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Association Between Tumor T-Cell Receptor Repertoire and Stromal Tumor-Infiltrating Lymphocytes in Breast Cancer: A Single-Center Observational Cohort Study

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Abstract

Background

Tumor-infiltrating lymphocytes (TILs) are known to correlate with treatment response and prognosis in breast cancer; however, their qualitative evaluation remains insufficient. T-cell receptor (TCR) repertoire analysis may reflect tumor immune responses, and its association with treatment efficacy has been reported in other malignancies. This study investigated the relationship between the tumor TCR repertoire and TILs in breast cancer.

Methods

We enrolled 29 patients aged ≥20 years with primary breast cancer (Stage 0–III) diagnosed at Kawasaki Medical School Hospital between January 2023 and March 2024 who provided consent for peripheral blood and tumor sampling prior to treatment. TCR repertoire analysis was performed on tumor tissue and peripheral blood to calculate diversity and clonality. The primary endpoint was the correlation between the tumor TCR repertoire and stromal TILs. Secondary endpoints included associations between the tumor repertoire and breast cancer subtype, clinical TNM stage, peripheral blood TCR repertoire, age, and BMI.

Results

Tumor TCR diversity was positively correlated with TILs (TRA: ρ=0.564, P=0.003; TRB: ρ=0.636, P<0.0010.0005). Tumor TCR clonality also correlated with TILs for TRA (ρ=0.516, P=0.007) but not for TRB (ρ=0.331, P=0.098). No significant correlation was observed between tumor and peripheral blood repertoires. Tumor TCR were not associated with stage, age, or BMI.

Conclusions

Both TCR diversity and clonality showed significant positive correlations with TIL levels, providing qualitative insights that complement quantitative TIL assessment. These features reveal deeper aspects of tumor immunity, potentially informing more precise personalized therapeutic strategies in breast cancer.

Introduction

Breast cancer remains the most frequently diagnosed malignancy among women worldwide, with a steadily increasing incidence in Japan. Despite significant advances in therapeutic strategies, clinical outcomes remain heterogeneous due to tumor biological complexity and variations in host immune responses. Consequently, identifying biomarkers that more accurately predict prognosis and treatment efficacy is a critical clinical priority.

In particular, the presence of tumor-infiltrating lymphocytes (TILs) has emerged as a robust prognostic and predictive biomarker in specific subtypes, such as triple-negative (TNBC) and HER2-positive breast cancers[1–3]. Based on these findings, TILs are increasingly being applied clinically as a representative histological marker of tumor immune responses[4]. However, the quantitative assessment of TILs has inherent limitations; abundant TILs may include immunosuppressive populations, while sparse TILs may harbor highly potent, tumor-specific activated T cells[5]. Thus, the "quantity" of TILs alone does not necessarily reflect the "quality" or functional repertoire of the antitumor immune response. To address this, a molecular-level analysis of T-cell composition and functional diversity is essential.

T-cell receptor (TCR) repertoire analysis, powered by next-generation sequencing (NGS), enables a comprehensive evaluation of T-cell clonality and diversity[6]. This approach provides deep insights into antigen recognition patterns within the tumor microenvironment (TME) and their relationship with systemic immune status. While associations between TCR diversity and clinical outcomes have been reported in other malignancies, such as melanoma and lung cancer[7, 8], research specifically linking TCR repertoire characteristics to histological TIL levels in breast cancer remains limited[9].

The aim of this study was to characterize the TCR repertoire in breast cancer and evaluate its association with histological TILs. We hypothesized that TCR diversity and clonality would provide distinct immunological information that complements traditional TIL scoring. By examining the relationship between these quantitative (TILs) and qualitative (TCR) markers, we sought to provide a more nuanced understanding of the breast cancer immune microenvironment.

Methods

Study Design and Setting

This single-center observational study was conducted as an exploratory analysis of an ongoing cohort study, "Study on the Relationship between Gut Microbiota and Breast Cancer". The study protocol was approved by the Institutional Review Board of Kawasaki Medical School Hospital (Approval No.5868-00). The analysis included patients enrolled in the parent study between January 1, 2023, and March 31, 2024.

