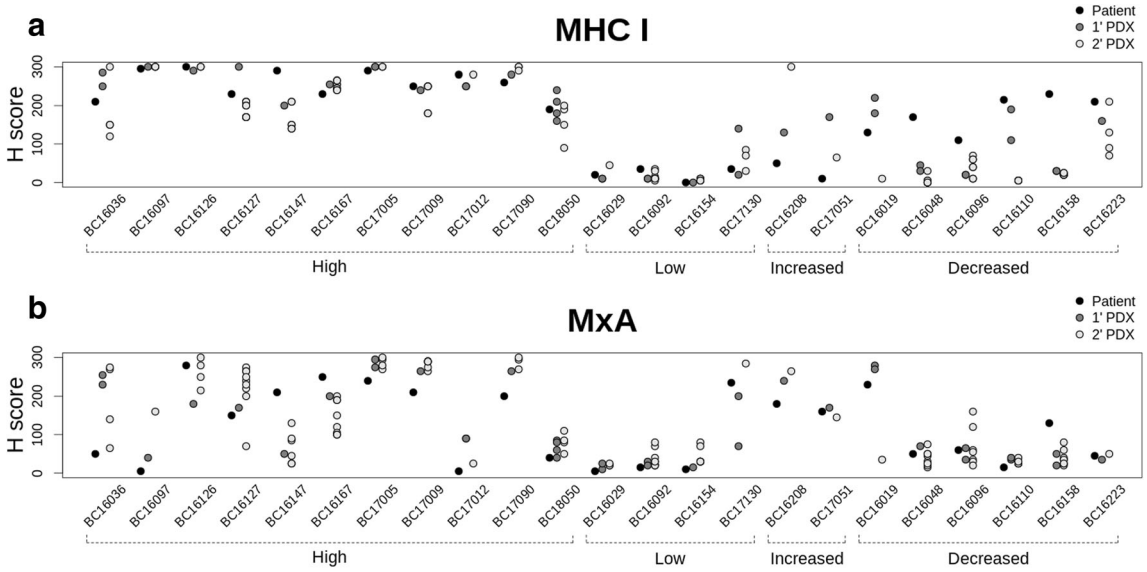

Fig. 1 Expression of MHC I and MxA in tumors from patient tumor tissues and primary (1') and secondary (2') PDXs

IFNB1, *IFNG*, *HLA-A*, *HLA-B*, and *HLA-C* expression ($\rho =$ 0.56, 0.33, 0.56, 0.62, and 0.50, respectively; $P = 0.03$, 0.05, 0.03, 0.02, and 0.03, respectively; Supplementary Fig. S1). *IFNB1* expression was positively correlated with *HLA-A* and *HLA-B* expression ($\rho = 0.36$ and 0.41, respectively; $P = 0.05$ and 0.04, respectively). *IFNG* expression was also positively correlated with *HLA-A*, *HLA-B*, and *HLA-C* expression ($\rho =$ 0.53, 0.60, and 0.41, respectively; $P = 0.03$, 0.03, and 0.04, respectively). *IFNA1* and *IFNA2* did not show statistically significant correlation with other interferon-related molecules and HLA molecules (data not shown).

Discussion

Breast cancer is a heterogeneous disease with various genotypes and phenotypes. The in vivo PDX model is a better tool for analyzing individual tumor responses to therapies than an in vitro cancer cell culture. Orthotopic PDX models such as those used in the present study, in which breast cancer tissues are implanted in the inguinal mammary fat pads of mice, can provide a similar breast stromal microenvironment for the growth of cancer cells [16]. Although PDX models showed rapid accumulation of copy number alterations and clonal selection during PDX passaging [17, 18], these models faithfully conserved the genetic patterns of the corresponding tumors, and their clinical responses to drugs were significantly associated with therapeutic outcomes of the corresponding PDXs to the same drugs in 87% of the patients (112/129 clinical correlation across 92 patients with cancer) [19].

To prevent the rejection of implanted tumors, PDX models are generated using immune-deficient mice, such as the NOD-SCID or NSG mice. Lack of a functioning immune system in these immune-deficient mice precludes the active engagement of PDX models in cancer immunotherapy evaluation. However, PDX models can be used to evaluate the efficacy of adoptive T cell immunotherapy [20]. Jespersen et al. compared the clinical and PDX responses to adoptive TIL therapy in patients with melanoma and found correlation between them [21]. Recently, humanized PDX models have been developed as well to improve the quality of human immune cell reconstitution. They showed a high success rate of tumor engraftment and are expected to enable in-depth oncoimmunological analysis [22, 23].

