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# CD103$^+$ Cells and Chemokine Receptor Expression in Breast Cancer

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**Conflict of Interest**
The authors declare no potential conflicts of interest.

## ABSTRACT

Mucosal environments harbour lymphocytes, which express several adhesion molecules, including intestinal homing receptors and integrin αE/β7 (CD103). CD103 binds E-cadherin, an integrin receptor expressed in intestinal endothelial cells. Its expression not only enables homing or retention of T lymphocytes at these sites but is also associated with increased T lymphocyte activation. However, it is not yet clear how CD103 expression is related to the clinical staging of breast cancer, which is determined by factors such as the size of the tumor (T), the involvement of nearby lymph nodes (N), and presence of metastasis (M). We examined the prognostic significance of CD103 by FACS in 53 breast cancer patients and 46 healthy controls enrolled, and investigated its expression, which contributes to lymphocyte recruitment in tumor tissue. Patients with breast cancer showed increased frequencies of CD103$^+$, CD4$^+$CD103$^+$, and CD8$^+$CD103$^+$ cells compared to controls. CD103 was expressed at a high level on the surfaces of tumor-infiltrating lymphocytes in patients with breast cancer. Its expression in peripheral blood was not correlated with clinical TNM stage. To determine the localisation of CD103$^+$ cells in breast tissue, tissue sections of breast tumors were stained for CD103. In tissue sections of breast tumors stained for CD103, its expression in T lymphocytes was higher compared to normal breast tissue. In addition, CD103$^+$ cells expressed higher levels of receptors for inflammatory chemokines, compared to CD103$^-$ cells. CD103$^+$ cells in peripheral blood and tumor tissue might be an important source of tumor-infiltrating lymphocyte trafficking, homing, and retention in cancer patients.

**Keywords:** CD103; Integrin αE/β7; E-cadherin; Tumor-infiltrating lymphocytes

## INTRODUCTION

Integrin αE/β7 (also known as cluster of differentiation 103 [CD103]) is important in lymphocyte retention in the intraepithelial compartment. It is expressed in tissues and shows no tendency to migrate to other sites (1). CD103 expression on dendritic cells (DCs) can affect the balance between effector and T regulatory cells in the intestine (2,3). CD103$^+$

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**Abbreviations**

APC, antigen presenting cell; CD, cluster of differentiation; DC, dendritic cell; TIL, tumor infiltrating lymphocyte.

**Author Contributions**

Conceptualization: Lee SH; Data curation: Song GY, Oh CS, Kim WS; Formal analysis: Seo EH, Song GY, Oh CS, Kim SH, Lee SH; Investigation: Lee SH; Writing - original draft: Seo EH, Song GY; Writing - review & editing: Oh CS, Kim SH, Kim WS.

DCs are present in diverse tissues and induce lymphocyte and CTL activity (4,5). In addition, $CD103^+$ DCs produce IL-6, inducing differentiation of Th17 cells (6). $CD103^+$ $CD8^+$ DCs expressing TLR3, TLR7, and TLR9 can produce IL-6. $CD103^+$ $CD8^+$ DCs induce a Th1 cell CTL response (7). $CD103^+$ T cells are important in immunity. The only known ligand for CD103 is the epithelial cell surface molecule E-cadherin, and adhesive interactions between CD103 and E-cadherin are responsible for the retention of antigen-specific lymphocytes in epithelial tissues (8-12). Interactions between CD103 and its ligand E-cadherin enable recognition of tumor cells by T cells in pancreatic cancer (1,10). $CD103^+$ $CD8^+$ cells reside in the epithelium of various tissues, including the gut (13), skin (14), and female reproductive tract (15). Intraepithelial $CD8^+$ tumor infiltrating lymphocytes (TILs), such as normal mucosal intraepithelial lymphocytes, might express the αE/β7 integrin subunit CD103 (8). $CD8^+$ $CD103^+$ TILs are strongly associated with increased survival in a variety of cancers (8,9). Furthermore, expression of CD103 in CTLs mediates the adherence of memory T cells to E-cadherin, which has been implicated in tumor-cell lysis and tumor rejection (16).

Weak CD103 expression is a risk factor for inflammatory skin diseases (17). CD103 is expressed by intraepithelial T cells, suggesting a role in the localisation of $CD8^+$ T cells to the epithelial surface (18). Interactions between CD103 and E-cadherin support antitumor CTL activity by triggering lytic granule polarisation and exocytosis (16). In addition, T-cell homing to tissues is critical for host protection. For inflammatory chemokine receptor expression, the site of priming imprints effector T cells with the ability to traffic to particular organs (19). Various chemokine receptors interact with $CD103^+$ cells (1,20,21). However, CD103 has only been investigated in terms of organisation and DCs. The presence of $CD103^+$ T cells in peripheral blood from breast cancer patients and their role in the anti-cancer immune response are unclear.

We evaluated the role of $CD103^+$ TILs in breast tumor tissue and analysed $CD103^+$ and $CD103^-$ cells in peripheral blood from breast cancer patients. We investigated $CD103^+$ expression in cell subsets via flow cytometry and immunohistochemical staining.

## MATERIALS AND METHODS

### Study population

This study was conducted in accordance with the Institutional Review Board of Konkuk University Medical Center (KUH1160091, KUH1210044), and written informed consent was obtained from all patients. Korean female patients undergoing elective breast cancer surgery were enrolled. Patients were excluded based on the following criteria: age <20 years, repeat surgery, history of cancer, other concurrent surgeries excluding breast reconstruction surgery, and history of drug abuse. Blood and tissue samples were obtained from patients who underwent surgery for primary breast cancer at Konkuk University Medical Center from July 2014 to October 2015.

### Blood and tissue samples

Venous blood was collected in ethylenediaminetetraacetic acid tubes. After tissue excision by the surgeon, the anatomical pathologist obtained samples of breast tumor tissue and adjacent normal tissue.


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### Flow cytometry analysis of CD103$^+$ cells

Single-cell suspensions were obtained from peripheral blood. PBMCs were isolated from heparinised venous blood by density-gradient centrifugation over a Biocoll gradient solution (Biochrom AG, Berlin, Germany). The cells were washed with phosphate-buffered saline. Single cells were washed with FACS buffer (phosphate-buffered saline, 1% bovine serum albumin, 0.01% $NaN_3$). The Abs used were fluorescein isothiocyanate anti-human CD103 (clone Ber-ACT8; eBioscience, San Diego, CA, USA), PE-cy7 anti-human CD45RO (clone UCHL1; eBioscience), antigen presenting cell (APC)-cy7 anti-human CD4 (clone OKT4; BioLegend, San Diego, CA, USA), and PE anti-human CD8 (clone RPA-T8; BD Pharmingen, Franklin Lakes, NJ, USA). Ab staining was performed for 30 min in darkness at room temperature. After washing with FACS buffer, at least 50,000 cells per sample were collected for analysis on a flow cytometer (BD FACS Aria™; Becton Dickinson, San Jose, CA, USA) and analysed in FlowJo™ software (Tree Star, Ashland, OR, USA).

