Interleukin-17-based cytokine signaling as a determinant for tumor immune microenvironment with prognostic value in triple-negative breast cancer

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Abstract

Background: Only a proportion of triple-negative breast cancer (TNBC) is immunotherapy-responsive. We hypothesized that the tumor microenvironment (TME) might influence the outcomes of TNBC and investigated the relevant signaling pathways.

Methods: RNA-seq data of 115 TNBC samples and 112 normal adjacent tissues were retrieved for ESTIMATE, CIBERSORTx, X2K, KEGG, and GSVA analyses. The immune score (IS) and stromal score (SS) were calculated and correlated with the overall survival (OS) in TNBC. Finally, we validated the altered transcription factor (TF) genes in the cBioPortal.

Results: The SS is a good predictor of the OS (better survival in SS-low patients; P = 0.0081). In line with these results, when compared with IS-low/SS-high patients, IS-high/SS-low patients showed a better OS (P = 0.045). Moreover, macrophages were polarized toward the M2 phenotypes in IS-low/SS-high patients (P < 0.001). Compared with IS-low/SS-high patients, CIBERSORTx also showed that IS-high/SS-low patients had an increased number of memory B cells, CD8+ T cells (14.8% vs. 3.7%, p = 0.0286), activated CD4+ memory T cells, follicular helper T cells, and activated NK cells in the TME; additionally, fewer resting NK cells were detected in the TME (P = 0.0108). Additionally, there were 284 upregulated and 367 downregulated DEGs (Differentially Expressed Genes) in IS-high/SS-low, and 187 upregulated and 183 downregulated DEGs in IS-low/SS-high patients. KEGG analysis further revealed that the DEGs were enriched in the IL-17 and cytokine-cytokine receptor interaction pathways. Of note, as per the cBioPortal platform, we discovered that 13% of ER-negative, HER2-unamplified BC patients harbored IL17RA deep deletions and 25% harbored TRAF3IP2 amplifications; interestingly, the nine altered TF genes were associated with significantly worse relapse-free survival and OS, in the context of 2,377 and 4,819 BC patients, respectively.

Conclusions: The TME with different immune cell components influences the survival of TNBC patients. IS-high/SS-low patients show a better overall survival. Further studies are required to examine whether an immune/stromal state also predicts the response to immunotherapy.

Introduction

In 2020, the global incidence of breast cancer was 2,261,419 diagnosed across 185 countries, accounting for 11.7% of all cancer types [1]. Triple-negative breast cancer (TNBC) is characterized as estrogen receptor-negative, progesterone receptor-negative, and a lack of HER2 expression or amplification and accounts for approximately 15%–20% of all breast cancers [2, 3]. TNBCs are heterogeneous (in terms of genomics, transcriptomics, and histopathology) and demonstrate the heterogeneity of response to anti-programmed death-1/ligand-1 (anti-PD-1/PD-L1) checkpoint inhibition immunotherapy [3-5].

In fact, recent studies by Schmid et al. revealed that PD-1 or PD-L1 checkpoint blockade immunotherapy combined with neoadjuvant chemotherapy (NAC) improved the pathological complete response (pCR) rate in the neoadjuvant setting; therefore, they proposed such an approach would probably improve the

progression-free survival (PFS) of patients with advanced or metastatic TNBC staining positive for PD-L1 in tumor-infiltrating immune cells, if used as the first-line therapy [6-8]. In contrast, other tumors are weakly immunogenic depending on the composition of the infiltrating immune cell populations and extrinsic factors (e.g. different metabolites or specific cytokines) enriched in the immune tumor microenvironment (TME). Of note, recent bioinformatics research, based on bulk tumor gene expression data demonstrated that a low abundance of regulatory CD4+ T cells (Treg) was significantly associated with an increased pCR rate in TNBC patients after NAC [9].

In a TNBC surgical specimen, untreated tumor cells typically represent approximately 60% of the cellular component, lymphoid and immune cells account for 20%, and stromal cells such as fibroblasts, histiocytes, endothelial cells, myofibroblasts, and adipocytes represent the remaining 20% [10]. The primary function of stromal cells is to establish an immune response. The TME creates a chemokine-rich milieu inside, promoting the encounter between the boundary tumor cells and a variety of surrounding infiltrating immune cells in addition to cancer-associated fibroblasts, neo-vessels, neo-neurites, and other supportive tissues [11]. Prior research has demonstrated that the prognosis of TNBC in terms of disease-free survival and disease-specific survival is worse in the basal-like immunosuppressed subtype and fare better in the basal-like immune-activated subtype, indicating that the immune TME plays a crucial role in the formation of either a tumor-permissive or tumor-expulsive milieu [4]. A clinicopathological study also demonstrated that TNBC with a high number of tumor-infiltrating CD56-positive natural killer (NK) cells was associated with a more favorable disease-free survival [12]. Additionally, research focusing on cancer-associated chemokines revealed that NK cells expressing abundant CXCR3 (also known as GPR9 and CD183) molecules on the cell surface are recruited by the chemokines CXCL9, CXCL10, or CXCL11 secreted by immune cells or stromal cells in the TME [13]. However, it is still unclear what driving force explains the immunogenic or immunosuppressive phenotype in the TME in patients with TNBC. Hence, here, we aimed to investigate which infiltrating immune cell populations would be associated with the immune phenotype, and which signaling pathways could determine a subgroup of TNBC that is strongly immunogenic, thus, having a better prognosis. Lastly, we also aimed to determine whether transcription factor signatures derived from the RNA-seq data have prognostic value.