Differentially expressed genes in PDX models compared with corresponding human tumor tissue have been analyzed [24, 25]. In general, gene expression is highly correlated for tumors and corresponding PDX models (Spearman's correlation coefficient 0.82–0.97; 58 patients with eight different tumor types). However, immune-related genes, including *HLA-DPA1*, *HLA-DPB1*, and *HLA-DRA*, were identified as differentially expressed between patients' tumor tissues and their PDX tissues [24]. We found abrupt changes in MHC I expression in approximately one-third of PDX models. We claim that since PDX models have great potential to assist medical caregivers in their selection of drugs specific to the needs of each patient, possible changes in the expression of MHC molecules in PDX models must be considered very carefully, especially when testing for immunotherapeutic agents that are dependent on the activity of T cells after engagement of TCRs with the peptide-MHC complex of tumor cells.

We also found changes in the expression of MxA, a protein involved in interferon signaling. We also found that MHC I expression in two PDX models was strongly increased after interferon injection. Activation of the interferon signaling pathway is associated with changes in various molecules important for immune response. The antigen presentation

mechanism, including MHC molecules, is also regulated by interferon [4], as are immunoproteasomes. Immunoproteasomes are specialized proteasomes which degrade intracellular protein, have different components than ordinary proteasomes, and have different preference of cleavage activity [26]. Since immunoproteasomes are induced by interferon, changes in the interferon signaling pathway may influence the presented antigen repertoire in PDX tumor models. Therefore, these changes in antigen presentation may also affect the results of immunotherapy agents in PDX models.

The significance of interferon signaling in the tumor microenvironment for the control of cancer was revealed over 40 years ago [27]. Loss of interferon signaling was associated with less effective immune cell function, including cytotoxic T cells, dendritic cells, and natural killer (NK) cells [28]. Interferon signaling also plays a role in the suppression of regulatory T cell (Treg) proliferation and accumulation of myeloid-derived suppressor cells [29]. Sources of interferon in the tumor microenvironment are primarily immune cells, such as plasmacytoid dendritic cells and T cells [30]. Recently, however, intrinsic secretion of interferon from tumor cells has been examined and shown to be an important factor in the efficacy of anticancer treatment [28, 31]. Our finding that approximately one-half of PDX tumors retained high levels of MHC I and MxA expression, as well as strong, significant correlation of MHC I and MxA expression in PDXs rather than patient tumor tissues, suggests the presence of tumor cell intrinsic interferon signaling without exogenous interferon from immune cells. However, cases with decreased expression of MHC I and MxA in PDXs compared with tumor tissues might be highly influenced by exogenous interferon