### Flow cytometry analysis of immune cells

We measured the frequencies of CD4$^+$ T cells, CD8$^+$ T cells, CD45RO$^+$ memory cells, DCs, NK cells, monocytes, and chemokine receptors. Single-cell suspensions were obtained from peripheral blood. PBMCs were isolated from heparinised venous blood by density-gradient centrifugation over a Biocoll gradient solution (Biochrom AG). PBMCs were washed with phosphate-buffered saline. Single cells were washed with FACS buffer (phosphate-buffered saline, 1% bovine serum albumin, 0.01% $NaN_3$). To detect DCs, we used a PerCP HLA-DR Ab (clone L243; BioLegend). To detect NK cells, PE-cy7 CD16 (clone CB16; eBioscience) and APC-CD56 (clone B159; BD Pharmingen) Abs were used. To detect monocytes, we used the APC-cy7 Ab CD14 (clone M5E2; BioLegend). To detect chemokine receptors, PerCP-cy5.5 anti-human CCR4 (clone 291H4; BioLegend), PE anti-human CCR6 (clone 11A9; BD Pharmingen), and APC anti-human CXCR3 (clone IC6; BD Pharmingen) Abs were used. Ab staining was performed for 30 min in darkness at room temperature. After washing with the FACS buffer, at least 50,000 cells per sample were collected for analysis on a flow cytometer (BD FACS Aria™; Becton Dickinson), and analysed in FlowJo™ software (Tree Star).

### Immunohistochemical staining of CD103$^+$ cells in breast tissue

This was performed using 3 μm sections of whole tumor tissues, which were deparaffinised and subjected to epitope retrieval using citrate buffer (pH 6). To prevent endogenous peroxidase activity, the slides were incubated in blocking solution for 1 h. Sections were rinsed 3 times for 5 min with 1× PBS. An anti-CD103 rabbit monoclonal Ab (Eptiomisclone EPR4166(2)) was applied (dilution 1:500) overnight at 4°C. An anti-E-cadherin mouse monoclonal Ab (HECD-1) was applied (dilution 1:500) overnight at 4°C. The sections were rinsed 3 times for 5 min with 1× PBS. The sections were incubated for 1 h with diluted biotinylated secondary Ab. The sections were rinsed 3 times for 5 min with 1× PBS. ABC Reagent (Vector, Newark, CA, USA) was applied for 1 h at 25°C followed by 3,30-diaminobenzidine (Vector). Sections were rinsed 3 times for 5 min with 1× PBS. The slides were counterstained with haematoxylin, rehydrated, and cover-slipped using mounting medium (Vector). Images were obtained using a microscope (Eclipse Ni; Nikon, Tokyo, Japan).

### Immunofluorescence staining

Immunofluorescence staining of breast tumor tissue and normal tissue sections was performed to detect CD103$^+$, CD4$^+$, and CD8$^+$ T cells. Tissue sections were deparaffinised and subjected to epitope retrieval using citrate buffer (pH 6). To prevent endogenous peroxidase activity, the slides were incubated in blocking solution for 1 h. The sections

were rinsed 3 times for 5 min with 1× PBS. For CD4 and CD103 double staining, anti-CD103 rabbit monoclonal (EpitomisClone EPR4166(2)) and anti-CD4 mouse monoclonal (clone BC/IF6) Abs were added for 1 h at 25°C. The sections were rinsed 3 times for 5 min with 1× PBS and incubated with the secondary Ab (Life Technologies, Carlsbad, CA, USA) for 1 h at 25°C in darkness. The sections were rinsed 3 times for 5 min with 1× PBS. The slides were counterstained with TOPRO3 (Invitrogen, Waltham, MA, USA) and cover-slipped using fluorescence mounting medium (Vector). For CD8 and CD103 double staining, anti-CD103 rabbit monoclonal (EpitomisClone EPR4166(2)) and anti-CD8 mouse monoclonal (clone 144B) Abs were added for 1 h at 25°C. The slides were rinsed 3 times for 5 min with 1× PBS and incubated with the secondary Ab (Life Technologies) for 1 h at 25°C in darkness. The sections were rinsed 3 times for 5 min with 1× PBS, counterstained with TOPRO3 (Invitrogen), and cover-slipped using fluorescence mounting medium (Vector). Images were obtained with a confocal laser scanning microscope (FV1000; Olympus, Tokyo, Japan).

### Statistical analysis

Student's *t*-test was used to analyse differences between groups. Differences in the other variables between groups were analysed using the *t*-test or Mann-Whitney *U* test after confirmation of normality using the Shapiro-Wilk test. Values of $p<0.05$ were considered indicative of significance. Prism software (ver. 5.01; GraphPad Software, La Jolla, CA, USA) was used for statistical analysis.

## RESULTS

The numbers of breast cancer patients and healthy controls enrolled were 53 and 46, respectively. The characteristics of the patients with breast cancer are listed in **Table 1**. The characteristics of the patients who underwent tissue staining are listed in **Table 2**.

### Increased circulating $CD103^+$ and $CD103^+$ T cells in patients with breast cancer

We analysed T lymphocytes in PBMCs by flow cytometry and evaluated the frequencies of $CD103^+$, $CD4^+$ $CD103^+$, and $CD8^+$ $CD103^+$ T cells. Representative flow cytometry plots of $CD103^+$ T cells from patients and healthy controls are shown in **Fig. 1**. The frequencies of circulating $CD103^+$, $CD4^+$ $CD103^+$, and $CD8^+$ $CD103^+$ T cells were significantly increased in patients with breast cancer compared to healthy controls ($CD103^+$ 1.24±0.99 vs. 0.73±0.58, p=0.0064; $CD4^+$ $CD103^+$ 2.55±1.85 vs. 1.63±1.05, p=0.0073; $CD8^+$ $CD103^+$ 3.50±2.33 vs. 1.23±0.78, p=0.0254). However, there were no significant differences in the frequency of $CD103^+$ $CD45RO^+$ memory cells between breast cancer patients and healthy controls ($CD103^+$ $CD45RO^+$ 0.97±0.73 vs. 0.88±0.54).

The $CD103^+$ cell frequency did not differ between early-stage patients and healthy controls (1.02±0.68 vs. 0.73±0.58, p=0.0622) but was significantly increased in late-stage breast cancer patients compared to healthy controls (1.46±1.25 vs. 0.73±0.58, p=0.0023) (**Fig. 2**). There were no significant differences in $CD103^+$ memory cell frequency between breast cancer patients and healthy controls. $CD4^+$ $CD103^+$ T cells were significantly increased in frequency in both early-stage (1.11±0.91) and late-stage (1.07±1.19) breast cancer patients compared to healthy controls (0.61±0.47, p= 0.0044 and p=0.0327, respectively). The $CD8^+$ $CD103^+$ T cell frequency was significantly increased in early-stage (2.78±2.08), but not late-stage (1.46±1.25) breast cancer patients compared to the healthy controls (1.80±0.74, p=0.0077 and p=0.0675).