Methods

Ethics statement and study design

This human data-based research study leveraged multiple publicly accessible RNA-seq datasets containing only mature, anonymous, and de-identified genetic and demographic data. The Institute Review Board of Kuang Tien General Hospital approved the study with a Certificate of Approval numbered KTGH-10458. This study was performed in accordance with the Declaration of Helsinki.

The schematic diagram (Fig. 1) shows the study design and methodology adopted in this study. First, we downloaded from TCGA, a breast cancer gene expression RNA-seq dataset (TCGA-BRCA). Using this

dataset, we sorted 115 patients with TNBC, characterized by the lack of estrogen receptors, progesterone receptors, and HER2 non-overexpression or HER2-FISH un-amplification.

Estimation of the immune/stromal scores

As mentioned above, the gene expression data and corresponding clinical information from a total of 1222 cases were retrieved from a publically available dataset (TCGA-BRCA) [14]. After extracting the ER, PgR, and HER2 information of each sample, a total of 115 TNBC cases, 112 normal cases, and 994 non-TNBC cases were identified. To predict TNBC purity, we applied the Estimation of STromal and Immune cells in MAlignant Tumor tissues using Expression data (ESTIMATE) algorithm to the normalized expression matrix to determine the immune/stromal scores of each TNBC patients. The immune score (IS; derived from the immune signature of 141 genes) and stromal score (SS; derived from the stromal signature of 141 genes) were calculated using the r-ESTIMATE package in R [15]. This algorithm rank-normalizes and rank-orders a set of gene expression values in a given sample and calculates the empirical cumulative distribution function from the signature gene set of the remaining genes; therefore, ESTIMATE can calculate the SS and IS from the RNA-seq data. The algorithm also allows the combination of these scores into an estimate score, used to infer tumor purity, as per the following formula: tumor purity = cos (0.6049872018 + 0.0001467884 × estimate score) [15].

Correlation analyses

The overall survival (primary prognosis endpoint-based) was estimated using Kaplan-Meier survival analysis. Based on the calculated SS and IS, the corresponding patients were classified into two groups, and their prognoses were individually examined. A previously published method (maximally selected rank statistics), was employed for optimal cutoff identification using the survival in R to explore the relationship between the overall survival and the two groups of TNBC cases.

CIBERSORT analysis

We used CIBERSORT X (https://cibersort.stanford.edu) to compare the proportion of infiltrating immune cells in the TME of IS-low/SS-high and IS-high/SS-low TNBC patients [16, 17]. The CIBERSORT accurately allows the evaluation of the relative levels of 22 immune cell phenotypes; such and analysis was performed using the immunedeconv package in R [18]. The immune cell fraction level divided by the cutoff value was 0 or 1 in the subsequent scoring formula.

Differential expression analysis

According to the ESTIMATE results, the intersection genes were selected based on the stromal/immune scores. The limma package in R was used to screen DEGs between normal and TNBC samples [19]. Genes with a p-value ≤ 0.0001 and absolute log2 fold-change ≥ 4 were considered to be differentially expressed and extracted further for network construction. Heatmaps were generated using the pheatmap package in R [20]. The generated heatmaps and volcano plots show the differentially expressed genes in either the IS-high/SS-low or IS-low/SS-high TNBC groups.

Gene Ontology and KEGG Pathway Enrichment Analyses

Using these DEGs defined as above for the two TNBC groups, we investigated the enriched signaling pathways based on GO terms and KEGG pathways. Functional enrichment analyses of GO terms, including cellular components, molecular functions, and biological processes, and KEGG pathways were performed using the clusterProfiler package in R [21]. The functions among genes of interest were adjusted with a cutoff criterion of $p < 0.05$.

eXpression2Kinases analysis

We utilized eXpression2Kinases (X2K), as reported elsewhere, to disclose the potential enrichment of transcription factors [22, 23].

Gene set variation analysis

Gene set variation analysis (GSVA) is a bioinformatics framework that organizes gene expression data in the form of a pathway or signature summary [24]. GSVA is a popular pathway-related immune infiltration and tumor mutational burden immune-related analysis. Here, we leveraged GSVA to provide an accurate definition of pathway enrichment between samples from different groups. We used the GSVA package in R to evaluate the t score and assigned the pathway activity conditions. We then used the pheatmap package in R to display the distinctions in pathway activation between normal tissues and those of IS-high/SS-low and IS-low/SS-high patients.

cBioPortal for cancer genomics

Finally, we validated our findings in a different, more extensive dataset of breast cancer via cBioPortal analysis [25, 26].

Results

From the TCGA Breast Cancer dataset, gene expression data for 115 patients with triple-negative breast cancer and 112 normal adjacent tissues were retrieved. Fig. 1 demonstrates the study design, flow, and methodology used in the current study. In this cohort of 115 TNBC patients, favorable overall survival as per the Kaplan-Meier curves was significantly correlated with an earlier disease stage ($P = 0.0012$; Fig. 2).