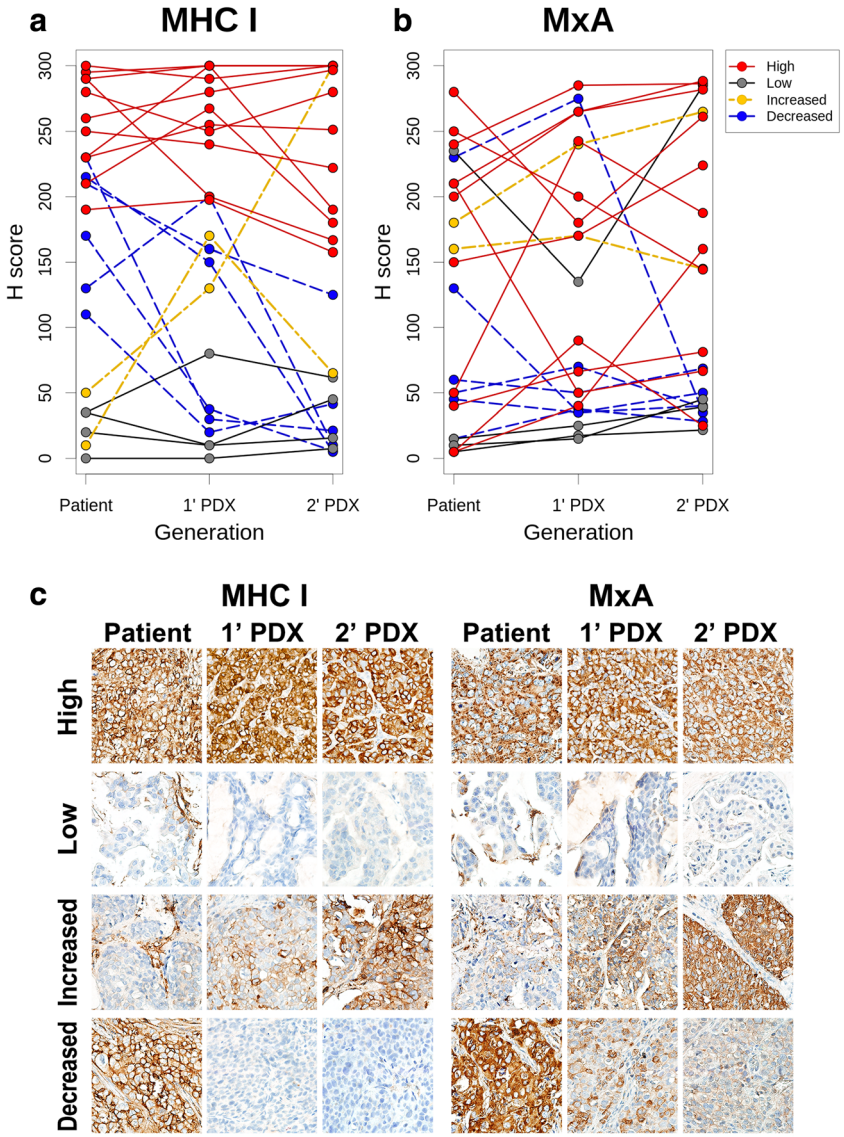

Fig. 2 Changes in tumor MHC I and MxA expression by PDX generation, and representative microscopic images of MHC I and MxA immunohistochemistry according to the expression changes between patient tumor and PDX generations

Table 2 Clinicopathological characteristics of the patients according to changes in MHC I expression in PDXs

	High ($n = 11$)	Low ($n = 4$)	Increased ($n = 2$)	Decreased ($n = 6$)
Age (years old)				
Median (range)	43 (33–51)	36 (33–57)	60 (51–69)	47 (33–62)
Neoadjuvant therapy				
No	3 (27.3%)	0 (0.0%)	0 (0.0%)	3 (50.0%)
Yes	8 (72.7%)	4 (100.0%)	2 (100.0%)	3 (50.0%)
Tissue source				
Primary tumor	11 (100%)	4 (100%)	2 (100%)	5 (83.3%)
Lymph node	0 (0%)	0 (0%)	0 (0%)	1 (16.7%)
Ki-67 (%)	80 (50–90)	75 (30–90)	70 (70–70)	75 (60–80)
Subtype				
HR+/HER2−	1 (9.1%)	0 (0%)	0 (0%)	1 (16.7%)
HR+/HER2+	0 (0%)	1 (25%)	0 (0%)	0 (0%)
TNBC	10 (90.9%)	3 (75%)	2 (100%)	5 (83.3%)
TIL (%)	20 (2–80)	2 (1–10)	10	10 (2–20)
MHC I H score				
Patient	260 (190–300)	27.5 (0–35)	30 (10–50)	190 (110–230)
1’ PDX	267.5 (197–300)	10 (0–80)	150 (130–170)	94 (20–200)
2’ PDX	251 (157–300)	30.3 (8–62)	182.5 (65–300)	16 (5–125)
MxA I H score				
Patient	200 (5–280)	12.5 (5–235)	170 (160–180)	55 (15–230)
1’ PDX	180 (40–285)	21 (15–135)	205 (170–240)	44 (35–275)
2’ PDX	187.5 (25–288)	42 (22–285)	205 (145–265)	40 (28–69)

HR, hormone receptor; *PDX*, patient-derived xenograft; *TIL*, tumor-infiltrating lymphocyte; *TNBC*, triple-negative breast cancer

in the tumor microenvironment, and loss of such cytokines in PDXs was associated with decreased expression of interferon-inducible molecules.

The low MHC I group shows the lowest MxA H score in patient tumor tissues and primary PDXs and the second lowest MxA H score in secondary PDXs following the decreasing MHC I group. The low MHC I group also shows lower levels of TILs in patient tumor tissues than all other groups. Therefore, these tumors are thought to be less influenced by interferon signaling in the tumor microenvironment. Since the presence of TILs and increased expression of MHC I are predictive factors for immunotherapy efficacy, we claim that efforts to change these immunologically cold tumors to hot tumors are necessary for these patients [3].

