Soluble IL-2R as a predictor of familial breast cancer

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Abstract

Background: The incidence of breast cancer has been increasing annually, and breast cancer-related diseases, such as breast cancer in the young, ovarian cancer, prostate cancer, and pancreatic cancer, have clearly and steadily increased in number and have become common among the family members of patients with breast cancer. Accordingly, an increase in the incidence of familial breast cancer (FBC) is anticipated in the future. Interleukin (IL)-2 is one of the cytokines that activate CTLs, which are important for cancer immunity. To search for the markers of increased risk for FBC, we examined the sIL-2R levels and immunologic factors in patients with breast cancer and nonfamilial breast cancer (NFBC).

Methods: Of the 106 untreated breast cancer patients who gave consent to participate in this study, 24 had FBC and 82 had NFBC. There were 11 healthy individuals included in this study. Serum and peripheral blood mononuclear cells were collected from all patients for the measurement of the levels of sIL-2R, IL-10, VEGF, IL-17, Tregs, and MDSC. Prognosis was assessed and compared, according to the sIL-2R levels (low vs. high). Tissue samples from postoperative patients with high sIL2 were stained with PD-LI and CD8.

Results: The sIL-2R level was significantly higher and had significantly better correlations with IL-10, VEGF, IL-17, Tregs, and MDSC levels in FBC than in NFBC. In cases with high sIL-2R level, the Tregs and MDSC levels were significantly higher and the OS and DFS rates were significantly worse in FBC than in NFBC. Among the FBC cases with high sIL-2R level, triple-negative breast cancer tissues stained well for PD-LI and CD8.

Conclusions: Compared with NFBC, FBC was associated with higher sIL-2R levels, Th2 significance, and less aggressive cancer immunosuppressive cells. We have identified sIL-2R as a biomarker that can predict the prognosis of FBC. The ability to prospectively identify patients who are less likely to have NFBC is a vital step in improving the overall survival of this population.

Background

Familial breast cancer (FBC) is a cluster of breast cancer patients within a family. Most cases of breast cancer occur sporadically in individuals with little to no family history of the condition. Approximately 5% to 10% of breast cancer cases are considered hereditary through an autosomal dominant mechanism (NIH National Center for Advancing Translational Sciences, GARD Genetic and Rare Diseases Information Center, https://rarediseases.info.nih.gov/diseases/10415/familial-breast-cancer). However, the diagnostic analysis of breast cancer-related genes, such as the *hereditary breast and ovarian cancer* gene, in patients and carriers, as well as the genetic testing of every single family member remain very difficult to perform in a hospital setting. Furthermore, an increase in the prevalence of FBC is anticipated in the future. Regardless, the fact that the number of young breast cancer patients has increased and that patients with recurrent breast cancer require prompt treatment remain.

Interleukin (IL)–2, which is one of the most important cytokines for lymphocyte development, proliferation, and function, is produced by helper cells that differentiated from naïve cells upon stimulation with interferon γ (IFN-γ) or IL–12. The actions of IL–2 include proliferation and activation of T cells, promotion of proliferation and antibody production of B cells, activation of monocytes/macrophages, and proliferation/activation of natural killer cells, to name a few [1]. In addition, IL–2 has been believed to be required for the maintenance of regulatory T cells (Tregs), which release the inhibitory cytokine IL–10 and exhibit immunosuppressive effects [2]. Breast cancer patients have been known to have increased blood levels of IL–2 and its soluble IL–2 receptor (sIL–2R) [3–6]. In this study, we examined the role of sIL–2R in patients with FBC.