Participants and Eligibility Criteria

Eligible participants were patients aged ≧ 20 years diagnosed with primary breast cancer (pathological Stage 0–III) who provided written informed consent prior to the initiation of treatment. Exclusion criteria included a history of other malignancies, antibiotic use within the previous 6 months, or endocrine therapy within the previous 12 months. From the parent cohort of 65 patients, those with available paired tumor and peripheral blood samples who consented to T-cell receptor (TCR) repertoire analysis were included in the present study. Although medical records were screened for factors potentially influencing immune responses—such as the use of systemic steroids, immunosuppressants, or active autoimmune

diseases—these were not used as exclusion criteria to maintain a representative real-world cohort. As this was an exploratory analysis, no a priori sample size calculation or power analysis was performed.

Sample Collection and TCR Repertoire Analysis

Tumor samples were obtained from surgical specimens. Briefly, a 0.5cm square tumor tissue block was macroscopically excised by the operating surgeon during surgery, and representative tumor areas were subsequently selected by experienced pathologists. Peripheral blood samples were collected preoperatively on the day of admission for surgery. Genomic DNA was extracted from both tumor tissues and blood for analysis. TCR repertoire analysis was performed by Takara Bio Inc. (Japan) using the SMARTer Human TCR α/β Profiling Kit v2 in accordance with the manufacturer's protocol. Repertoire diversity was evaluated using the Chao1 index, and clonality was assessed based on the distribution of unique T-cell clones.

Assessment of TILs and Breast Cancer Subtypes

Stromal tumor-infiltrating lymphocytes (TILs) were evaluated in accordance with the International TIL Working Group guidelines[10]. Based on the distribution within the cohort, patients were dichotomized into TIL < 10% and TIL ≧ 10 groups[11]. Breast cancer subtypes were determined using immunohistochemistry (IHC) and fluorescence in situ hybridization (FISH) following the ASCO/CAP guidelines. Estrogen receptor (ER) and progesterone receptor (PgR) positivity were defined as expression in ≧ 1% of tumor cells. Additionally, the neutrophil-to-lymphocyte ratio (NLR) was calculated using preoperative peripheral blood samples by dividing the absolute neutrophil count by the absolute lymphocyte count.

Statistical Analysis

The primary endpoint was the correlation between tumor TCR repertoire indices (Chao1 index for diversity and unique clone distribution for clonality) and histological stromal TIL levels. Secondary endpoints included the associations between tumor TCR repertoire features and various clinicopathological factors, such as breast cancer subtypes, age, body mass index (BMI), and peripheral blood immune markers including the (NLR).

All statistical analyses were conducted using JMP Student Edition 18 (SAS Institute, Cary, NC, USA). Continuous variables were compared between groups using the Wilcoxon rank-sum test, and associations between continuous variables were evaluated using Spearman's rank correlation coefficient. Statistical significance was defined as a two-sided p-value < 0.05. In accordance with established benchmarks for exploratory biomarker studies, a correlation coefficient (ρ) ≧ 0.4 was considered to indicate a clinically meaningful association[12].

Results

Study Participants

Of the 65 patients enrolled in the parent study, 49 provided consent for repertoire analysis and had samples suitable for processing. Among these, 29 patients with available paired tumor and peripheral blood samples were included in the final analysis. The remaining 20 patients were excluded because tumor TCR repertoire data were unavailable for the following reasons: failure to collect tumor specimens during surgery (n = 11; due to omission of protocol-defined sampling), sequencing library preparation failure (n = 8), and patient death prior to admission, which prevented sample collection (n = 1). While the comprehensive TCR repertoire analysis was conducted for all 29 patients, TIL-related analyses were restricted to 26 patients with invasive carcinoma; three cases of ductal carcinoma in situ (DCIS) were excluded from these specific analyses because TIL scoring is standardized only for invasive components.