We analyzed the expression of MHC I and MxA in different secondary PDX tumors. Although a few cases showed considerable differences (for example, secondary PDXs from BC16036 showed difference of MxA expression of up to 215 in H score.), the difference was generally low. Eirew et al. explored breast cancer PDX models using single-cell

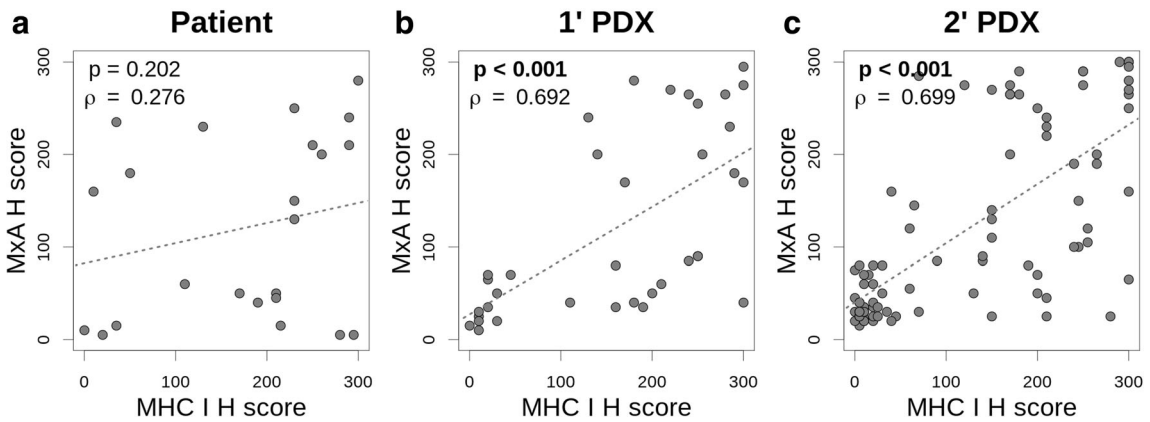

Fig. 3 Correlation of MHC I and MxA expression in patient tumor tissues and primary (1') and secondary (2') PDXs

sequencing methods [18]. The majority of cases showed highly skewed clonal dynamics in which initially minor prevalence clones expanded to dominant clones following PDX passages. However, different tumors in different mice from the same tumor population (the same passage) showed similar clonal dynamics, suggesting the presence of a shared deterministic mechanism for clonal expansion.

A limitation of this study is we cannot assess interferon itself. Because interferons are secretory molecules, secretion of interferons is detected by ELISA or intracellular staining of interferons after blocking secretory activity to detect interferon level. Only formalin-fixed paraffin-embedded tissue samples for primary tumors were available, and immunohistochemistry cannot detect secreted molecules; thus, MxA was used as a surrogate marker of the interferon signaling pathway.

In conclusion, we identified retained or changed expression of MHC I in breast cancer PDXs. Our study suggests that consideration of the MHC I expression level in PDX tumor models is necessary when using these models to evaluate cancer immunotherapy efficacy in a preclinical setting. Further research including the injection of human immune cells to PDX models would be beneficial to understand the effect of interferon signaling to the tumor MHC I expression.

Funding information This work was supported by the Korean Health Technology R&D Project, Ministry of Health & Welfare (HI15C0708 and HI17C0337); Basic Science Research Programs through the National Research Foundation of Korea (NRF) funded by the Ministry of Science, ICT & Future Planning, Republic of Korea (NRF-2016R1D1A1B03935367 and NRF-2018R1D1A1B07040612); and the Asan Institute for Life Sciences, Asan Medical Center, Seoul, Korea (2016-169).

Compliance with ethical standards

Conflict of interest The authors declare that that they have no conflict of interest.

Ethical approval This study was performed in accordance with the Declaration of Helsinki and was approved by the Institutional Review Board of Asan Medical Center (IRB#2015-0438) and the International Animal Care and Use Committee (IACUC) of the Laboratory of Animal Research at the Asan Medical Center, Seoul, Republic of Korea.

Informed consent Informed consent was obtained from all individual participants included in the study.

