Overexpression of CD155 associated with PD-1 and PD-L1 expression on immune cells, rather than tumor cells in microenvironment of breast cancer

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Research

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Abstract

Objective This study was performed to investigate the expression status of CD155 and the association with exhausted CD4 + helper and CD8 + cytotoxic tumor-infiltrating lymphocytes (TILs) and programmed cell death-ligand 1 (PD-L1) in breast cancer microenvironment.

Methods 126 breast cancer patients with invasive ductal breast cancer were recruited into this study consecutively. Immunohistochemistry was used to detect the expression CD155, PD-L1 and programmed cell death protein 1 (PD-1) on tumor-infiltrating immune cells and tumor cells in the microenvironment.

Results The proportion of patients with CD155 expression was higher in triple negative breast cancer (72.7%) than Luminal A patients (22.2%, $p < 0.05$). Patients with positive CD155 expression had higher percentage of CD4 + /PD-1 + helper TILs (30%) than patients with negative CD155 expression (21%, $p < 0.05$). Patients with positive CD155 expression also had higher cell counts of exhausted CD4+ TILs (47 vs. 20/HPF) and unexhausted CD8+ TILs (30 vs. 17/HPF) than patients with negative expression ($p < 0.05$). CD155 expression was correlated with an increased PD-L1 expression in immune cells, 0.8% and 0.02% immune cells expressing PD-L1 in patients with positive and negative CD155 expression, respectively ($p < 0.05$).

Conclusions CD155 was related with an inhibitory immune microenvironment of breast cancer. CD155 was associated with high proportion of exhausted CD4 + and unexhausted CD8 + TILs and high PD-L1 expression in immune cells.

Introduction

Breast cancer (BC) is the most common malignant tumor among Chinese women, and new cases of breast cancer accounted for 15% of all female cancer patients in 2015[1]. The clinicopathological characteristics of Chinese women with BC are different from western women, lower the expression rate of hormone receptors and higher expression rate of human epidermal growth factor receptor 2[2].

CD155 is one ligand of T cell immunoglobulin and ITIM domain (TIGIT) expressed in various cell types, including antigen-presenting cells and tumor cells[3] and the interaction limits cell function through feedback inhibition[4]. Normal tissues have no or low expression of CD155 but malignancies have upregulated expression [5–7], which plays a key role in tumor cell invasion and migration.

PD-L1 is mainly expressed on the membrane surface of mature immune cells and various tumor cells[8]. PD-1 is an immune checkpoint molecule and the interaction inhibits biological functions of effector T-cells. PD-1 expression on BC TILs was related with different clinicopathological characteristics [9][10]. However, whether the exhausted phenotypes of effector TILs and PD-L1 is related to the CD155 expression on BC cancer has not been reported. Therefore, this study was performed to investigate the distribution of CD155 expression and their relationship with PD-L1 and phenotypes of exhausted CD4⁺

and CD8⁺ effector TILs to illustrate the effect of CD155 expression on immune microenvironment of BC in Chinese patients.

Methods

Ethical approval and informed consent

All procedures performed in this study involving human participants were approved by the ethical committee of Beijing Shijitan Hospital, Capital Medical University, in accordance with the ethical standards of the 1964 Helsinki declaration and its later amendments. This study was under a retrospective study and the formal consent was waived.

Patients

126 patients with invasive ductal BC were recruited into this cohort study from January 1, 2012 to December 31, 2013 consecutively. Patients were diagnosed with operable BC and received surgical treatment at the Department of Breast Surgery, Beijing Shijitan Hospital, Capital Medical University. All the cases were diagnosed with primary invasive BC from pathology test.

Tissue preparation

The surgical specimen from all patients was fixed by 4% neutral formaldehyde, embedded by paraffin (FFPE), and stained by hematoxylin and eosin. A serial 4-μm thickness sections from each specimen were used to determine the histopathological features. Nottingham modification of the Bloom–Richardson system was used to classify histological grading of BC.