The ESTIMATE algorithm computationally estimates the fraction of stromal and immune cells in the TME. The SS was high (SS-high) in 10 patients, and the IS was high (IS-high) in the ten other patients. There were no significant correlations between the SS or IS and the cancer stage (Suppl. Fig. 1). However, as per the Kaplan-Meier analyses, SS-low patients showed a higher overall survival (OS) than that of SS-high patients (P = 0.0081; Suppl. Fig. 2), while IS-high patients showed a higher OS than that of IS-low patients (P = 0.2; too few cases in the IS-high; Suppl. Fig. 3). Of note, a strong correlation between both the SS and IS and the patients' overall prognosis was observed. Expectedly, when compared with IS-low/SS-high patients, IS-high/SS-low patients showed a better OS (P = 0.045) (Fig. 3). Cytoscape

revealed the immune cell infiltration levels between samples grouped by IS-high/SS-low or IS-low/SS-high (Suppl. Fig. 4). Interestingly, grouped linear regression showed a statistically significant increase in M2 macrophages in TNBC patients with the IS-high/SS-low phenotype. In line with these results, in the tumor microenvironment (TME), a higher proportion of M2 was also found in IS-low/SS-high patients, as estimated by CYBERSORTx ($P < 0.001$) (Suppl. Fig. 5). Additionally, significantly more CD8+ cytotoxic T-cells, memory B cells ($P = 0.0304$), activated CD4+ memory T cells ($P = 0.0056$), follicular helper T cells ($P = 0.0044$), and activated NK cells ($P = 0.0511$) were also observed in IS-high/SS-low patients (14% vs. 5.3%, $p = 0.0143$) (Table 1). On the other hand, more resting NK cells were observed in IS-low/SS-high patients ($P = 0.0108$).

Moreover, DEG analysis showed 651 DEGs (284 upregulated, and 367 downregulated) in IS-high/SS-low patients, and 370 DEGs (187 upregulated, and 183 downregulated) in IS-low/SS-high patients (Suppl. Fig. 6). Heatmaps and volcano plots of the DEGs in the context of these two groups are shown in Fig. 4A-D. KEGG pathway enrichment analysis showed that the overexpressed DEGs from both phenotypes were enriched in the IL-17 signaling pathway and viral protein interaction cytokine and cytokine receptor genes. Notably, the overexpressed DEGs in the context of the SS-low/IS-high phenotype were also enriched in other two cytokine-related pathways: cytokine-cytokine receptor interactions pathway and chemokine signaling pathway (Fig. 4E).

Using X2K, we also inferred the transcription factors associated with the two immune phenotypes. In SS-high/IS-low TNBC patients, the inferred TFs were PPARG, HNF4A (also known as farnesoid X receptor, FXR), NR1H4, NR0B2, and MLXIPL, whereas the TF signature of SS-low/IS-high TNBC patients was composed of the PPARG, CEBPA, and MLXIPL TFs (Fig. 5). Of note, the OncoScore analysis did not perform as well in the search for the TF signatures (Suppl. Table 1). Additionally, we performed a manual search on PubMed and discovered nine TF genes linked to IL-17-mediated signaling, including PPARG, CEBPA, MEOX1, KLF15, CD36, ZNF750, EZH2, HNF4A, and NR0B2 (Table 2).

Importantly, GSVA suggested the involvement of additional pathways, including the JAK-STAT signaling, T cell receptor signaling, B cell receptor signaling, cytokine-cytokine receptor interaction, TGF-β signaling, and PPAR signaling pathways (Fig. 6; the upper panel shows the GSVA of the 370 DEGs of the SS-high/IS-low subgroup and the lower panel shows the GSVA of the 651 DEGs of the SS-low/IS-high subgroup).

Validation of the results in the cBioPortal

IL-17 is a proinflammatory cytokine that signals mainly via the TRAF3 Interacting protein 2 (TRAF3IP2), as reported previously [27, 28]. TRAF3IP2 is an inflammatory mediator and upstream regulator of several crucial transcription factors, such as AP-1 and NF-κB [28]. Act1, an essential component in IL-17 signaling complex, is encoded by the gene TRAF3IP2. Finally, we used the Oncoprint from cBioPortal to validate the frequency of IL-17 genes in TNBC. We discovered that 13% of ER-negative and HER2-FISH unamplified breast cancers harbored IL17RA deep deletions and 25% harbored TRAF3IP2 amplifications (Suppl. Fig 7).

Interestingly, we also discovered using the cBioPortal platform that aberrations in the nine TF genes mentioned above are associated with a worse prognosis, as per the relapse-free survival of 2,377 patients (log-rank, P = 0.00007) and the overall survival of a larger group of 4,819 patients (log-rank, P = 0.001697) (Fig. 7).