Cases of FBC probably possess a functional failure of the *mismatch repair (MMR)* gene, such as the *BRCA1/2.* A deficient DNA mismatch repair function due to *MMR* gene mutation increases the number of somatic gene mutations and tumor mutational burden (TMB) and leads to the release of neoantigens from cancer cells that have a large number of gene mutations. Dendritic cells incorporate and degrade neoantigens, which are long peptides that bind to Human leukocyte antigen (HLA) class II and are presented on the cell surface. Neoantigens are recognized by naïve cluster of differentiation (CD)4+ T cells and produce IL–2. The simultaneously incorporated and degraded short peptides bind to HLA class I, presented on the cell surface, recognized by naïve CD8+ T cells, and express IL–2R. IL–2 binds to IL–2R to induce differentiation and proliferation of cytotoxic T lymphocytes (CTL), which are cancer cell-specific immune cells. Upon activation, CTLs express immune checkpoint molecules, such as programmed cell death 1 (PD–1), on the surface. In addition, due to the IFN-γ produced by CTLs for cancer cell attack, cancer cells express programmed cell death ligand 1 (PD-L1) and suppress CTL activity.

Eventually, CTLs become immune tolerant and incapable of functioning. In such cases, identification of the genes associated with increased TMB would enable the prediction of the sensitivity to immune checkpoint inhibitors and the more efficacious therapy for FBC. In this study, we measured and compared the levels of sIL–2R, IL–10, vascular endothelial growth factor (VEGF), IL–17, immunosuppressive Tregs, and myeloid-derived suppressor cells (MDSC) between FBC and nonfamilial breast cancer (NFBC). Furthermore, CD8+ and PD-L1 staining of tissues was evaluated and compared between FBC and NFBC.

Methods

Study subjects

The present study enrolled 11 healthy volunteers and 106 preoperative patients who had histologically confirmed breast cancer and were treated at the Department of Breast Cancer Surgery of Fukushima Medical University (Fukushima, Japan) between January 2011 and June 2016. Staging was done in accordance with the National Comprehensive Cancer Network (NCCN) Clinical Practice Guidelines in Oncology: Breast Cancer Screening and Diagnosis [7, 8].

FBC was defined as a proband with breast cancer and (1) histologically proven breast cancer or ovarian cancer in at least 3 relatives, 1 of whom should be a 1st degree relative, including the mother or a sister, and with bilateral breast cancer, prostate cancer, and pancreatic cancer in the family or 2) at least 2 successive generations diagnosed as breast or ovarian cancer in at least 1 of the relatives at an age younger than 45 years and with bilateral breast cancer, prostate cancer, and pancreatic cancer in the family. NFBC was defined as only the proband having breast cancer.

After collection of blood samples from the study population, a total of 1×10^6 peripheral blood mononuclear cells (PBMCs) were isolated using the Ficoll density gradient centrifugation method; aliquots of PBMCs were cryopreserved in a freezing medium. Plasma was separated by centrifugation and stored at 80 °C until flow cytometry analysis.

Cytokine production

The serum concentrations of sIL–2R, IL–10, VEGF, and IL–17 in the supernatants were measured using as enzyme linked immunosorbent assay kit (Quantikin; R&D Systems, Inc., Minneapolis, MN, USA), according to the manufacturer's protocol.

Flow cytometry analysis of Tregs

A 3-color flow cytometry analysis was performed using a mixture of antibodies, including fluorescent isothiocyanate (FITC)-conjugated antihuman CD4, phycoerythrin-conjugated antihuman FoxP3, and allophycocyanin-conjugated antihuman CD25. Data acquisition and analysis were performed on a FACS Aria II flow cytometer (BD Biosciences, San Jose, CA, USA) using Flow Jo v10.2 software (Becton, Dickinson and Co., Becton Drive, Franklin Lakes, NJ). The percentage of Tregs was calculated as a fraction of the total number of PBMCs.

Flow cytometry analysis of MDSC

A 3-color flow cytometry analysis was performed with a mixture of antibodies, including FITC-conjugated antiCD14, phycoerythrin-conjugated antiCD11b, and phycoerythrin cyanin 5.1-conjugated antiCD33. Data acquisition and analysis were performed on a FACS Aria II flow cytometer using Flow Jo v10.2 software. The percentage of MDSCs was calculated as a fraction of the total number of PBMCs.