References

1. Zheng PP, Kros JM, Li J. Approved CAR T cell therapies: ice bucket challenges on glaring safety risks and long-term impacts. Drug Discov Today. 2018. https://doi.org/10.1016/j.drudis.2018.02.012.
2. Gong J, Chehrazi-Raffle A, Reddi S, Salgia R. Development of PD-1 and PD-L1 inhibitors as a form of cancer immunotherapy: a comprehensive review of registration trials and future considerations. J Immunother Cancer. 2018;6(1):8. https://doi.org/10.1186/s40425-018-0316-z.
3. Chae YK, Oh MS, Giles FJ. Molecular biomarkers of primary and acquired resistance to T-cell-mediated immunotherapy in cancer: landscape, clinical implications, and future directions. Oncologist. 2018;23(4):410–21. https://doi.org/10.1634/theoncologist.2017-0354.
4. Agrawal S, Kishore MC. MHC class I gene expression and regulation. J Hematother Stem Cell Res. 2000;9(6):795–812. https://doi.org/10.1089/152581600750062237.
5. Silginer M, Nagy S, Happold C, Schneider H, Weller M, Roth P. Autocrine activation of the IFN signaling pathway may promote immune escape in glioblastoma. Neuro-Oncology. 2017;19(10):1338–49. https://doi.org/10.1093/neuonc/nox051.
6. Hu JL, Hua YJ, Chen Y, Yu B, Gao S. Structural analysis of tumor-related single amino acid mutations in human MxA protein. Chin J Cancer. 2015;34(12):583–93. https://doi.org/10.1186/s40880-015-0055-1.
7. Lee HJ, Song IH, Park IA, Heo SH, Kim YA, Ahn JH, et al. Differential expression of major histocompatibility complex class I in subtypes of breast cancer is associated with estrogen receptor and interferon signaling. Oncotarget. 2016;7(21):30119–32. https://doi.org/10.18632/oncotarget.8798.
8. Kim YA, Lee HJ, Heo SH, Park HS, Park SY, Bang W, et al. MxA expression is associated with tumor-infiltrating lymphocytes and is a prognostic factor in triple-negative breast cancer. Breast Cancer Res Treat. 2016;156(3):597–606. https://doi.org/10.1007/s10549-016-3786-z.
9. Whittle JR, Lewis MT, Lindeman GJ, Visvader JE. Patient-derived xenograft models of breast cancer and their predictive power. Breast Cancer Res. 2015;17:17. https://doi.org/10.1186/s13058-015-0523-1.
10. Salgado R, Denkert C, Demaria S, Sirtaine N, Klauschen F, Pruneri G, et al. The evaluation of tumor-infiltrating lymphocytes (TILs) in breast cancer: recommendations by an International TILs Working Group 2014. Ann Oncol. 2015;26(2):259–71. https://doi.org/10.1093/annonc/mdu450.
11. Harvey JM, Clark GM, Osborne CK, Allred DC. Estrogen receptor status by immunohistochemistry is superior to the ligand-binding assay for predicting response to adjuvant endocrine therapy in breast cancer. J Clin Oncol. 1999;17(5):1474–81. https://doi.org/10.1200/JCO.1999.17.5.1474.
12. Wolff AC, Hammond ME, Hicks DG, Dowsett M, McShane LM, Allison KH, Allred DC, Bartlett JM, Bilous M, Fitzgibbons P, Hanna W, Jenkins RB, Mangu PB, Paik S, Perez EA, Press MF, Spears PA, Vance GH, Viale G, Hayes DF, American Society of Clinical O, College of American P (2014) Recommendations for human epidermal growth factor receptor 2 testing in breast cancer: American Society of Clinical Oncology/College of American Pathologists clinical practice guideline update. Arch Pathol Lab Med 138 (2):241–256. doi:https://doi.org/10.5858/arpa.2013-0953-SA.
13. WHO classification of tumours of the breast (2012). 4th. International Agency for Research on Cancer
14. Cerami E, Gao J, Dogrusoz U, Gross BE, Sumer SO, Aksoy BA, et al. The cBio cancer genomics portal: an open platform for exploring multidimensional cancer genomics data. Cancer Discov. 2012;2(5):401–4. https://doi.org/10.1158/2159-8290.CD-12-0095.
15. Gao J, Aksoy BA, Dogrusoz U, Dresdner G, Gross B, Sumer SO, et al. Integrative analysis of complex cancer genomics and clinical profiles using the cBioPortal. Sci signal. 2013;6(269):pl1-pl1. https://doi.org/10.1126/scisignal.2004088.
16. Fleming JM, Miller TC, Meyer MJ, Ginsburg E, Vonderhaar BK. Local regulation of human breast xenograft models. J Cell Physiol. 2010;224(3):795–806. https://doi.org/10.1002/jcp.22190.