Immunohistochemistry (IHC)

Expression of CD155, PD-L1 and PD-1 was detected by IHC on 4 μm-thick FFPE sections. Monoclonal antibody against CD155 (rabbit anti-human, # 81254) was purchased from Cell Signaling Technology. Monoclonal antibody against CD155 (rabbit anti-human, # SP142) was Roche. Monoclonal antibody against PD-1 (mouse antihuman, # UMAB199) CD4 (rabbit anti-human, # EP204) and CD8 (# SP16) were purchased from Beijing Zhong Shan Golden Bridge Biotechnology Co. Ltd. Sections were baked for dehydration in an oven at 60 °C for 60 min, dewaxed for 20 min and washed in 100%, 100%, 95% and 75% alcohol for 2 min respectively. Then the sections were washed in PBS for 2 min and the wash was repeated by 5 times. EnVision™ FLEX Target Retrieval Solutions were used for antigen retrieval for 2 min 30 sec. Sections were settled at room temperature for 20 min, washed in PBS by 2 min, repeated the wash by 5 times. The sections were incubated in 3% H₂O₂ at room temperature for 15 min; washed in PBS by 2 min, repeated the wash by 5 times and then sealed with 5% serum at 37°C for 15 min. The supernatant was discarded and the primary antibody was added at 4°C for a night. The PBS wash was conducted for 2 min by 5 times and DAB was added and reacted for 5–10 min. PD-L1, PD-1 and CD155 detection was visualized with DAB whereas CD4 and CD8 with AP-red. Slides were counterstained with hematoxylin.

IHC scoring

TILs locating within the borders of the invasive tumor, excluding tumor zones with crush artifacts, necrosis, regressive hyalinization and biopsy site were evaluated by two pathologists to estimate the average level. All mononuclear cells (including lymphocytes and plasma cells) were scored, and polymorphonuclear leukocytes are excluded. The average number of TILs was counted in 10 high-power fields (HPF, $\times 400$) in IHC sections, selected randomly.

Positive CD155 expression was recorded as brown membrane in tumor cells. Positive CD155 tumor cells were defined as complete weak or incomplete strong staining on cell membrane. Positive PD-L1 expression was recorded as brown cytoplasm and/or cytomembrane in immune and tumor cells. Positive PD-1 expression was recorded as brown cytoplasm in lymphocytes. CD4 and CD8 were expressed on the cytomembrane of lymphocytes with the color of red. Double staining of CD4/PD-1 and CD8/PD-1 showed red cytomembrane and brown cytoplasm of lymphocytes. We counted the PD-1, CD4 or CD8 positive cells in 100 TILs and calculated the expression rate.

Statistical analysis

All analyses were conducted with SPSS software (version 17.0). Correlation of age and CD155 expression was analyzed by Wilcoxon rank sum test. Histological grade and TNM stage were analyzed with CD155 expression by Spearman correlation test. The relationship between CD155 expression and molecular subtype was estimated under Chi-square test. Percentage and cell counts of phenotypic CD4⁺ and CD8⁺ effector TILs were analyzed with CD155 expression by Wilcoxon rank sum test. The percentage of tumor and immune cells expressing PD-L1 were analyzed with CD155 expression by Wilcoxon rank sum test. All analyses were two sided and the significance level was 0.05.

Results

Patients' age did not have any relationship with CD155 expression (Table 1). BC patients classified by histological grades and TNM stages had comparable expression of CD155 ($p > 0.05$, Table 1). Molecular subtypes were correlated with CD155 expression that 22% Luminal A BC patients were detected with positive CD155 expression, compared with 73% TNBC patients ($p < 0.05$, Table 1).

CD155 expression was not associated with percentage of CD4⁺ helper TILs (Table 2). However, patients with positive CD155 expression had higher level of CD4⁺/PD-1⁺ TILs and lower CD4⁺/PD-1⁻ TILs ($p < 0.05$, Table 2). CD155 expression was not related with percentage of phenotypic CD8⁺ TILs (Table 2).

The expression of CD155 was related with higher cell counts of CD4⁺ helper TILs (87 vs. 54/HPF, Table 3). The increasing cell counts of exhausted, not unexhausted CD4⁺ helper TILs were related with CD155 expression (47 vs. 20/HPF, Table 3). CD155 expression was associated with a higher cell counts of CD8⁺ TILs and the unexhausted CD8⁺ TILs was increased by 76% among patients with positive CD155 expression ($p < 0.05$, Table 3).

CD155 expression was correlated with higher proportion of immune cells expressing PD-L1 (Fig. 1). The rate of immune cells with PD-L1 expression was 0.02% and 0.8% between patients with negative and positive CD155 expression ($p < 0.05$, Fig. 1A, 1B). PD-L1 expression rates were 0.6% and 0.8% in tumor cells with negative and positive CD155 expression, and no significant relationship was observed.