Statistical analysis

All data were presented as mean ± standard deviation. Differences between groups were determined using Student's t-test. Relationships between 2 variables were quantified using Spearman's rank correlation coefficient test. For the assessment of overall survival (OS) and disease-free survival (DFS),

data that were available until the last follow-up date or at 2,500 days were censored. The prognoses of the patients were analyzed using Kaplan–Meier method, and the log rank test was used to determine the univariate significance of the variables. Multivariate Cox regression analysis of the survival of patients with preoperative breast cancer was performed, according to the tumor molecular subtype and Ki67 status, as defined in the NCCN Clinical Practice Guidelines in Oncology [7, 8]. Cox proportional hazards model was used to examine the simultaneous effects of multiple covariates on survival. The effect of each variable was described by the hazard ratio, with 95% confidence interval. A p value of <0.05 was considered to indicate statistical significance. SAS software version 9.2 (SAS Institute Inc., Cary, NC, USA) was used for statistical analysis.

Staining for PD-L1 and CD8

Slides were deparaffinized in toluene, rehydrated in graded alcohols, then heat-induced epitope retrieval was performed, followed by a CD8 IHC protocol on clone (C8/144B)Monoclonal Mouse (Dako, Agilent Technologies, Santa Clara, CA) and a PD-L1 IHC protocol on clone E1L3N Rabbit (Cell Signaling Technology, Danvers, MA).

Results

The present study included a total of 106 patients; of these 24 had FBC and 82 had NFBC (Table 1). The median age of the patients was 42.0 years (range, 30–63 years) for FBC and 61.0 years (range, 46–88 years) for NFBC. The FBC group included 3 patients in stage I, 13 in stage II, 2 in stage III, and 6 in stage IV; whereas the NFBC group included 16 patients in stage I, 35 in stage II, 10 in stage III, and 21 in stage IV. None of the preoperative patients received prior anticancer treatment.

Serum sIL–2R levels

As shown in Fig. 1, the sIL–2R levels were higher in the preoperative patients (FBC and NFBC, 1014.2 ± 79.2 U/mL) than in the healthy volunteers (667.5 ± 42.5 U/mL). The sIL–2R level of FBC patients (1255.8±176.2) was significantly higher, compared with that of healthy volunteers (p = 0.04) and NFBC patients (865.4 ± 58.8 U/mL, p = 0.01).

sIL–2R correlations

As shown in Fig. 2, the sIL–2R level of preoperative FBC patients had significant positive correlations with IL–10 production (r = 0.48, p = 0.01); VEGF level (r = 0.80, p<0.0001); IL–17 production (r = 0.77, p<0.0001); Tregs level (r = 0.80, p<0.0001); and MDSC level (r = 0.70, p = 0.0002).

The sIL–2R level was classified as high or low, based on a cutoff value of 700 U/mL, high serum sIL–2R levels in FBC patients significantly increased the levels of Tregs (p = 0.0008) and MDSC (p = 0.01) (Fig.

3). The upper limit of 700 U/mL was chosen as the cutoff value for classifying sIL–2R as high or low, based on the median sIL–2R value of 667.50 ± 42.54 U/mL in healthy subjects.

sIL–2R survival possibility

As shown in Fig. 4, FBC patients who had high sIL–2R levels (>700 U/mL), compared with those with low sIL–2R levels, had significantly worse rates of OS (p = 0.0091) and DFS (p = 0.0038).

The Kaplan–Meier plot of DFS was dichotomized, based on sIL–2R expression above and below the median value of 700 U/mL. The Kaplan–Meier method was used to obtain the survival curves, which were analyzed by the log-rank test.

sIL–2 immunostaining

As shown in Fig. 5, immunohistochemistry of the core needle biopsy specimens from the FBC patients before treatment (T4N1M1 stage IV, ER–, PgR–, HER2–)demonstrated relatively more expressions of PD-L1 and CD8 when the sIL–2R level was high.