17. Ben-David U, Ha G, Tseng YY, Greenwald NF, Oh C, Shih J, et al. Patient-derived xenografts undergo mouse-specific tumor evolution. Nat Genet. 2017;49(11):1567–75. https://doi.org/10.1038/ng.3967.
18. Eirew P, Steif A, Khattra J, Ha G, Yap D, Farahani H, et al. Dynamics of genomic clones in breast cancer patient xenografts at single-cell resolution. Nature. 2015;518(7539):422–6. https://doi.org/10.1038/nature13952.
19. Izumchenko E, Paz K, Ciznadija D, Sloma I, Katz A, Vasquez-Dunddel D, et al. Patient-derived xenografts effectively capture responses to oncology therapy in a heterogeneous cohort of patients with solid tumors. Ann Oncol. 2017;28(10):2595–605. https://doi.org/10.1093/annonc/mdx416.
20. Everson RG, Antonios JP, Lisiero DN, Soto H, Scharnweber R, Garrett MC, et al. Efficacy of systemic adoptive transfer immunotherapy targeting NY-ESO-1 for glioblastoma. Neuro-Oncology. 2016;18(3):368–78. https://doi.org/10.1093/neuonc/nov153.
21. Jespersen H, Lindberg MF, Donia M, Soderberg EMV, Andersen R, Keller U, et al. Clinical responses to adoptive T-cell transfer can be modeled in an autologous immune-humanized mouse model. Nat Commun. 2017;8(1):707. https://doi.org/10.1038/s41467-017-00786-z.
22. De La Rochere P, Guil-Luna S, Decaudin D, Azar G, Sidhu SS, Piaggio E. Humanized mice for the study of immuno-oncology. Trends Immunol. 2018;39(9):748–63. https://doi.org/10.1016/j.it.2018.07.001.
23. Capasso A, Lang J, Pitts TM, Jordan KR, Lieu CH, Davis SL, et al. Characterization of immune responses to anti-PD-1 mono and combination immunotherapy in hematopoietic humanized mice implanted with tumor xenografts. Journal for ImmunoTherapy of Cancer. 2019;7(1):37. https://doi.org/10.1186/s40425-019-0518-z.
24. Li H, Zhu Y, Tang X, Li J, Li Y, Zhong Z, et al. Integrated analysis of transcriptome in cancer patient-derived xenografts. PLoS One. 2015;10(5):e0124780. https://doi.org/10.1371/journal.pone.0124780.
25. Wang D, Li JR, Zhang YH, Chen L, Huang T, Cai YD. Identification of differentially expressed genes between original breast cancer and xenograft using machine learning algorithms. Genes (Basel). 2018;9(3). https://doi.org/10.3390/genes9030155.
26. Kaur G, Batra S. Emerging role of immunoproteasomes in pathophysiology. Immunol Cell Biol. 2016;94(9):812–20. https://doi.org/10.1038/icb.2016.50.
27. Gresser I, Maury C, Brouty-Boye D. Mechanism of the antitumour effect of interferon in mice. Nature. 1972;239(5368):167–8.
28. Brockwell NK, Parker BS. Tumor inherent interferons: impact on immune reactivity and immunotherapy. Cytokine. 2018. https://doi.org/10.1016/j.cyto.2018.04.006.
29. Weiss JM, Subleski JJ, Back T, Chen X, Watkins SK, Yagita H, et al. Regulatory T cells and myeloid-derived suppressor cells in the tumor microenvironment undergo Fas-dependent cell death during IL-2/αCD40 therapy. Journal of immunology (Baltimore, Md : 1950). 2014;192(12):5821–9. https://doi.org/10.4049/jimmunol.1400404.
30. Sisirak V, Faget J, Gobert M, Goutagny N, Vey N, Treilleux I, et al. Impaired IFN-α production by plasmacytoid dendritic cells favors regulatory T-cell expansion that may contribute to breast cancer progression. Cancer Res. 2012;72(20):5188–97. https://doi.org/10.1158/0008-5472.CAN-11-3468.
31. Sistigu A, Yamazaki T, Vacchelli E, Chaba K, Enot DP, Adam J, et al. Cancer cell-autonomous contribution of type I interferon signaling to the efficacy of chemotherapy. Nat Med. 2014;20(11):1301–9. https://doi.org/10.1038/nm.3708.