Discussion

Several recent studies have shed light on the pivotal role of the TME in the shaping of the tumor behavior in TNBC [29-34]. Given the paucity of studies investigating the mechanism behind the strikingly different prognoses of immunosuppressive or immunogenic TNBCs, our study aimed to explore the differences in stromal cells (particularly immune cells) within the TME, as well as in the enriched DEGs in the search for the driving force behind the formation of these two different phenotypes. Interestingly, we discovered that the composition of immune cells reacting to TNBC tumor cells was strikingly different in the highly immunogenic-weak stromal TME and the strong stromal-weak immunogenic TME. Additionally, we discovered that the immunosuppressive type (SS-high/IS-low) showed gene signatures enriched in the IL-17, and viral-cytokine and cytokine receptor interactions' pathways. Of note, this study is unique considering the demonstration that altered TF genes derived from IL-17-mediated signaling showed strong significance with respect to both the relapse-free and overall survival, as per the analysis of another extensive dataset.

More than 1,600 known (or likely) human TF genes represent approximately 8% of the human genes [35]. Mutations in TF genes are often highly deleterious in humans [35]. Nine TF genes, *PPARG, CEBPA, MEOX1, CD36, ZNF750, KLF15, EZH2, HNF4A,* and *NR0B2,* were enriched in our bioinformatics analysis. Importantly, this is the first time that the alteration in one or more of the nine TF in the context of TNBC was linked to the IL-17 signaling pathway and associated with significantly poorer prognosis in terms of relapse-free survival and overall survival in a large number of breast cancer patients, excluding ER-positive and HER2-amplified cases.

A plethora of studies have revealed increased IL-17A levels in ER-negative or triple-negative breast cancer [36]. In fact, the upregulation of IL-17A signaling is associated with increased expression of programmed cell death protein 1 (PD-1) and programmed death-ligand 1 (PD-L1) in breast cancer with low ER expression, which may elevate the infiltration of CD8+ T cells in the tumor tissues [36]. Of note, CD4+ T cells among tumor-infiltrating lymphocytes in the TME are the main source of IL-17 [37, 38]. IL-17-mediated downstream signaling plays a critical role in the TME, inducing the expression of different genes either to switch on pro-tumor effector cytokines or to inhibit tumor growth in a context- and system-dependent manner [37]. For example, IL-17 induces the production of IL-6, which in turn induces STAT3 (signal transducer and activator of transcription 3) [37]; in fact, IL-6 orchestrates both the increase in the recruitment of suppressive tumor-associated myeloid cells and impacts their ability to inhibit anti-tumor T-cell responses [39]. Early on, a recent study uncovered how an IL-17–mediated paracrine network could recruit immature myeloid cells into the TME, causing tumor progression; this study demonstrated that through NF-κB and ERK signaling, tumor-infiltrating T helper type 17 (Th17) cells and IL-17 would induce

granulocyte colony-stimulating factor expression, which is crucial for the mobilization of immature myeloid cells into the TME [30]. In contrast, Bianchini et al. demonstrated that a subset of highly proliferative, ER-negative breast cancers with high expression of a B-cell/plasma cell stromal metagene corresponding to immune functions and extracellular matrix components were associated with a favorable prognosis [10]. These studies lend support to the composition of cellular constituents in the TME, and their association with the patient's prognosis.

Still regarding the TF genes associated with TNBC, a previous study suggested that EZH2 plays decisive roles in immune cells (e.g., T cells, NK cells, dendritic cells, and macrophages) in the tumor microenvironment [40]. Liu and colleagues demonstrated a novel function of PPARγ in lymphocyte trafficking and the cross-talk between Th17 and B cells [41].

Additionally, investigators from the Institut Curie and INSERM found that a high Th17 metagene was associated with a good prognosis in T cell non-inflamed type TNBC, suggesting Th17 is a novel prognostic composite biomarker. Altogether, these studies clearly support the notion that integrating immune cells and tumor molecular diversity is an efficient strategy for the prognostic stratification of cancer patients [42].

The purpose of the current research was neither to develop a predictive molecular panel biomarker, nor to identify innate resistance *versus* sensitive phenotypes to checkpoint inhibitor immunotherapy; we aimed to investigate the factors behind the TME's immune-stromal state and to look for the systemic functions of the involved molecular signaling pathways. However, this study is not without limitations. One of them is the available low number of cases used to separate tumors into two extreme immune-stromal phenotypes.

Conclusions

Overall, our data suggest that the disclosure of the distinct molecular anatomy of the TME in patients with TNBC (not of the cancer cells *per se*) will assist in the determination of the ultimate tumor behavior. Of note, the results of this integrated *in silico* study can only be generalized to approximately 17% of patients with TNBC, in which infiltrating stromal cells and immune cells play a determinant prognostic role. Remarkably, our data suggest that the determining factor for the differences in the tumor immune microenvironment (immunogenic versus immunosuppressive) can be explained by IL-17 signaling and its paracrine network.

Declarations

Ethics approval and consent to participate

This human data research leveraged multiple publicly accessible RNA-seq datasets containing only mature, anonymous, and de-identified genetic and demographic data. The Institute Review Board of

Kuang Tien General Hospital approved the study with a Certificate of Approval numbered KTGH-10458. This study was performed in accordance with the Declaration of Helsinki.

Consent for publication

Not applicable

Availability of data and materials

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

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

VCK conceived the study. VCK and CCW designed the study. SHL and DLC performed the experiments. All authors analyzed and interpreted the results, and VCK was the major contributor to the writing of the manuscript. All authors read and approved the final manuscript.

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Tables

Table 1. Comparison of the immune cell profiles in the tumor microenvironment (TME) of IS-high/SS-low and IS-low/SS-high triple-negative breast cancer patients, estimated using CYBERSORTx.