Discussion

Breast cancer is a heterogeneous disease that comprises multiple molecular subtypes. In this study, we found no significant difference in tumor molecular subtype between FBC and NFBC patients (Table 1). In several malignancies, serum sIL–2R levels are high, compared with those in healthy individuals. Although sIL–2R is not organ-specific, except for malignant lymphoma, measuring its serum level was shown to be valuable for stage evaluation and monitoring during treatment [9]. Breast cancer patients have been known to have increased serum levels of sIL–2R [10], as well as IL–10, VEGF, IL–17, Tregs, and MDSC [11–15]. Others have reported the correlation of sIL–2R in cancer with IL–10, VEGF, and IL–17 and the relationship between Tregs and MDSC [15–18].

Consistent with the previously reported results, the results of this study showed increased sIL–2R level in all breast cancer patients, compared with that in healthy subjects. Overall, sIL–2R level was significantly higher in FBC than in NFBC. Compared with NFBC, FBC comprises cancer cells that contain more mutations and release neoantigens and has a microenvironment of CD8+ T cells that express IL–2 receptors and release more sIL–2Rs in blood. Moreover, in FBC, there are simultaneous increases in the level of IL–10 and number of cells that suppress cancer immunity, such as Tregs and MDSC. In this study, the sIL–2R level in FBC patients was significantly and positively correlated with IL–10, VEGF, IL–17, Tregs, and MDSC. In particular, the significant positive correlation of sIL–2R level with IL–10 indicated disruption of the Th2>Th1 balance, which leads to Th2 predominance and suppression of cellular immunity. The significant positive correlation of sIL–2R level with VEGF and IL–17 suggested further tumor progression. The high levels of both VEGF and IL–17 further increased the number of cancer

immunosuppressive cells. On the other hand, our results showed no significant and positive correlation of sIL–2R with IL–10, VEGF, IL–17, Tregs, or MDSC in NFBC patients. These results suggested significantly more dramatic inflammatory and immune responses in the cancer microenvironment of FBC than that of NFBC.

High sIL–2R significantly increased the Tregs and MDSC in FBC patients but not in NFBC patients. This result implied the importance of preventing the growth of cancer immunosuppressive cells, such as Tregs and MDSC, in FBC patients. Moreover, high sIL–2R led to significantly worse prognosis in FBC but not in NFBC. The elevated sIL–2R level may have predisposed the FBC patients to cancer growth rather than cancer suppression. Notably, proliferation of CTLs does not guarantee the removal of all cancer cells. IL–2 is a systemically administered treatment that can lead to serious side effects, but decreasing its dose may render it ineffective [19, 20].

PD-L1 is expressed in 20% of TNBCs, suggesting PD-L1 as a therapeutic target in TNBCs. Because PTEN loss is one mechanism regulating PD-L1 expression, agents targeting the PI3K pathway may increase the antitumor adaptive immune responses [21].

In this study, immunohistochemical analysis of untreated preoperative cases confirmed tissue expressions of PD-L1 and CD8 in FBC with high sIL–2R and less CD8 staining in NFBC. These results indicated similar expressions of PD-LI in both FBC and NFBC, but in the case of FBC, Th2>Th1 balance or Tregs and MDSC in cancer microenvironment suppressed, on the way, the expression of CD8+ T cells.Although the direct relationship between IL–2 and PD-L1 is unknown, a combination of IL–2 and PD-L1 inhibitors has been the treatment regimen for dominant infections and cancer [22]. The duration of the proliferative contact between CD8+ T cells and antigen-presenting cells is influenced by the sensitivity of individual CD8+ T cells to activation signals and by the concentration of IL–2 in the extracellular environment [23]. Regardless of the level of Tregs or MDSC, immune checkpoint inhibitors should be effective, as long as CD8+ T cells proliferate. Treatment of patients with FBC seems necessary, while determining the relationship among genetics, immunity, and cancer. In the future, making Th1/Th2 balance towards Th1-dominant to inactivate cancer immunosuppressive cells may enhance the treatment efficacy of immune checkpoint inhibitors for patients with FBC.