Discussion

CD155, originally identified as a poliovirus receptor, has similar characteristics of conserved amino acid and domain with the immunoglobulin superfamily[11]. For the similar domain to nectin, CD155 is designated as the fifth member of the nectin-like molecular family, so it is referred to as necl-5[12]. Up-regulated expression of CD155 can promote cell migration and enhances growth factor-induced cell proliferation[13].

CD155 expression was increased in malignant tumor tissues. In this study, CD155 expression was correlated with molecular subtypes of BC, and the positive rate of triple-negative breast cancer is higher than Luminal A. Studies[6, 7] have shown that CD155 is less expressed in normal tissues, but significantly increased in various malignant tumor tissues, and its overexpression was associated with tumor progression and poor prognosis. In addition, plasma soluble CD155 was significantly higher in cancer patients than that in healthy people, and the level in patients of advanced stage was even higher than that in patients of early stage[14]. These studies suggest that CD155 may serve as a biomarker for tumor progression and prognosis.

CD155 expression is reported to be regulated by activating signaling pathways of Raf-MEK-ERK-AP1[15], sonic hedgehog[16], and the TLR4 [17]. Overexpression of CD155 inhibited tumor cell apoptosis through the AKT/ bcl-2 signaling pathway in colon cancer[18]. In addition, DNA damage is one of the important mechanisms to induce CD155 expression. Reactive oxygen species (ROS) or reactive nitrogen species (NOS) can induce the expression of CD155 in multiple myeloma cells[19]. Therefore, CD155 expression in tumor tissues is increased under the influence of multiple factors.

T cell activation is initiated after T cell receptor (TCR) recognition of antigens, and the co-signaling molecules affect T cell activation, subsets differentiation and survival[20]. Co-stimulatory and co-inhibitory receptors determine the functional outcome of TCR signaling[21]. TIGIT, like PD-1 and CTLA-4, is a co-inhibitory receptor that can be expressed by CD4⁺T cells, CD8⁺T cells, natural killer (NK) cells and other immune cell surface[22]. CD155 can regulate the function of immune cells. In this study, patients with CD155 overexpression had higher level of CD4⁺/PD-1⁺ TILs and higher cell counts of CD4⁺, CD8⁺ TILs. The lymphocytes, T-cells, B-cells, macrophages or NK cells, left the vasculature and localized in tumor stroma were called TILs[23]. The immune system, especially TILs in the epithelium, plays a broad role in controlling the growth of virtually all solid tumors[24]. TILs in the microenvironment reportedly affect cancer development, prognosis, and treatment efficacy. The existence of TILs has been determined to be a positive prognostic factor in a number of solid cancers including, but not limited to, colon cancer[25] and BC[26]. Although CD8⁺ or CD4⁺ T lymphocytes have been shown to recognize cancer

antigens and inhibit the development of cancer, but some cancer cells can thwart immune recognition and response[27]. CD155 can interact with its receptors on immune cells to regulate immune function. TIGIT, CD96 and CD226 are common receptors for CD155. When CD155 binds with the co-stimulatory molecule CD226 on the surface of T cells or NK cells, these immune cells are activated to secrete cytokine and kill tumor cells, however, when CD155 interacted with co-inhibitory molecule TIGIT or CD96, the function of immune cells is inhibited[28]. The interaction between CD155 and CD226 down-regulated the expression of CD226 in T cells and NK cells[29]. Contrary to CD226, TIGIT is significantly upregulated on TILs, and its expression parallels with that of other coinhibitory receptors, most notably PD-1[30]. It is now clear that co-signalling molecules have a crucial role in regulating T cell activation, subset differentiation, effector function and survival.

In this study, proportion of immune cells with PD-L1 expression was correlated with CD155 expression score of tumor cells. Studies have confirmed that during the activation process of T cells, IFN- γ molecules will be secreted to up-regulate the expression of PD-L1 on DC cells, and its binding with PD-1 on T cells will generate inhibitory signals and inhibit the proliferation of T cells[31]. Moreover, in tumor tissues, IFN- γ secretion induced by activation of TLR4 signaling pathway induced CD155 expression[17]. The common IFN- γ pathway shared by PD-L1 expression in immune cells and CD155 expression in tumor cells might explain co-high expression.

In this study, although CD155 was observed to be correlated with the molecular phenotype of BC, and there was a significant correlation with TILs and PD-L1, but the mechanism was still unclear. The unclear expression of TIGIT, CD96 and CD226 on TILs cells was the main limitation in this study. The relevant signaling pathways are not discussed in this paper.