Immune cell type in the TME	Immune score-high/stromal score-low TNBC (n = 10)	Immune score-low/stromal score-high TNBC (n = 10)	*P*-value*
naive B cells	0.0363 ± 0.0532	0.0258 ± 0.0263	0.9288
memory B cells	0.0168 ± 0.0121	0.0061 ± 0.0153	**0.0304**
plasma cells	0.0162 ± 0.0376	0.0148 ± 0.0397	0.7599
CD8+ T cells	0.1403 ± 0.0934	0.0531 ± 0.0616	**0.0143**
naive CD4+ T cells	0	0	-
resting CD4+ memory T cells	0.0956 ± 0.0557	0.1604 ± 0.1036	0.2176
activated CD4+ memory T cells	0.0441 ± 0.0316	0.0176 ± 0.0159	**0.0056**
follicular helper T cells	0.0700 ± 0.0235	0.0379 ± 0.0305	**0.0044**
regulatory T cells	0.0517 ± 0.0409	0.0271 ± 0.0219	0.8767
gamma delta T cells	0.0421 ± 0.0361	0.0259 ± 0.0268	0.2454
resting NK cells	0	0.0199 ± 0.0395	**0.0108**
activated NK cells	0.0360 ± 0.0257	0.0178 ± 0.0256	**0.0511**
Monocytes	0.0009 ± 0.0030	0.0164 ± 0.0428	0.2105
M0 macrophages	0.1196 ± 0.0540	0.1979 ± 0.1377	0.2799
M1 macrophages	0.1497 ± 0.0789	0.1357 ± 0.0670	0.8534
M2 macrophages	0.1043 ± 0.0628	0.1534 ± 0.0939	0.1431
resting dendritic cells	0.0395 ± 0.0663	0.0211 ± 0.0178	0.5889
activated dendritic cells	0.0001 ± 0.0004	0.0008 ± 0.0026	0.5
resting mast cells	0.0344 ± 0.0214	0.0681 ± 0.0466	0.123
activated mast cells	0	0	-
eosinophils	0.0008 ± 0.0028	0	> 0.9999
neutrophils	0.0013 ± 0.0032	0.0002 ± 0.0006	0.582

(%) Mean±SD; *using Mann-Whitney U test.

CYBERSORTx: https://cibersortx.stanford.edu/index.php

Table 2. Selection of the transcription factors linked to interleukin-17-mediated signaling.		
Transcription Factor (TF)	Investigated Relationship	PMID
PPARG	PPARγ-induced SOCS3 expression prevents IL-17-mediated cancer growth.	23619236
CEBPA (=C/EBPα)	Transcription factor that coordinates proliferation arrest and the differentiation of myeloid progenitors, adipocytes, hepatocytes, and cells of the lung and the placenta. IL-17 suppresses expression of several pro-adipogenic TFs, including PPARγ and CEBPA.	23332504 11242107
MEOX1	TGF-β1 transcriptionally regulates MEOX1 expression via Smad2/3 in adult human dermal fibroblasts, thus promoting cell migration.	32241049
KLF15	Specifically, IL-17 suppresses KLF15, a pro-adipogenic TF, and enhances expression of KLF2 and KLF3, which are anti-adipogenic. Thus, IL-17 suppresses adipogenesis at least in part through the combined effects of TFs that regulate adipocyte differentiation.	23332504
CD36	In the presence of palmitic acid (PA), IL-17a could directly increase the cellular uptake of PA, leading to the proliferation of ovarian cancer cells via the IL-17a/IL-17RA/p-STAT3/FABP4 axis rather than via CD36.	31802182
ZNF750	ZNF750 is the p63 target gene. The levels of inflammatory cytokines (IL17d and Tnfsf15) were significantly reduced by Rbm38 deficiency in senescence-resistant Rbm38-/-;TAp63+/- mouse livers and MEFs. Rbm38 and p63 function as intergenic suppressors in aging and tumorigenesis.	29520104 24441097
EZH2	EZH2 positively regulate the expression of IL-17a and IL-17f. The inducible binding of EZH2 at the IL-17a promoter was dependent on signaling pathways downstream of the TCR. IL-17f bears 50% homology to IL-17a and has recently been suggested to play a role in inflammation.	21674483 31850514
HNF4A (= farnesoid X receptor, FXR)	In addition to the classical Jak-Stat antiviral signaling pathway, IFN-λ1 inhibits hepatitis C virus replication through the suppression of miRNA-122 transcription via an inflammatory Stat 3-HNF4A feedback loop. Inflammatory feedback circuits activated by IFNs during chronic inflammation expose non-responders to the risk of hepatocellular carcinoma.	26657215
NR0B2 (= SHP)	This TF is a tumor suppressor. Both FXR (-/-) and NR0B2(-/-) mice develop spontaneous hepatocellular carcinoma. Upregulated NR0B2 will regulate the IL-6-dependent pathway.	23811326 22349108