Conclusions

Serum sIL–2R appeared to be a reliable marker in FBC. The suppression of cellular immunity and increased number of cancer immunosuppressive cells brought about by high sIL–2R may be related to immunologic mechanisms. The possibility of clinical control by targeting molecular mechanisms, such as IL–2R expression and immune checkpoint, needs to be investigated in future research.

Abbreviations

FBC: familial breast cancer; NFBC: non-familial breast cancer; HBOC: hereditary breast and ovarian cancer; IL: interleukin; IL–2R: interleukin–2 receptor; sIL–2R: soluble interleukin–2 receptor; VEGF: vascular endothelial growth factor; Treg: regulatory T cell; MDSC: myeloid-derived suppressor cell; CTL: cytotoxic T lymphocyte; MMR: mismatch repair gene; TMB: tumor mutational burden; PD-L1: programmed cell death ligand 1; PD–1: programmed cell death 1; OS: overall survival; DFS: disease free survival

Declarations

Ethics approval and consent to participate

The institutional review board and the local ethics committee (Ethical Review Board of Fukushima Medical University, 2011–2016) approved the study. All procedures were performed in accordance with the ethical standards of the responsible committee on human experimentation at Fukushima Medical University and with the Helsinki Declaration of 1975, as revised in 2000. Each author certifies that all investigations were conducted in conformity with the ethical principles.

Consent for publication

Written informed consent for the publication of this case report and any accompanying images was obtained from all patients. A copy of the written consent is available for review by the Editor-in-Chief of this journal.

Availability of data and materials

The datasets used and analyzed during the current study are available from the corresponding author upon reasonable request.

Competing interests

The authors declare that they have no financial or nonfinancial competing interests.

Funding

There is no funding to declare.

Authors’ contributions

KG performed patient recruitment and clinical investigation. KG, SH, YM, KS, and ST conceived and designed the study and drafted the manuscript. All authors read and approved the final manuscript.

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Table 1

Table 1 The clinical features in patients, according to molecular subtype. P values were determined using the Student's t-test.

Due to technical limitations, Table 1 is only available as a download in the supplemental files section.