The patient characteristics (N = 29) are summarized in Table 1. The median age was 67 years (range, 45–87), and the median body mass index (BMI) was 23.2 kg/m^2 (range, 17.7–31.5). The cohort predominantly consisted of hormone receptor-positive (HR+) and HER2-negative (HER2-) cases (27/29, 93%), with one HER2-positive/HR-negative case. Most patients had early-stage disease, with 65% (19/29) having tumor sizes 2 cm or less and 93% (27/29) showing no lymph node metastasis. Among the 26 cases of invasive carcinoma evaluated for TILs, the median TIL level was 5% (range, 5–70%).

Association between TILs and Tumor TCR Repertoire

The correlation between the tumor TCR repertoire and TILs is shown in Fig. 1. TCR alpha-chain (TRA) diversity in tumor showed a significant positive correlation with TIL levels ($\rho = 0.564$, $p = 0.003$), and TCR beta-chain (TRB) diversity similarly demonstrated a robust positive correlation ($\rho = 0.636$, $p < 0.001$). Furthermore, TRA clonality was positively correlated with TILs ($\rho = 0.516$, $p = 0.007$). In contrast, the correlation between TRB clonality and TILs did not reach statistical significance ($\rho = 0.331$, $p = 0.098$).

When comparing tumor TCR repertoires between the two groups (TIL < 10% vs. TIL ≧ 10%; Fig. 2), both TRA and TRB diversity were significantly higher in the high-TIL group. TRA and TRB clonality also tended to be higher in the high-TIL group. These findings suggest that the diversity and, to a lesser extent, the clonality of the tumor TCR repertoire increase in association with local TIL density.

Secondary Analyses and Peripheral Blood Repertoire

We evaluated the association between the tumor TCR repertoire and the peripheral blood TCR repertoire; however, no significant correlations were found (TRA diversity: $\rho = 0.243$, $p = 0.204$; TRB diversity: $\rho =$

0.188, p = 0.328) (**Supplemental Fig. 1**). Similarly, no significant associations were observed between the tumor TCR repertoire and NLR, clinical stage, or age (**Supplemental Figs. 2–4**).

Notably, BMI showed a significant positive correlation with tumor TRB clonality (ρ = 0.42, p = 0.023) (**Supplemental Fig. 5**).

Discussion

In this study, we analyzed the characteristics of the intratumoral T-cell receptor (TCR) repertoire in breast cancer and its association with histological tumor-infiltrating lymphocytes (TILs), thereby gaining new insights into the qualitative aspects of local tumor immunity. Our analysis revealed that both tumor TCR diversity and clonality exhibited a positive correlation with TILs, with TCR diversity showing a significant association (ρ = 0.564–0.636). In the two-group comparison based on TIL levels, both diversity and clonality were significantly higher in the high-TIL group. Furthermore, the lack of correlation between the tumor and peripheral blood TCR repertoires suggests that a tumor-specific immune response is locally established. These findings suggest that the tumor immune response in breast cancer is not solely defined by the quantitative abundance of TILs, but that qualitative aspects, such as the diversity and clonality of the local T-cell population, are also critical. High TCR diversity reflects the presence of T-cell groups capable of recognizing diverse tumor antigens, suggesting a broad local antigen response[13]. Conversely, increased TCR clonality signifies the expansion of reactive clones against specific antigens, serving as an indicator of the strength of antigen-specific responses[14]. The most noteworthy finding of this study is the simultaneous high values of both TCR diversity and clonality observed in TIL-high cases. Generally, diversity and clonality are often viewed as opposing concepts. However, we observed an unexpected pattern where both measures increased concurrently in the high-TIL group. This phenomenon may reflect a “dual immune activation state” in the breast tumor microenvironment, where both a diverse T-cell response and the concentration of specific clones coexist. Although this dual state has been reported in highly immunogenic tumors like lung cancer[15, 16], its demonstration in hormone receptor-positive breast cancer, which is typically considered less immunogenic, is highly novel. TCR repertoire analysis can thus serve as an effective method for capturing these qualitative differences. Therefore, combining TIL evaluation with TCR repertoire analysis may complementarily capture the qualitative aspects of the tumor immune response. Furthermore, we found no clear correlation between the tumor TCR repertoire and the peripheral blood TCR repertoire or the neutrophil-to-lymphocyte ratio (NLR). These results suggest that the TCR structure in the tumor tissue is independent of systemic circulation, indicating that local immune dynamics are governed by the tumor microenvironment itself[17, 18]. Notably, BMI showed a significant positive correlation specifically with tumor TRB clonality (ρ = 0.42, p = 0.023). While obesity is associated with chronic low-grade inflammation, accumulating evidence suggests an "obesity paradox" in cancer immunotherapy. Obesity has been reported to promote the expansion of specific T-cell clones and increase the expression of exhaustion markers like PD-1, potentially resulting in a more clonally skewed but therapeutically responsive immune profile. Consistent with this, studies in multiple malignancies including melanoma and non-small cell lung cancer have shown that obese patients (BMI ≧ 30) exhibit significantly longer progression-free and overall survival