Conclusion

CD155 was related with a suppressive immune microenvironment in breast cancer patients. High CD155 expression was associated with high level of exhausted CD4⁺ helper TILs and PD-L1 expression in immune cells. Further studies were warranted.

Abbreviations

TILs
Tumor-infiltrating lymphocytes
PD-L1
Programmed cell death-ligand 1
PD-1
Programmed cell death 1
BC
Breast cancer
TIGIT

T cell Immunoglobulin and ITIM domain
FFPE
Formalin-fixed and paraffin-embedded
IHC
Immunohistochemistry
TCR
T cell receptor
NK
Natural killer

Declarations

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Contributions

YCL, BZ and QKS designed the study. RBW, SZL, KYY, YJZ and JPW performed the experiments. QKS collected and analyzed the data. QZ reviewed the histopathology and IHC results. BZ provided critical comments on the manuscript. All authors read and approved the final manuscript. RBW and QKS drafted the manuscript.

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Ethics declarations

Ethical approval and consent to participate

All procedures performed in this study involving human participants were approved by the ethical committee of Beijing Shijitan Hospital, Capital Medical University, in accordance with the ethical standards of the 1964 Helsinki declaration and its later amendments. This study was under a retrospective study and the formal consent was waived.

Consent for publication

Not applicable.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Competing interests

The authors declare that they have no competing interests.

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Tables

Table 1. Relationship between CD155 expression and pathological characteristics			
	CD155 expression		p
	Negative (n=78)	Positive (n=48)	
Age*, mean±SD	58.2±13.87	57.8±13.26	0.914
Histological grade**, n(%)			0.112
□	11 (15.3)	2 (4.3)	
□	47 (65.3)	32 (69.6)	
□	14 (19.4)	12 (26.1)	
TNM stage**, n(%)			0.662
□	20 (27.0)	10 (21.3)	
□	39 (52.7)	28 (59.6)	
□	12 (16.2)	6 (12.8)	
□	3 (4.1)	3 (6.4)	
Molecular subtype***, n(%)			0.002
Luminal A	49 (77.8)	14 (22.2)	
Luminal B	16 (51.6)	15 (48.4)	
HER2 over-expression	5 (50.0)	5 (50.0)	
Triple negative	3 (27.3)	8 (72.7)	
* Wilcoxon rank sum test, ** Spearman correlation test, ***Chi-square test			

Table 2. Association between CD155 expression and percentage of TILs phenotypes*			
	CD155 expression		p
	Negative (n=78)	Positive (n=48)	
Percentage of CD4 ⁺ TILs, mean±SD	60%±22%	61%±22%	0.788
Percentage of CD4 ⁺ /PD-1 ⁺ TILs, mean±SD	21%±20%	30%±19%	0.004
Percentage of CD4 ⁺ /PD-1 ⁻ TILs, mean±SD	39%±20%	31%±19%	0.032
Percentage of CD8 ⁺ TILs, mean±SD	23%±13%	24%±11%	0.342
Percentage of CD8 ⁺ /PD-1 ⁺ TILs, mean±SD	4%±5%	5%±5%	0.280
Percentage of CD8 ⁺ /PD-1 ⁻ TILs, mean±SD	19%±10%	20%±9%	0.437
* Wilcoxon rank sum test			

Table 3. Association between CD155 expression and cell counts of TILs phenotypes*			
	CD155 expression		p
	Negative (n=78)	Positive (n=48)	
Cell counts of CD4 ⁺ TILs, mean±SD	54±46	87±93	0.041
Cell counts of CD4 ⁺ /PD-1 ⁺ TILs, mean±SD	20±24	47±57	0.002
Cell counts of CD4 ⁺ /PD-1 ⁻ TILs, mean±SD	34±30	41±45	0.658
Cell counts of CD8 ⁺ TILs, mean±SD	21±20	37±44	0.040
Cell counts of CD8 ⁺ /PD-1 ⁺ TILs, mean±SD	4±5	7±13	0.069
Cell counts of CD8 ⁺ /PD-1 ⁻ TILs, mean±SD	17±17	30±35	0.040
* Wilcoxon rank sum test			

Figures

Figure 1

The relationship between PD-L1 expression in immune cells and CD155 expression in tumor cells. A. low expression of PD-L1 among patients with negative CD155; B. higher expression of PD-L1 among patients with positive CD155.