Figures

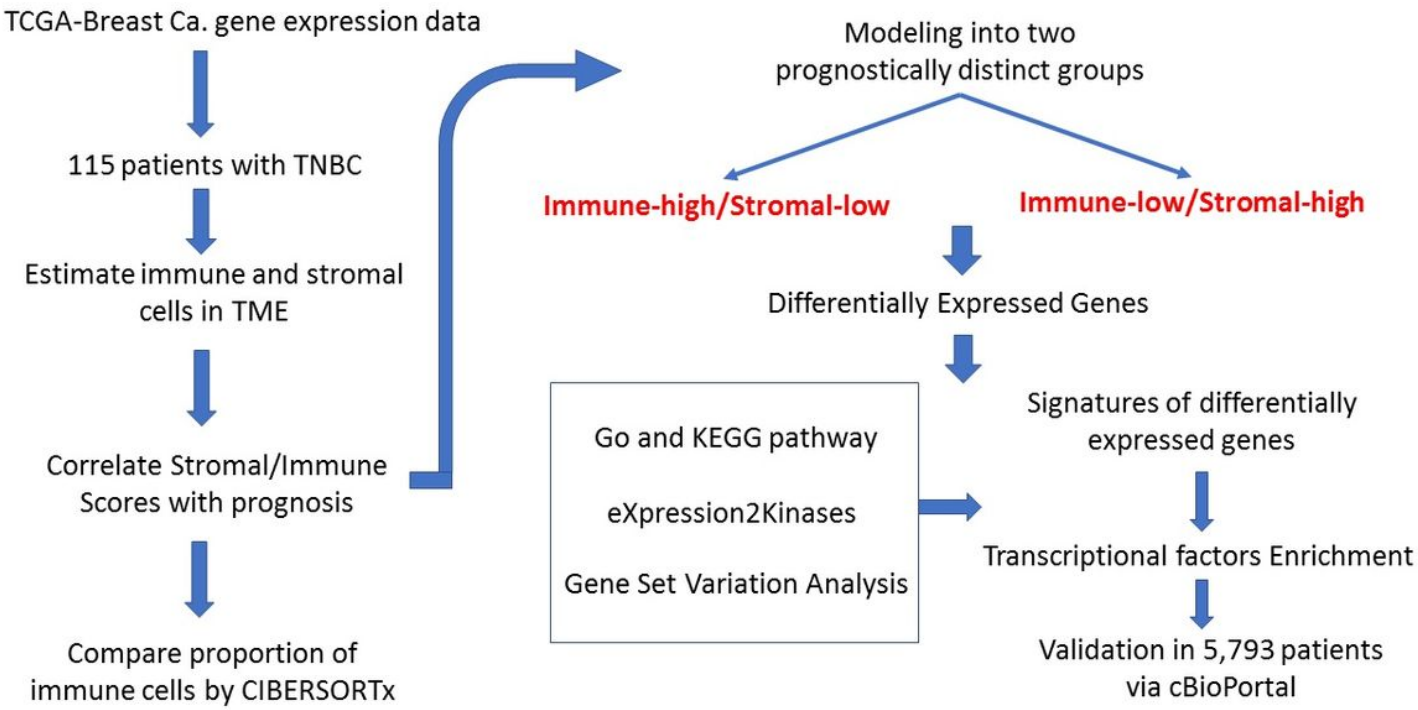


Figure 1

Study flow diagram. The schematic diagram represents the study design and methodology adopted.