following PD-1/PD-L1 blockade[19]. Our findings of increased TRB clonality in higher BMI patients may reflect this obesity-related immune remodeling within the breast tumor microenvironment.

Limitations

This study has several limitations. First, this analysis was an exploratory study conducted at a single institution with a relatively small number of cases. The predominance of hormone receptor-positive, HER2-negative cases may limit the generalizability of these findings to more immunogenic subtypes. Second, the TCR repertoire indices used in this study do not directly reflect the antigen specificity or functional status of T cells. Another important limitation is the lack of direct evaluation of clinical outcomes, such as pathological complete response (pCR) after neoadjuvant chemotherapy or recurrence. While TILs have been suggested in various studies to be associated with clinical outcomes, they may not yet serve as direct indicators of clinical benefit in the patient population examined here. Therefore, the relationship between TCR repertoire features and actual clinical outcomes remains unclear in this study.

Conclusion

Our study demonstrates that tumor TCR repertoire diversity and clonality provide essential qualitative information that complements the quantitative assessment of histological TILs in breast cancer. The coexistence of high TCR diversity and clonality in high-TIL cases reveals a complex immune microenvironment, even in less immunogenic hormone receptor-positive subtypes. These findings suggest that integrating TCR repertoire analysis with conventional TIL scoring offers a more nuanced understanding of tumor immunity, potentially contributing to the refinement of personalized therapeutic strategies.

Declarations

Acknowledgments

This manuscript was prepared with the assistance of ChatGPT (version [e.g., 4], OpenAI) for language editing.

Author contributions

NT and TI contributed to the study conception and design. NT, HK, KT, TN, YK, RO, YF, AJ, CK, AS, TI and YN prepared the material preparation and collected the data. TS performed pathological studies of the specimens in this study. YN and TI performed the formal analysis. YN, NT, AS, CK, AJ, YF, RO, YK, TN, KT, DH, HK and TI contributed to the interpretation and discussion of the results. YN wrote the first draft of the manuscript, and all authors commented on the previous versions of the manuscript. All authors read and approved the final manuscript.

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Competing interests

KT: Lecture fee: Eisai, Lilly and Ono. The other authors declare no conflicts of interest associated with this manuscript.

Ethics approval

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. This study was performed in line with the principles of the Declaration of Helsinki. Approval was granted by the Ethics Committee of Kawasaki Medical School (5868-00) and the respective institution, and each participant provided written informed consent.

Consent to participate and publish

Informed consent was obtained from all individual participants included in the study.All authors consent to the publication of this manuscript. The patient informed consent also included a section on consenting to the publication of the patients' anonymized data.

Data availability statement

The datasets generated during and/or analyzed during the current study are not publicly available due to concerns regarding the protection of the personal information of included patients but are available from the corresponding author on reasonable request.