**Table 1.** General characteristics of patients with breast cancer

| Variable | No. of patients (%) | CD103+ in PBMC (%) |
|---|---|---|
| Age (yr) | | |
| 21–30 | 1 (1.88) | 1.06 |
| 31–40 | 14 (26.41) | 1.04±0.17 |
| 41–50 | 18 (33.96) | 1.26±0.84 |
| 51–60 | 17 (32.07) | 1.17±0.35 |
| >60 | 3 (5.66) | 1.43±0.75 |
| T stage | | |
| Tris | 12 (22.64) | 1.34±0.44 |
| 1 | 21 (39.62) | 1.42±0.62 |
| 2 | 17 (32.07) | 1.21±0.20 |
| 3 | 2 (3.77) | 1.27±0.11 |
| 4 | 1 (1.88) | 1.52 |
| N stage | | |
| X | 9 (16.98) | 1.16±0.37 |
| 0 | 34 (64.15) | 1.40±0.24 |
| 1 | 7 (13.20) | 1.18±0.40 |
| 2 | 3 (5.66) | 1.28±0.12 |
| TNM stage | | |
| 0 | 12 (22.64) | 1.04±0.74 |
| I | 17 (32.07) | 1.35±0.50 |
| IIA | 16 (30.18) | 1.22±0.43 |
| IIB | 3 (5.66) | 1.34±0.11 |
| IIIA | 4 (7.54) | 1.23±0.15 |
| IIIB | 1 (1.88) | 1.30 |
| ER | | |
| Negative | 8 (15.09) | 1.22±0.41 |
| Positive | 45 (84.90) | 1.25±0.37 |
| PR | | |
| Negative | 18 (33.96) | 1.14±0.46 |
| Positive | 35 (66.03) | 1.21±0.35 |

Data are expressed as incidence (percentage). Data are expressed as mean ± standard deviation.
Tris, tumor *in situ*; TNM stage, tumor, node, metastasis stage; ER, estrogen receptor; PR, progesterone receptor.

**Table 2.** Demographic information breast cancer tissues

| Subject identification | Age (yr) | Tumor size (cm) | TNM stage | Cancer stage | PR/ER/Her2 |
|---|---|---|---|---|---|
| 1 | 73 | 2.6 | T2N1M0 | IIB | +/+/– |
| 2 | 53 | 2.4 | T2N0M0 | IIA | +/+/– |
| 3 | 53 | 1.2 | T1N0M0 | IA | –/+/– |
| 4 | 48 | 2.4 | T2N1M0 | IIB | +/+/– |
| 5 | 56 | 2.1 | T2N1M0 | IIB | –/+/– |

TNM stage, tumor, node, metastasis stage; PR, progesterone receptor; ER, estrogen receptor; Her2, receptor tyrosine-protein kinase erbB-2.

There were no significantly different clinical presentations, such as clinical tumor, node, metastasis stage, oestrogen receptor, and progesterone receptor (data not shown).

## Correlations among CD103$^+$, CD4$^+$ CD103$^+$, and CD8$^+$ CD103$^+$ T cells in peripheral blood

We found a correlation between the frequencies of CD103$^+$ and CD4$^+$/CD8$^+$ PBMCs. There were no significant relationships among CD103$^+$, CD4$^+$ CD103$^+$, and CD8$^+$ CD103$^+$ T cells in the healthy controls (CD103$^+$ and CD4$^+$ CD103$^+$ cells, r=0.1176, p=0.4638; CD103$^+$ and CD8$^+$ CD103$^+$ cells, r=0.1075, p=0.5035; CD4$^+$ CD103$^+$ and CD8$^+$ CD103$^+$ cells, r=0.2475, p=0.1188) (**Fig. 3**). By contrast, there were significant positive correlations between CD103$^+$ and CD4$^+$ CD103$^+$ T cells (r=0.4599, p=0.0009) and between CD103$^+$ and CD8$^+$ CD103$^+$ T cells (r=0.3780, p=0.0074) in patients with breast cancer. However, there was no correlation between CD4$^+$ CD103$^+$ and CD8$^+$ CD103$^+$ T cells (r=0.2066, p=0.1543) in patients with breast


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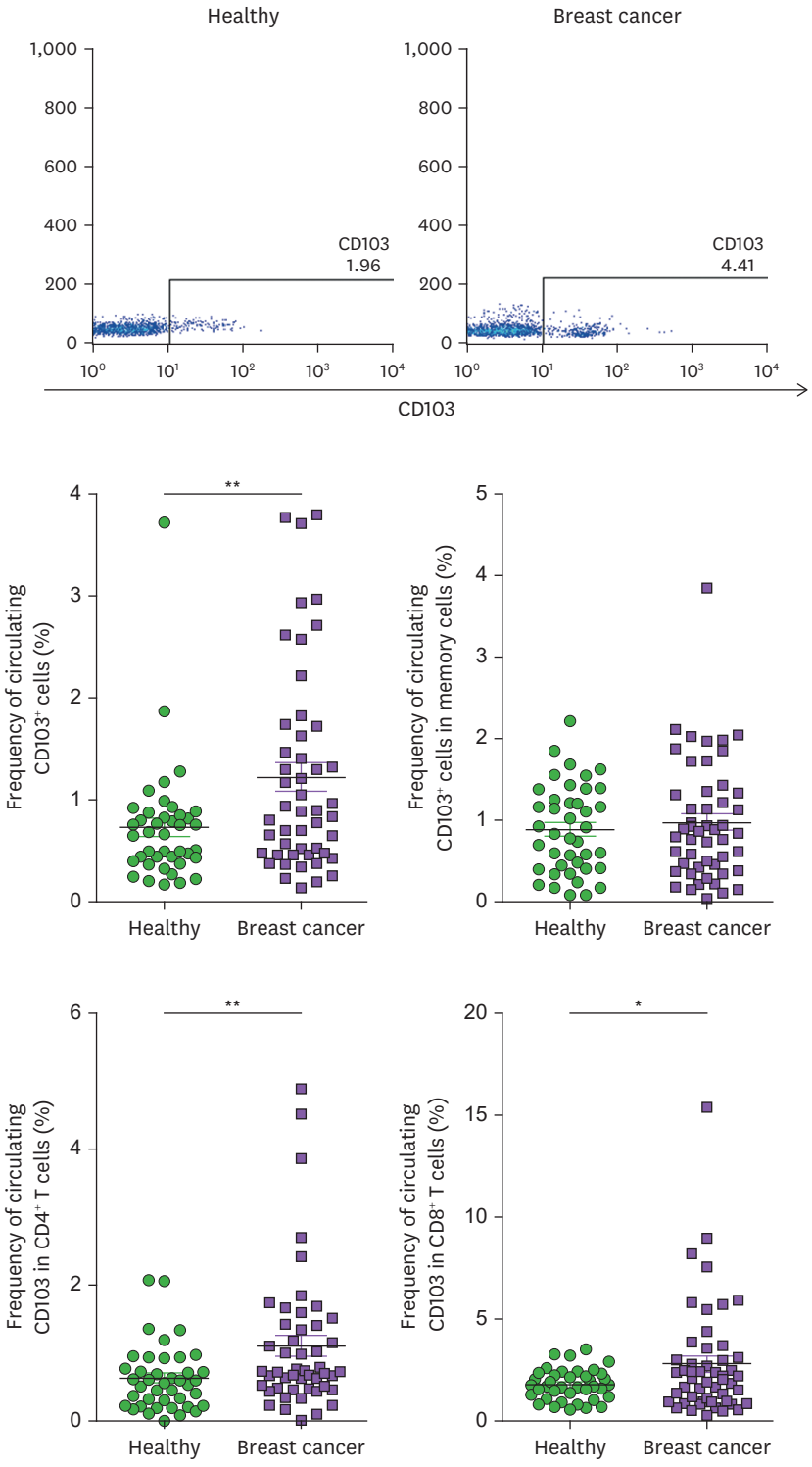


**Figure 1.** Frequencies of CD103⁺ cells in breast cancer patients and healthy controls. PBMCs stained for CD4, CD8, CD45RO, and CD103 and subjected to flow cytometry. Experiments were repeated more than 3 times with similar results.
*p<0.05; **p<0.01.