Figures

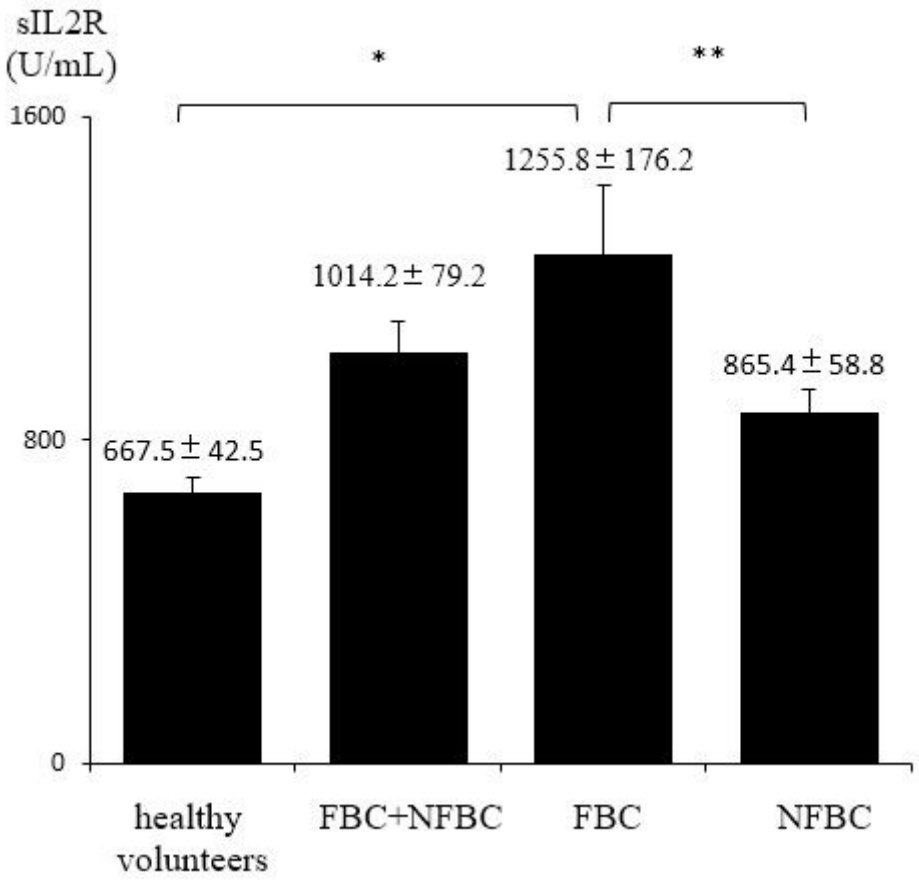

Figure 1

Results of sIL-2R evaluation in healthy volunteers and in patients with FBC + NFBC, FBC alone, and NFBC. The sIL-2R levels were higher in the preoperative patients (FBC + NFBC) than in the healthy volunteers. The sIL-2R level of FBC patients was significantly higher, compared with that of healthy volunteers (*p=0.0414) and NFBC patients (**p=0.0155). Data are represented as mean ± SD. P values were determined using the Student's t-test.

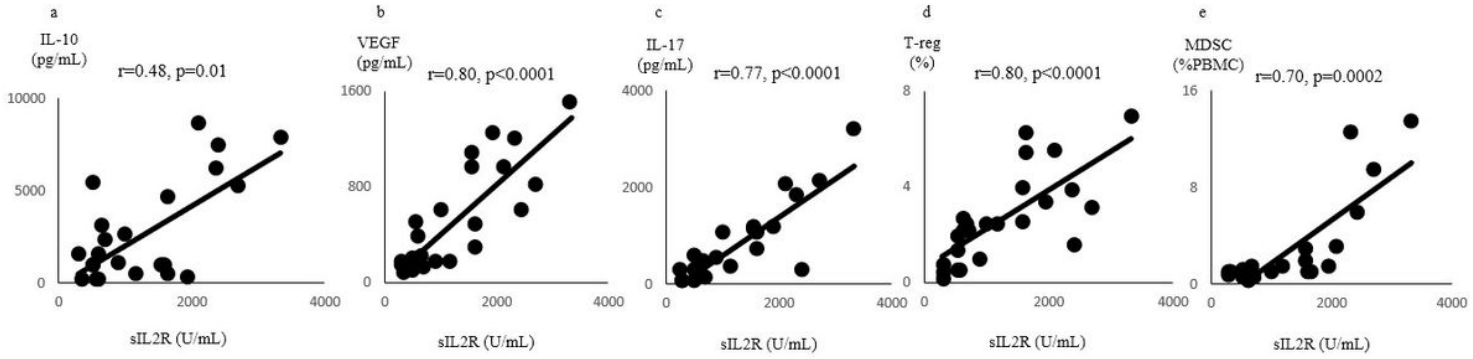

Figure 2

Correlations of sIL-2R with IL-10, Tregs, VEGF, IL-17, and MDSC. The sIL-2R levels of preoperative FBC patients are significantly positively correlated with (a) IL-10 production, (b) VEGF levels, (c) IL-17

production, (d) Tregs levels, and (e) MDSC levels. The relationship between 2 variables was quantified by Spearman's rank correlation coefficient.