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Figures

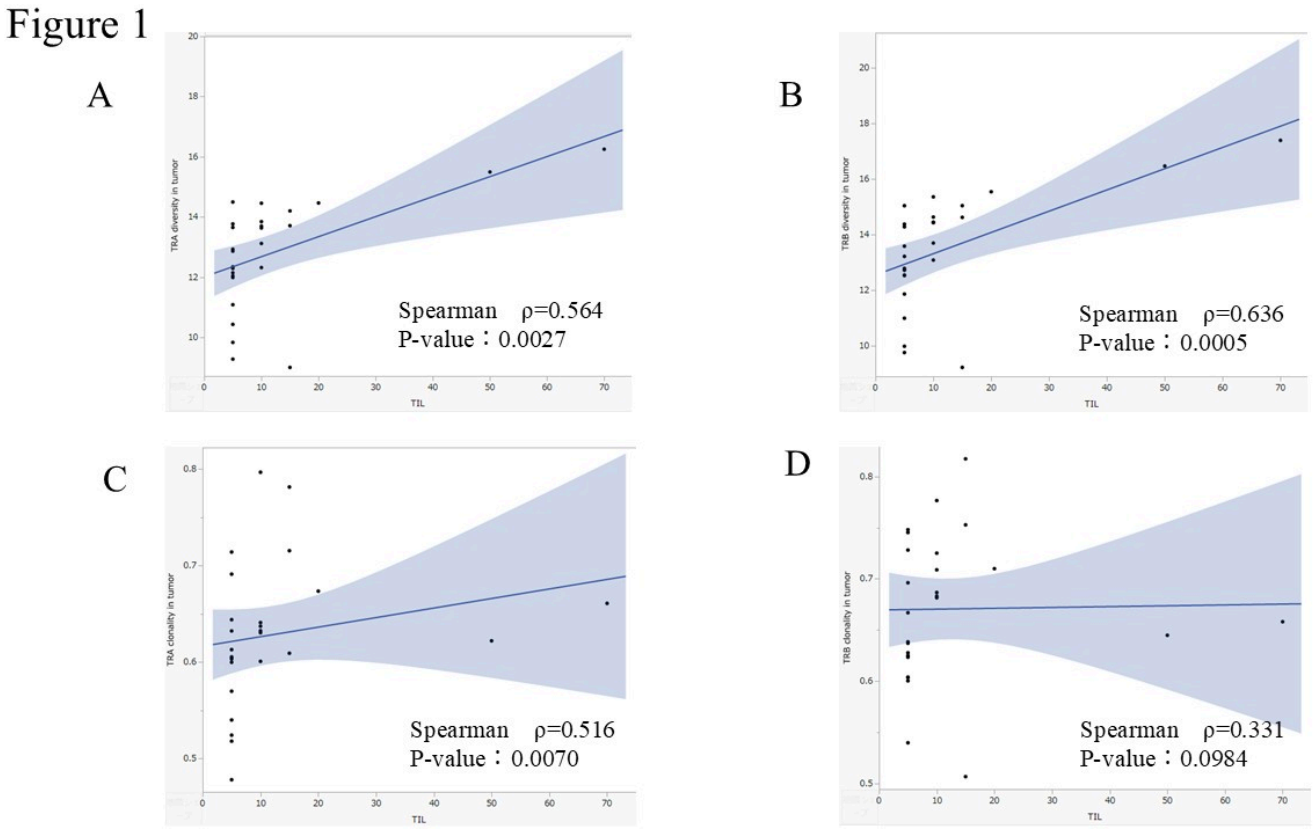


Figure 1

Correlation between tumor TCR repertoire and TILs. Spearman's correlation analysis was performed. (A) Correlation between TRA diversity in tumor and TIL. (B) Correlation between TRB diversity in tumor and TIL. (C) Correlation between TRA clonality in tumor and TIL. (D) Correlation between TRB clonality in tumor and TIL. The blueline represents the regression line, and blue shading indicates the 95% confidence interval. TCR: T-cell receptor; TIL: tumor infiltrating lymphocytes, TRA: T-cell receptor α; TRB: T-cell receptor β.