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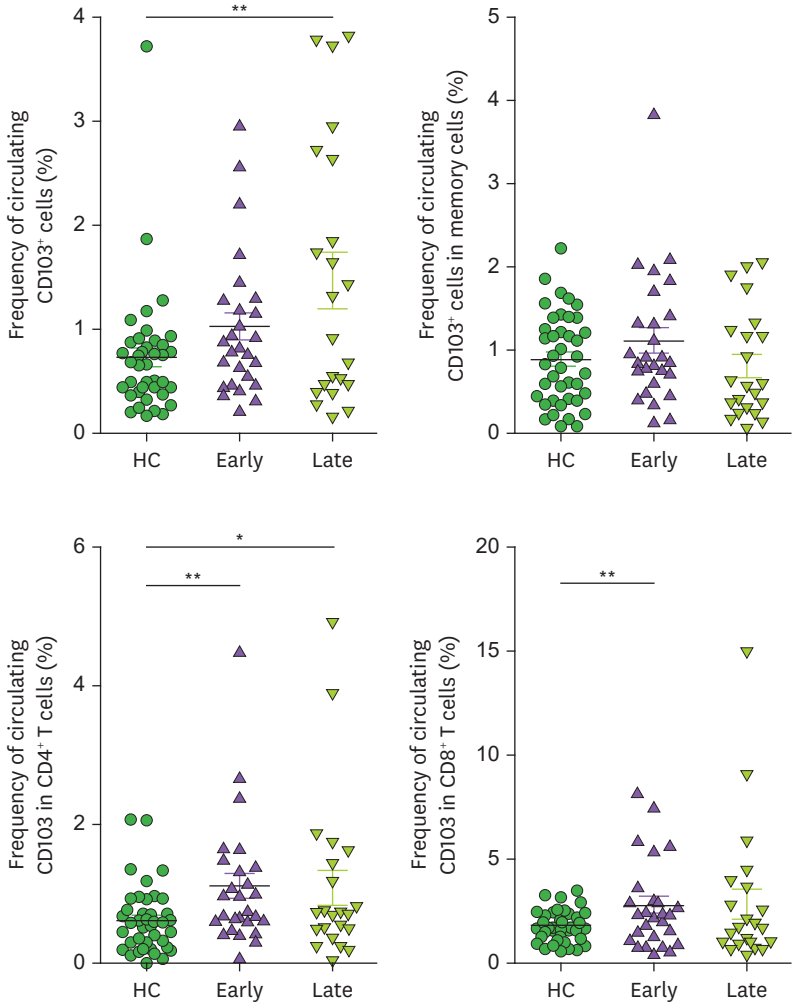


**Figure 2.** Frequencies of CD103$^+$ cells and CD103$^+$ T cells in the peripheral blood of patients with early-stage and late-stage breast cancer and healthy controls (early-stage, TNM stage 0 and 1; late-stage, TNM stage 2 and 3). PBMCs were stained for CD4, CD8, CD45RO, and CD103 and subjected to flow cytometry. Experiments were repeated more than 3 times with similar results.
TNM stage, tumor, node, metastasis stage.
*p<0.05; **p<0.01.

cancer. Therefore, the numbers of CD4$^+$ CD103$^+$ and CD8$^+$ CD103$^+$ T cells in healthy controls were not significantly affected by CD103$^+$ cells. By contrast, the numbers of CD4$^+$ CD103$^+$ and CD8$^+$ CD103$^+$ T cells in patients with breast cancer were significantly affected by CD103$^+$ cells. However, there were no significant correlations between CD4$^+$ CD103$^+$ and CD8$^+$ CD103$^+$ cell numbers in patients with breast cancer.

## Circulating immune cells in peripheral blood

The frequencies of circulating CD4$^+$ and CD8$^+$ cells were nonsignificantly increased in breast cancer patients compared to healthy controls (CD4$^+$ 38.92±8.17 vs. 36.88±8.4; CD8$^+$ 20.80±7.91 vs. 19.67±7.02) (**Fig. 4**). The patients had a significantly increased number of circulating CD45RO$^+$ lymphocytes compared to the healthy controls (44.04±9.59 vs. 38.08±10.07,p=0.0068). The frequencies of circulating DCs and NK cells showed a nonsignificant increase in breast cancer patients compared to healthy controls (HLA-DR 22.29±7.59 vs. 19.59±8.3; CD16$^+$ CD56$^+$ 15.30±6.77 vs. 16.80±10.52). Breast cancer patients


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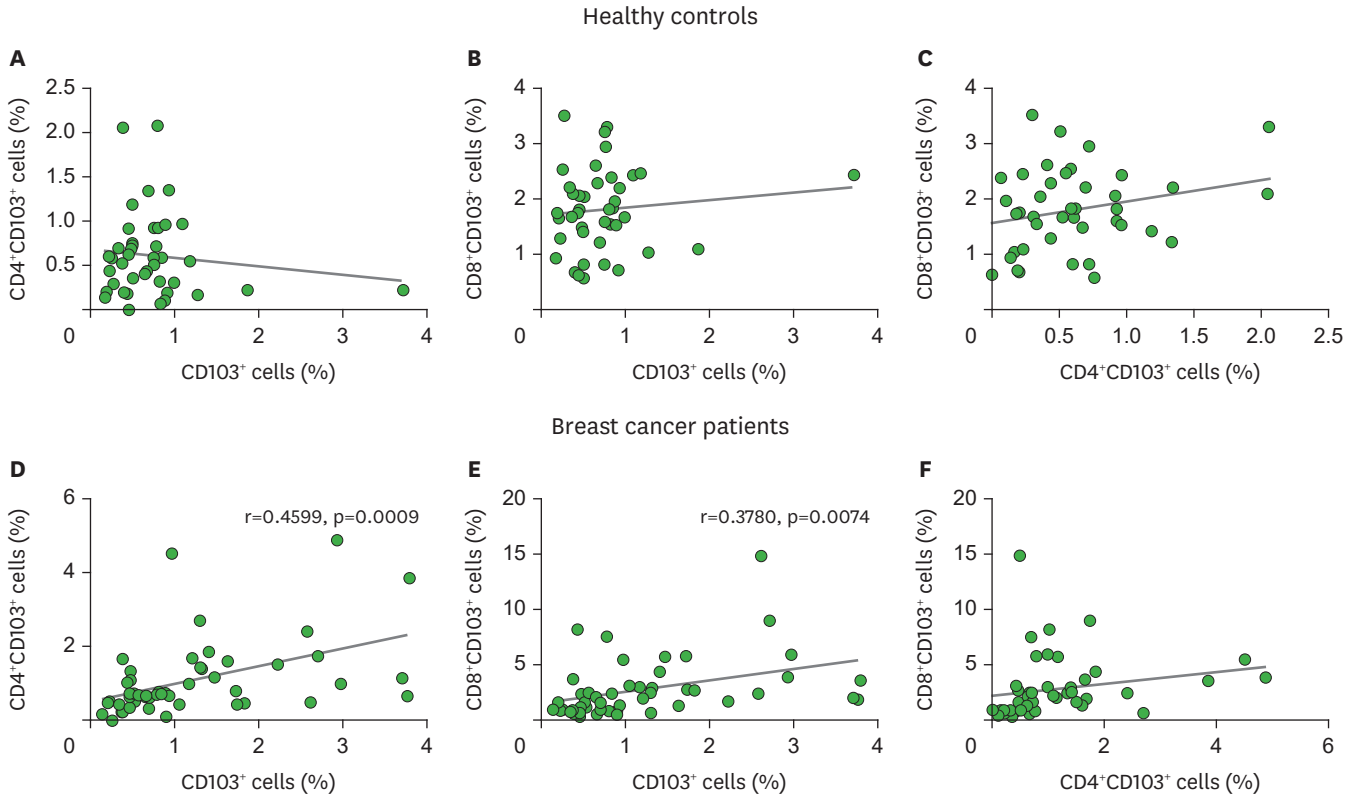