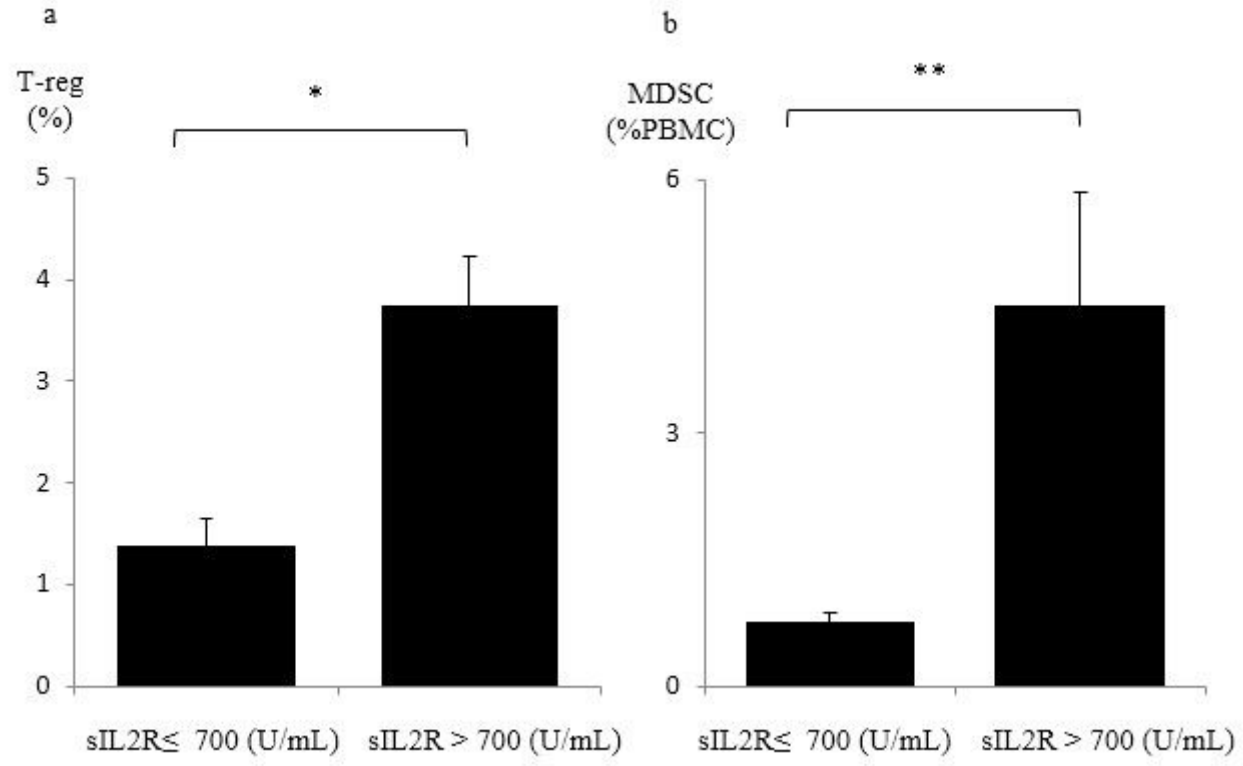

Figure 3

Tregs and MDSC levels according to the sIL-2R level in FBC patients. Based on a cutoff value of 700 U/mL, the levels of (a) Tregs (*p=0.0008) and (b) MDSC (**p=0.01) are significantly higher in patients with high sIL-2R than in those with low sIL-2R. Data are represented as mean ± SD. P values were determined using the Student's t-test.