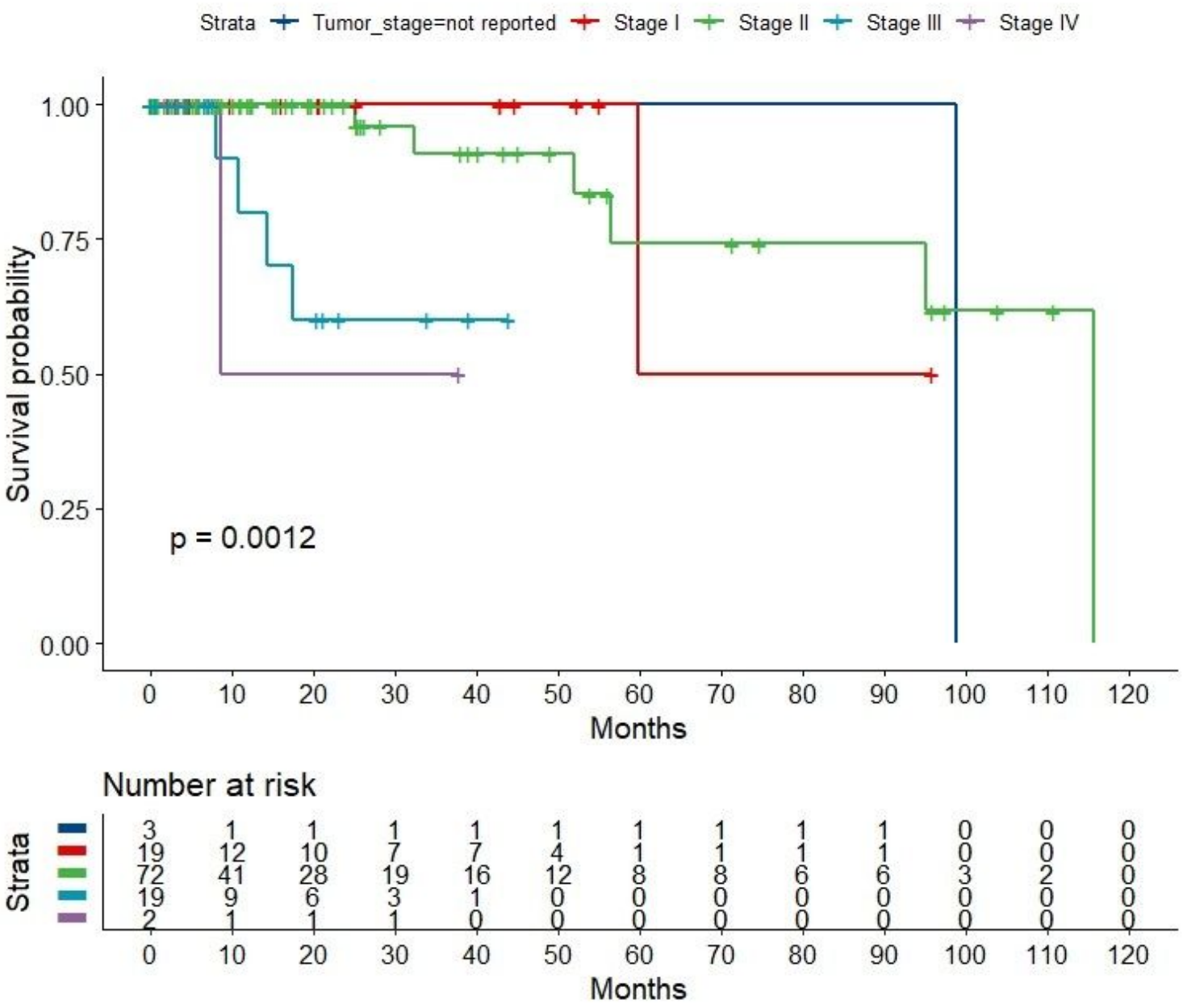


Figure 2

Overall survival of 115 patients with triple-negative breast cancer stratified by cancer stage at diagnosis. Kaplan-Meier curves referring to different overall TNBC stages. The higher stage correlates significantly with poorer survival (P = 0.0012).