**Figure 3.** Correlation between CD103$^+$ cells and CD103$^+$ T cells in the peripheral blood of healthy controls and breast cancer patients. CD103 expression on T cell surfaces in peripheral blood analysed according to flow cytometry. (A-C) There were no significant correlations between CD103$^+$ cells and CD103$^+$ T cells. (D-F) There was a significant correlation between CD103$^+$ and CD103$^+$ T cells. Experiments were repeated more than 3 times with similar results. The flow cytometry results for CD103$^+$ cells were evaluated using a Spearman's rank correlation test.

had a significantly higher number of circulating monocytes compared to healthy controls (CD14$^+$ 16.13±6.19 vs. 12.31±6.74, p=0.0279). We detected CXCR3, CCR4, and CCR6 in the peripheral blood of healthy controls and breast cancer patients. The numbers of CCR4$^+$ and CXCR3$^+$ cells in peripheral blood were higher in breast cancer patients than healthy controls (CCR4$^+$ 20.05±6.13 vs. 13.82±8.77, p=0.0001; CXCR3$^+$ 16.86±10.50 vs. 14.84±7.73, p=0.0034). However, the number of CCR6$^+$ cells in peripheral blood was not significantly different between breast cancer patients and healthy controls (14.84±7.73 vs. 12.11±7.07).

## Expression of chemokine receptors on CD103$^+$ cells in patients with breast cancer

We designed multicolour flow cytometry panels to simultaneously assess T-cell phenotype and chemokine receptor expression in PBMCs. CD103$^+$ cells promote the expression of chemokine receptors such as CCR4, CXCR3, and CCR6 compared to CD103$^-$ cells. The number of CCR4$^+$ CD103$^+$ cells was significantly increased in the breast cancer patients compared to CD103$^-$ cells (37.44±12.12 vs. 19.59±6.01, p<0.0001) (**Fig. 5**). The number of CXCR3$^+$ CD103$^+$ cells was significantly increased in patients with breast cancer compared to CXCR3$^+$ CD103$^-$ cells (32.63±11.38 vs. 16.57±10.76, p<0.0001). The number of CCR6$^+$ CD103$^+$ cells was significantly increased in patients with breast cancer compared to CCR6$^+$ CD103$^-$ cells (11.81±7.02 vs. 23.27±16.83, p<0.0001).


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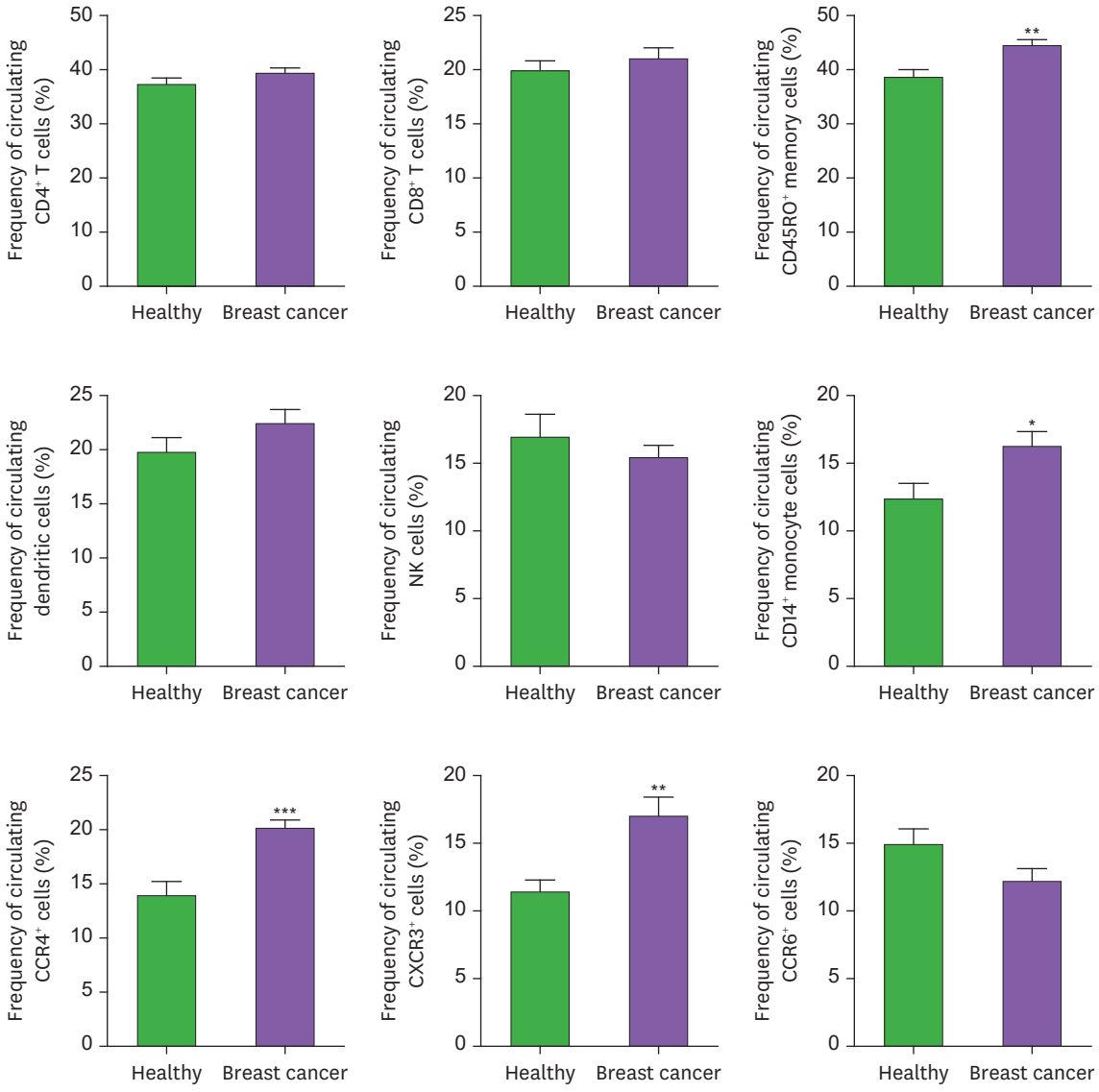


**Figure 4.** Numbers of immune cells in breast cancer patients and healthy controls. PBMCs were stained for CD4, CD8, CD45RO, HLA-DR, CD16, CD56, CD14, CCR4, CXCR3, and CCR6 and subjected to flow cytometry. Experiments were repeated more than 3 times with similar results.
*p<0.05; **p<0.01; ***p<0.001.