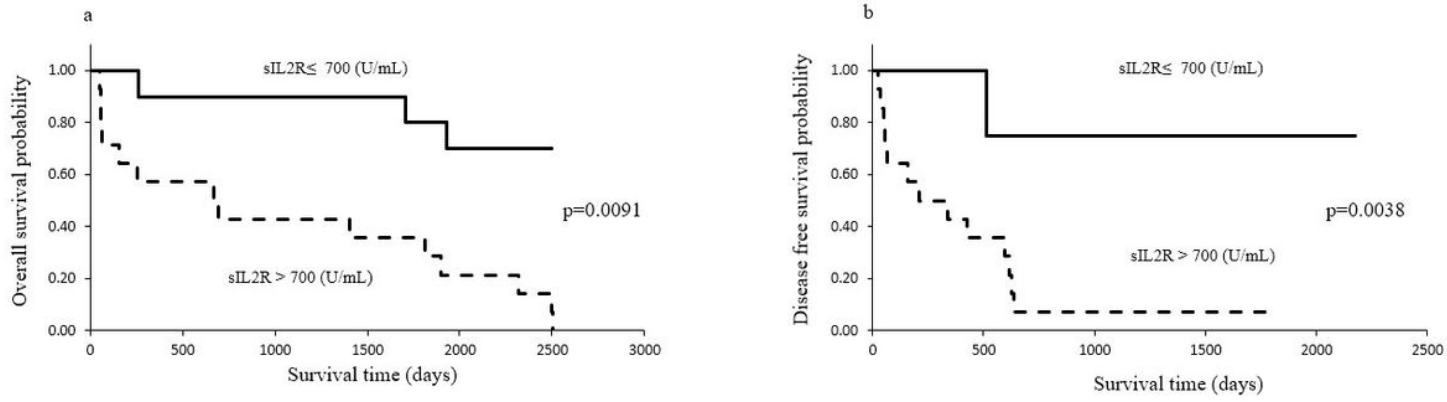

Figure 4

Kaplan–Meier estimates of overall and disease-free survival rates, according to surveillance status among FBC cases. The (a) OS and (b) DFS rates of FBC patients are significantly worse with high levels

of sIL-2R (>700 U/mL) than with low levels of sIL-2R (<700 U/mL).

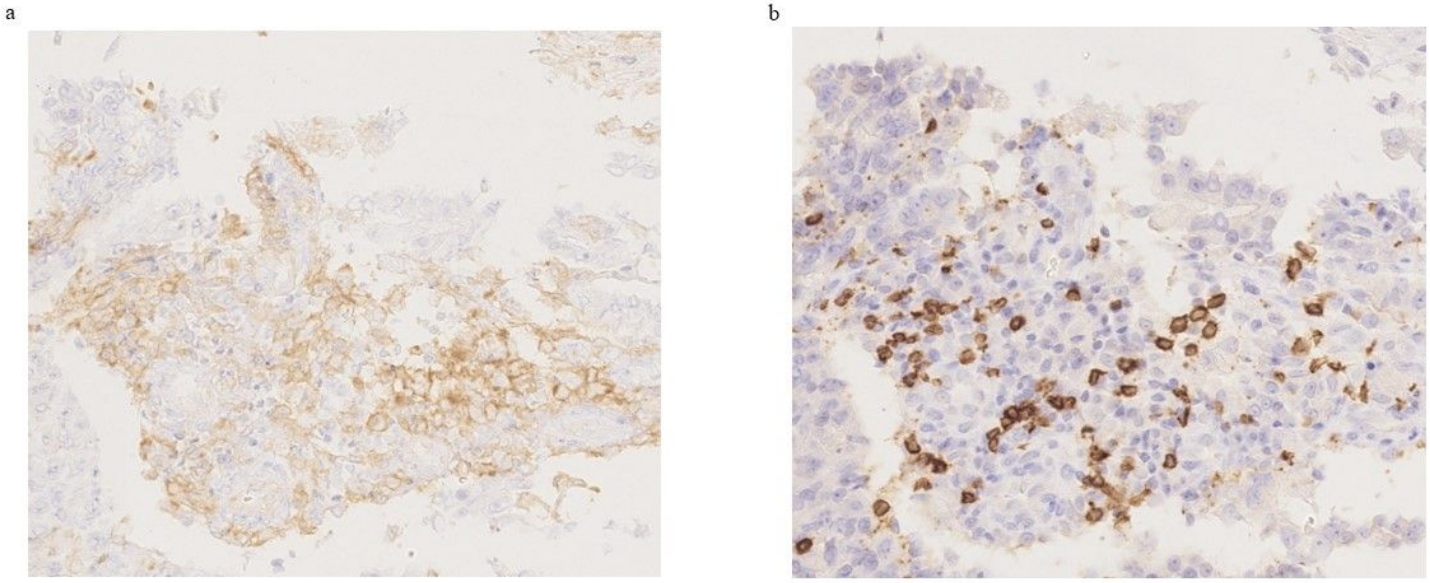

Figure 5

Representative images of immunohistochemistry for PD-L1 and CD8. (a) The epithelial compartment is positive for PD-L1 (×10), whereas (b) the infiltrating immune cells are positive for CD8 (×10).

Supplementary Files

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- Table1.jpg