Figure 2

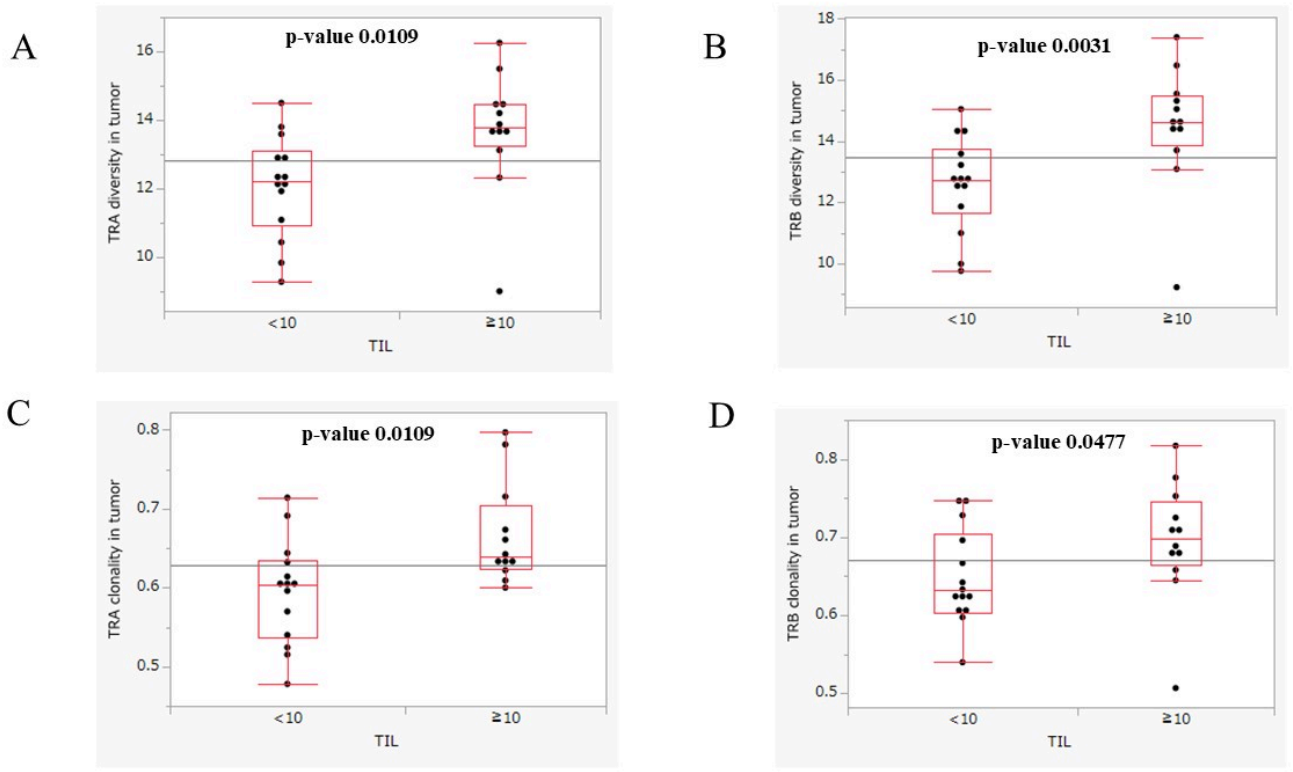


Figure 2

Relationship between TCR repertoire and TIL levels. The Wilcoxon rank-sum test was used to compare the TIL <10% and ≥10% groups. (A) Relationship between TRA diversity in tumor and TIL levels. (B) Relationship between TRB diversity in tumor and TIL levels. (C) Relationship between TRA clonality in tumor and TIL levels. (D) Relationship between TRB clonality in tumor and TIL levels. TCR: T-cell receptor; TIL: tumor infiltrating lymphocytes; TRA: T-cell receptor α; TRB: T-cell receptor β.

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