## CD103$^+$ cells in breast tumor and normal tissues

We performed immunohistochemical staining to evaluate the tissue homing of circulating CD103 cells (**Fig. 6**). Few CD103$^+$ cells were detected in normal breast tissue (CD103$^+$ TILs, 5.20±3.34). A larger number of CD103$^+$ cells was detected in breast cancer tissue compared to normal breast tissue (CD103$^+$ TILs 71.40±51.58). The number of CD103$^+$ cells was significantly higher in breast cancer tissue than in normal breast tissue (CD103$^+$ TILs normal vs. tumor 5.20±3.34 vs. 71.40±51.58, p=0.021). These results were similar to those for peripheral blood.

## Relationship between E-cadherin expression and CD103$^+$ cell number

E-cadherin is the only known ligand for CD103. To evaluate its expression in tissues of breast cancer patients, immunohistochemical staining was performed. The number of CD103$^+$ cells was not correlated with E-cadherin expression. Also, there were no significant differences


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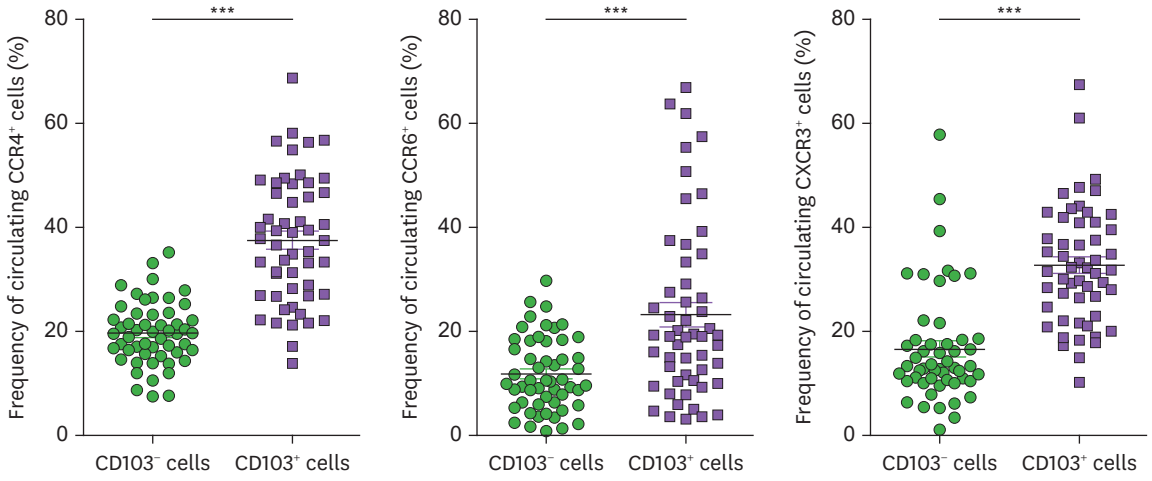


**Figure 5.** Expression of chemokine receptors on CD103$^+$ cells from patients with breast cancer and healthy controls. PBMCs were stained for CCR4, CXCR3, CCR6, and CD103 and subjected to flow cytometry. CD103$^+$ cells were distinguished from CD103$^-$ cells. Higher percentages of CD103$^+$ cells expressed CCR4, CXCR3, and CCR6 compared to CD103$^-$ cells. Experiments were repeated more than 3 times with similar results.
***p<0.001.

in its expression and CD103$^+$ cell number in normal and tumor tissues (**Fig. 7**). E-cadherin expression in tissues had no effect on CD103$^+$ cell number. In the normal breast tissue samples, E-cadherin expression was observed at varying levels, with some samples showing high expression and others low or undetectable. Similarly, in breast cancer tissue samples, E-cadherin expression was highly variable, with some tumors showing strong expression and others showing reduced or absent expression. These findings indicate that E-cadherin expression is not consistently upregulated or downregulated in breast cancer but rather exhibits a high degree of variability both within and among individual tumors.

### Immunofluorescence staining of CD103, CD4, and CD8

Immunofluorescence staining revealed the development of CD4$^+$ T cells and CD103$^+$ cells in tumor tissue, and the development of CD8$^+$ T cells and CD103$^+$ cells in tumor tissue (**Fig. 8**). CD4$^+$ and CD8$^+$ T cells with CD103$^+$ cells were highly expressed in tumor tissues compared to normal breast tissues.

## DISCUSSION

The number of CD103$^+$ cells, including both the CD4$^+$ and CD8$^+$ T cell subsets, in peripheral blood was significantly higher in breast cancer patients than healthy controls. Therefore, these cells contribute to the population of functional and tumor-reactive CD4$^+$ and CD8$^+$ T cells in the peripheral blood of breast cancer patients. The number of CD103$^+$ T cells was higher in breast tumor tissue than normal tissue.

CD103$^+$ cells are present at a high frequency in mucous membranes, skin, eyes, and bowels but at a lower frequency in serum (11). However, we found that its expression on the surface of circulating T-lymphocytes was higher in breast cancer patients than healthy controls. Therefore, CD103$^+$ T lymphocytes influence the tumor environment. CD103 is involved in the tissue trafficking and retention of CD4$^+$ and CD8$^+$ T lymphocytes (22). In our study, there was a correlation between CD103 expression and the number of CD4$^+$ CD103$^+$ or CD8$^+$ CD103$^+$ cells in the serum of breast cancer patients but not healthy controls. CD8$^+$ CD103$^+$ T

