Analyzing tumor microenvironment difference between TNBC subtypes by meta-analysis of 7 breast cancer scRNAseq studies

Kyungsoo Kim[1]
Min Ji Kim[1], Seung Eun Lee[1], Sohyun Moon[1], Jung Hyun Kim[1], Yoonwon Kook[1], Seung Ho Baek[1], Jee Hung Kim[1], Soong June Bae[1], Sung Gwe Ahn[1] and Joon Jeong[1]
[1] Institute for Breast Cancer Precision Medicine, Yonsei University College of Medicine

Background & objectives

Breast cancer can be classified into several types. Among them, triple-negative breast cancer (TNBC) is breast cancer that does not express estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER2). Since targeted therapy for breast cancer relies on the three receptors, treatment outcomes have been worst in TNBCs. However, the clinical benefit of immune-checkpoint inhibitors (ICIs) with chemotherapy over chemotherapy alone was demonstrated in several trials, opening a new avenue for patients with metastatic PD-L1+ TNBC. After several studies with ICIs, researchers found that the tumor microenvironment affects the response rate of immunotherapies.

Therefore, to unravel the heterogeneity of the tumor microenvironment of TNBC patients, we collected 7 public single cell RNA sequencing breast cancer studies.

Methods

32 TNBC samples from 7 public single cell RNA sequencing breast cancer studies that used a 10X Chromium platform were collected. From 32 TNBC samples, we extracted 141,068 cells which were used in downstream analysis.

Those samples were analyzed to identify Baylor-proposed TNBC molecular subtypes.

The tumor microenvironment was analyzed through Seurat, Monocle2, and CellChat from R to analyze the difference between TNBC subtypes.

Results

From 141,068 cells populating tumors and tumor-microenvironment (TMEs), we first identified epithelial cells separately. Using whole cells including epithelial and immune-stromal cells, we identified the Baylor-proposed TNBC molecular subtype of each patient. This approach with epithelial cells revealed that 19 basal-like immune-activated (BLIA), 5 basal-like immune-suppressed (BLIS), 2 luminal-androgen receptor (LAR), 3 mesenchymal (MES), and 3 unclassified subtypes.

By comparing cell number and cell-cell interactions, the heterogeneity of TME-consisting cell populations between the TNBC subtypes was analyzed. Analysis of tumor-infiltrating lymphocytes revealed their difference in activation, expansion, and exhaustion programs across patients. Among the subtypes, the BLIA subtype had greater amount of exhausted CD8+ T cells and regulatory T cells. This finding was consistent with cell-cell interaction analysis. In the case of the BLIA subtype, the interaction between regulatory T cells and CD8 T cells was more active than in other subtypes. Also, the interaction between cytotoxic cells (T cells and NK cells) and regulatory T cells were more active in the BLIA subtype. Additionally, tumor cells in the BLIA subtype seem to inhibit cytotoxic cells to a greater extent.

Among the interactions between epithelial cells and exhausted CD8+ T cells, MDK interaction was higher in the BLIA subtype. Additionally, we found that the survival of patients differs due to the expression of the MDK gene.

Conclusion

This extensive meta-analysis of public single cell RNA sequencing analysis enhances our knowledge about the heterogeneity and dynamics of the tumor microenvironment. These results offer insights about potential diagnostic and therapeutic targets for TNBC depending on their subtypes.