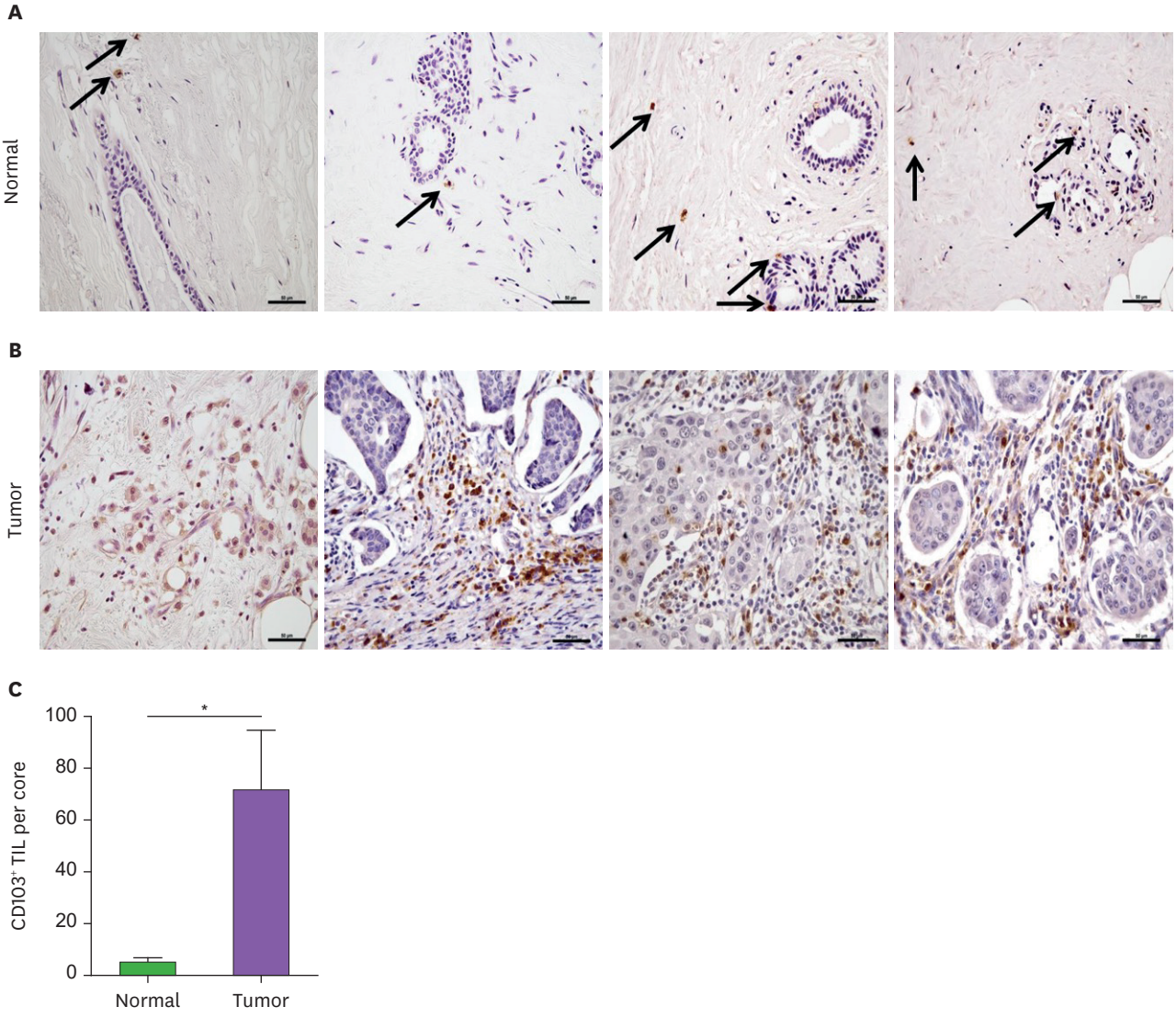


**Figure 6.** Expression pattern of CD103 in breast tissue. (A, B) Immunohistological detection of CD103$^+$ cells in breast tumor tissue and normal breast tissue. (C) Statistical analysis of CD103$^+$ cell frequencies in breast tumor and normal tissues. Black arrow, CD103$^+$ TILs. The number of CD103$^+$ cells was higher in breast tumor tissue than in normal tissue. Scale bar, 50 μm.
*p<0.05.

cells differ markedly from other CD103$^+$ cells in that they do not migrate (9), and they have predominantly cytotoxic rather than regulatory functions (11). CD103 expression differs according to cancer type (8). This suggests that the differences in CD103 expression between normal breast tissue and breast cancer tissue are specific to breast cancer, rather than a general feature of all types of cancer. This could provide insight into the mechanisms of CD103 regulation in breast cancer. We detected CD103$^+$ cells in breast cancer tissue but not in normal breast tissue. Therefore, these cells are implicated in the immune response of patients with breast cancer. Collectively, our data suggest that CD103$^+$ and CD103$^-$ cells are different cell populations with distinct functions. Furthermore, our findings suggest that the expression of chemokine receptors on the surface of CD103$^+$ cells could affect their ability to infiltrate tissues.


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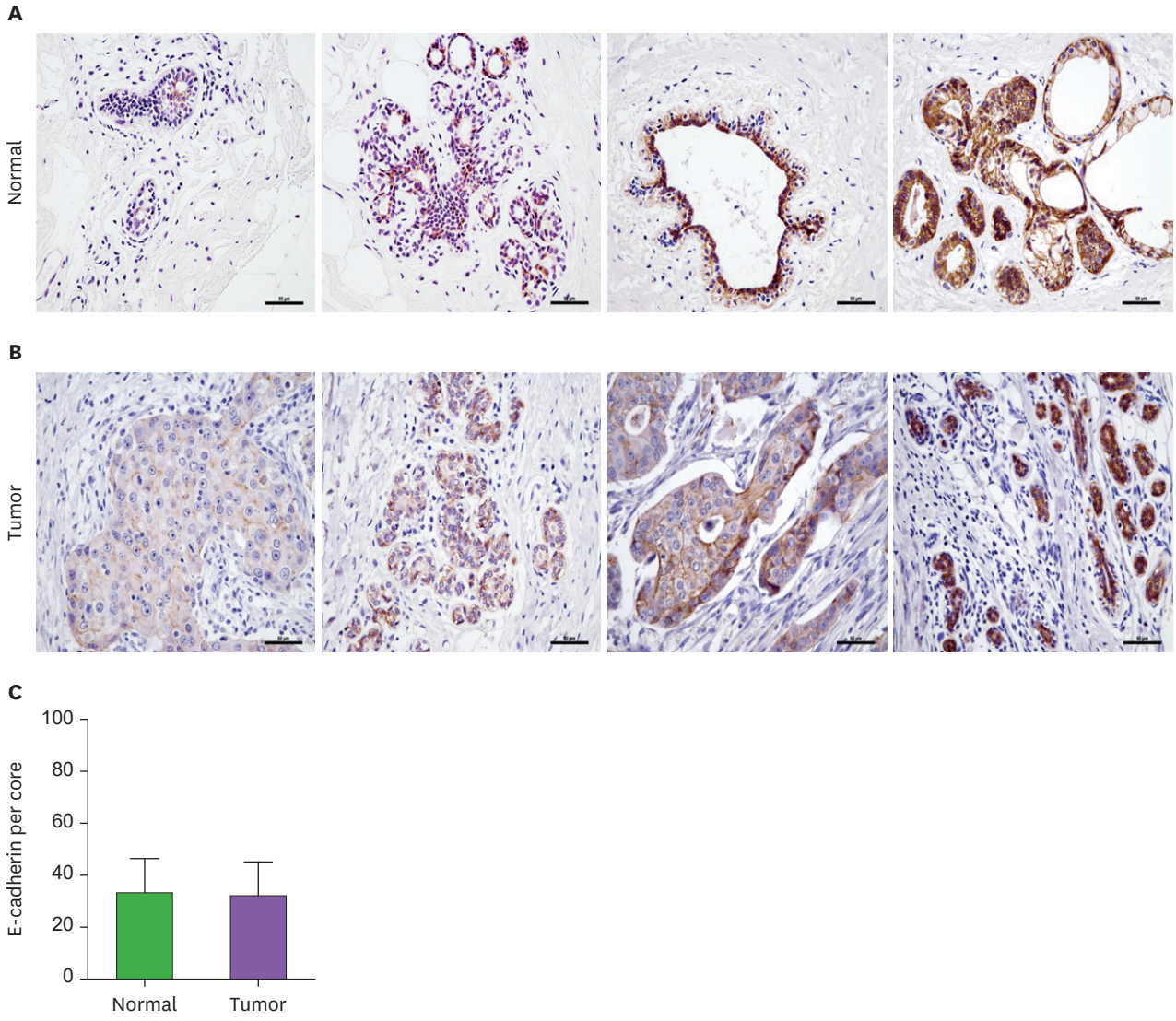


**Figure 7.** Expression pattern of E-cadherin in breast tissue. (A, B) Immunohistological detection of E-cadherin in breast tumor tissue and normal breast tissue. (C) Statistical analysis of E-cadherin frequencies in breast tumor and normal tissues. $CD103^+$ cell frequency was not correlated with E-cadherin expression in breast tumor tissues. Scale bar, 50 μm.

E-cadherin expression is variable in ovary cancer (23). E-cadherin expression was highly variable in normal breast and breast cancer tissue in this study. Although E-cadherin is the ligand for CD103 (3), we did not find a significant correlation between CD103 and E-cadherin expression in normal breast and breast cancer tissue. E-cadherin expression in tissues did not affect CD103.

Chemokine receptors such as CCR4 and CXCR3 were more highly expressed in breast cancer patients than healthy controls. Immune cell migration has many similarities to tumor cell trafficking and is regulated by chemokines and their receptors (24,25). In addition, the migration of T lymphocytes is affected by chemokine receptors in tumor tissue (26).

$CD103^+$ cells and $CD103^-$ cells have different functions and surface receptor patterns (1,9). $CD103^+$ DCs, but not $CD103^-$ DCs, promote the expression of the gut-homing receptor CCR9


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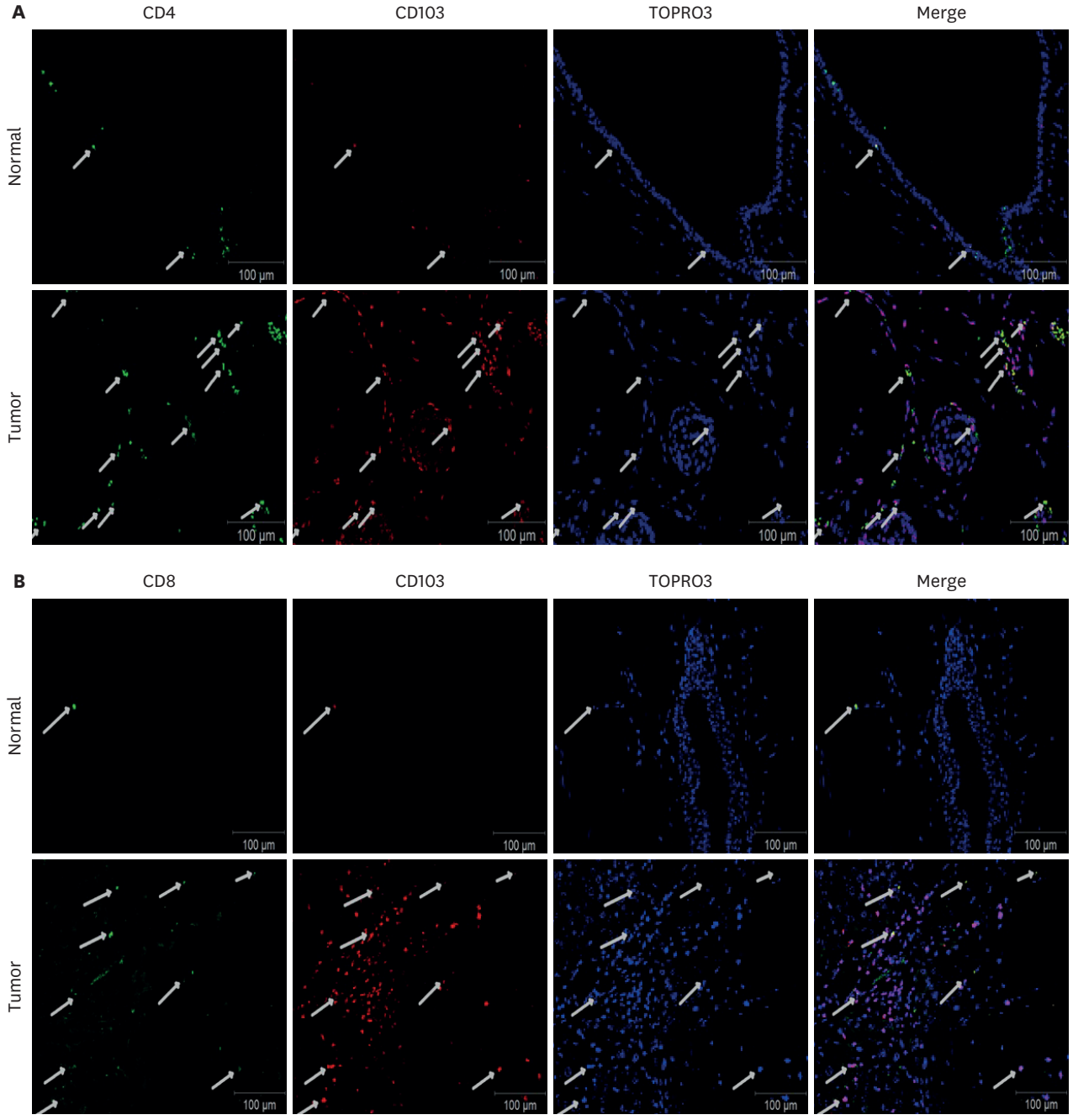


**Figure 8.** Immunofluorescence staining of CD4/CD103 and CD8/CD103 in normal breast tissue and tumor tissue. $CD103^+$ T cells in normal and tumor breast tissue. (A) Normal breast tissue was stained for CD103 (red), CD4 (green), and TOPRO3 (blue). Accumulation of $CD4^+$ and $CD103^+$ T cells in breast tissue. $CD103^+$ was expressed by $CD4^+$ tumor-infiltrating lymphocytes. Scale bar, 100 μm. (B) Normal breast tissue stained for CD103 (red), CD8 (green), and TOPRO3 (blue). $CD8^+$ and $CD103^+$ T cell accumulation in breast tissue. $CD103^+$ was expressed by $CD8^+$ tumor-infiltrating lymphocytes. White arrows, cells co-expressing CD103 (red), CD8 (green), and TOPRO3 (nuclei; blue). Scale bar, 100 μm.

on T cells (1). Therefore, high expression of chemokine receptors on $CD103^+$ cells promotes their infiltration. CD103-knockout mice show reduced T cell migration, implicating $CD103^+$ cells in T cell migration (22,27). The majority of $CD103^+$ cells in tumor tissue are not DCs but rather $CD3^+$ $CD8^+$ T lymphocytes (9). This suggests that $CD103^+$ T cells, particularly $CD8^+$ T cells, are involved in the immune response to breast cancer. This finding is consistent with previous reports of the importance of $CD8^+$ T cells in antitumor immunity. $CD103^+$ cells are

involved in lymphocyte retention and migration. There might be a relationship between $CD8^+$ $CD103^+$ effectors and the destruction of graft epithelial elements, suggesting that CD103 promotes the migration of $CD8^+$ T cells into epithelial compartments (22).

In conclusion, the numbers of $CD103^+$, $CD4^+$ $CD103^+$, and $CD8^+$ $CD103^+$ cells in peripheral blood were increased in patients with breast cancer and showed strong tumor tissue-specific infiltration. In peripheral blood, the expression levels of chemokine receptors on $CD103^+$ cells were high, promoting their infiltration. In addition, CD103 promoted the recruitment of $CD4^+CD8^+$ TILs in epithelial tumor islets. Further studies should investigate the influence of tissue $CD103^+$ cells on T cells and the functional differences between $CD103^+$ and $CD103^-$ cells.

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