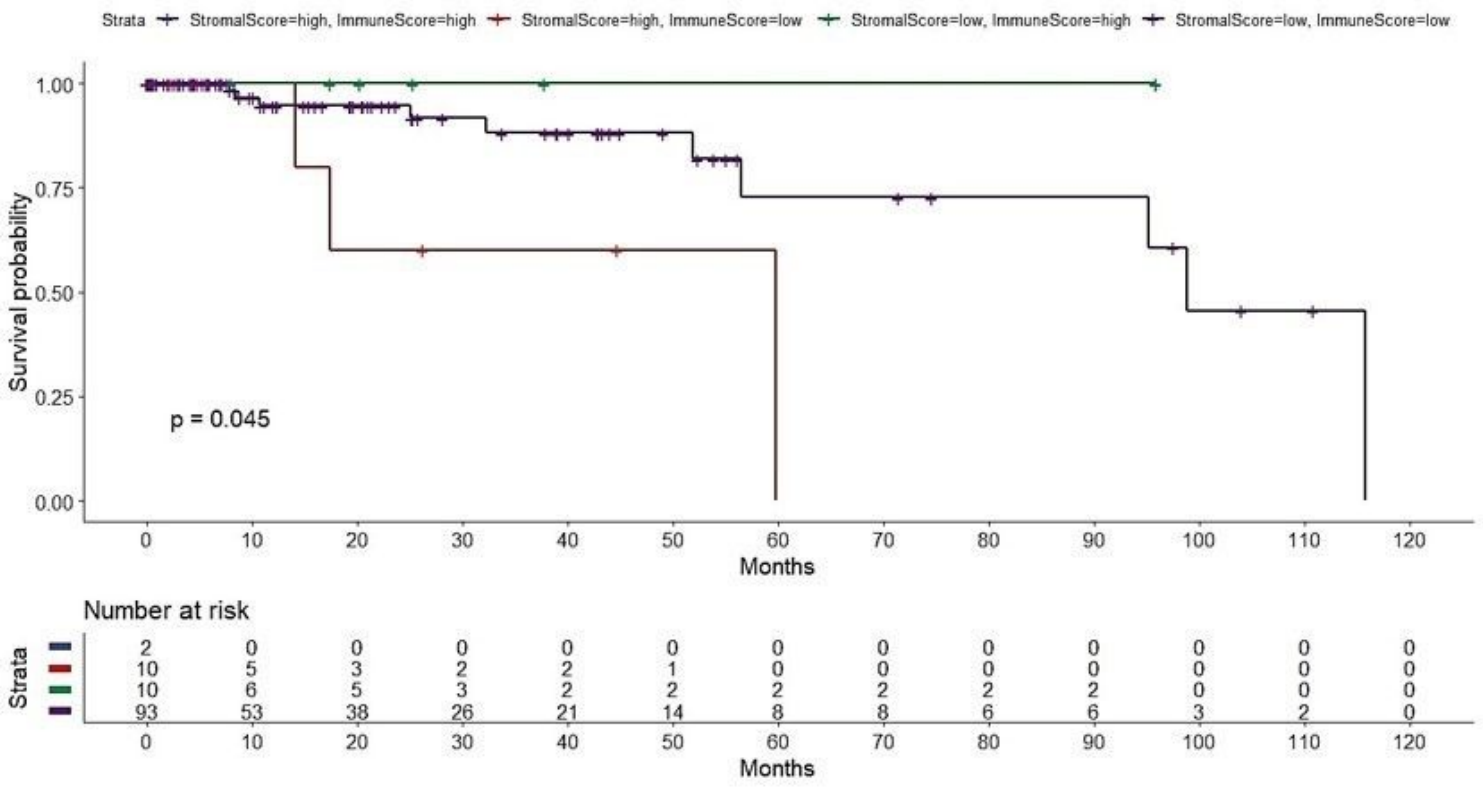


Figure 3

Overall survival of 115 patients with triple-negative breast cancer stratified by the combination of the Stromal Score and Immune Score. Both scores were inferred by the ESTIMATE gene expression signatures. SS-low/IS-high patients were associated with excellent overall survival, whereas SS-high/IS-low patients showed the worst overall survival.

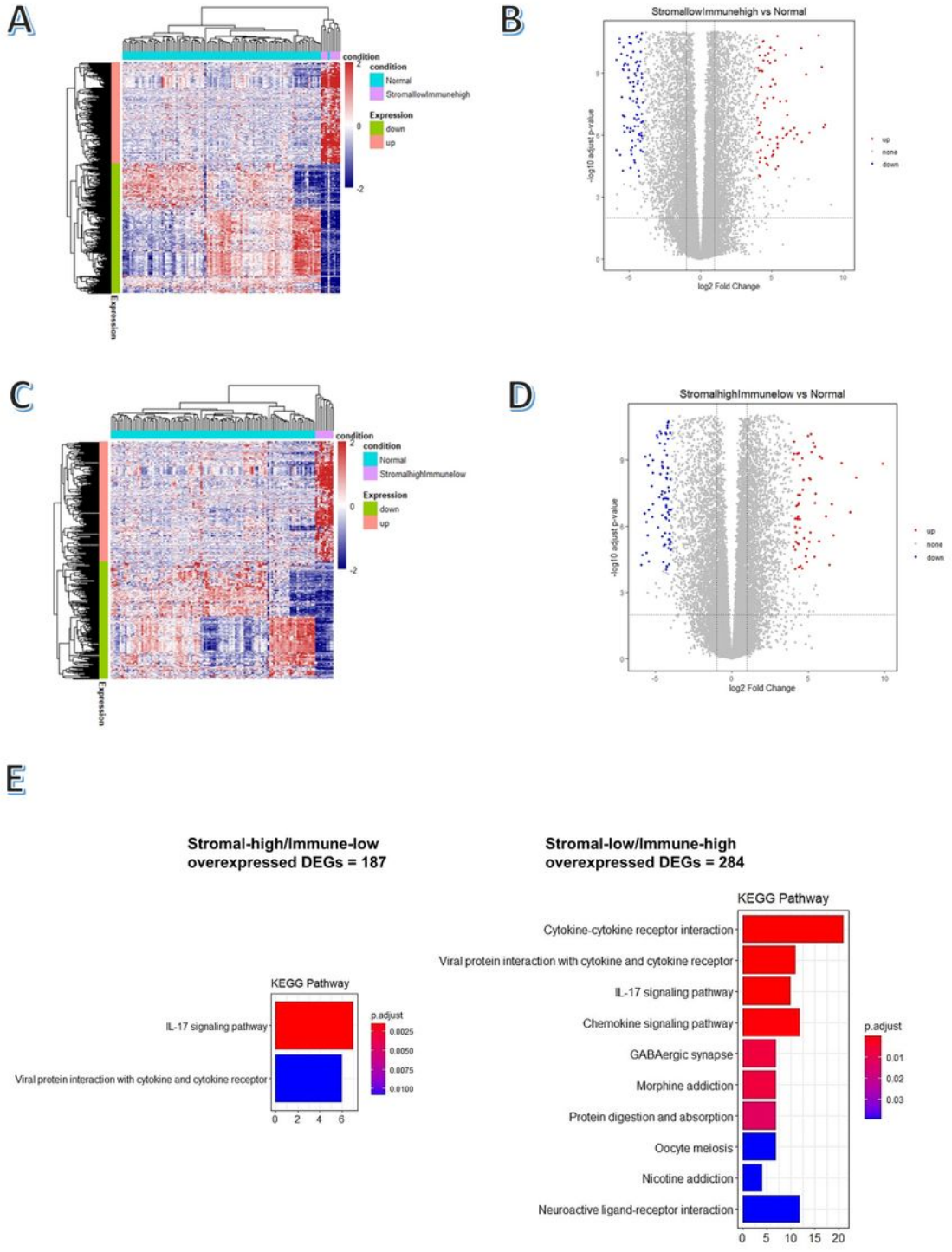


Figure 4

Heatmaps and volcano plots of the differentially expressed genes in SS-low/IS-high (A, B) and SS-high/IS-low (C, D) TNBC patients, and the corresponding KEGG pathway analysis (E). The overexpressed DEGs in the context of both phenotypes are enriched in the IL-17 and cytokine-cytokine receptor interactions' signaling pathways.

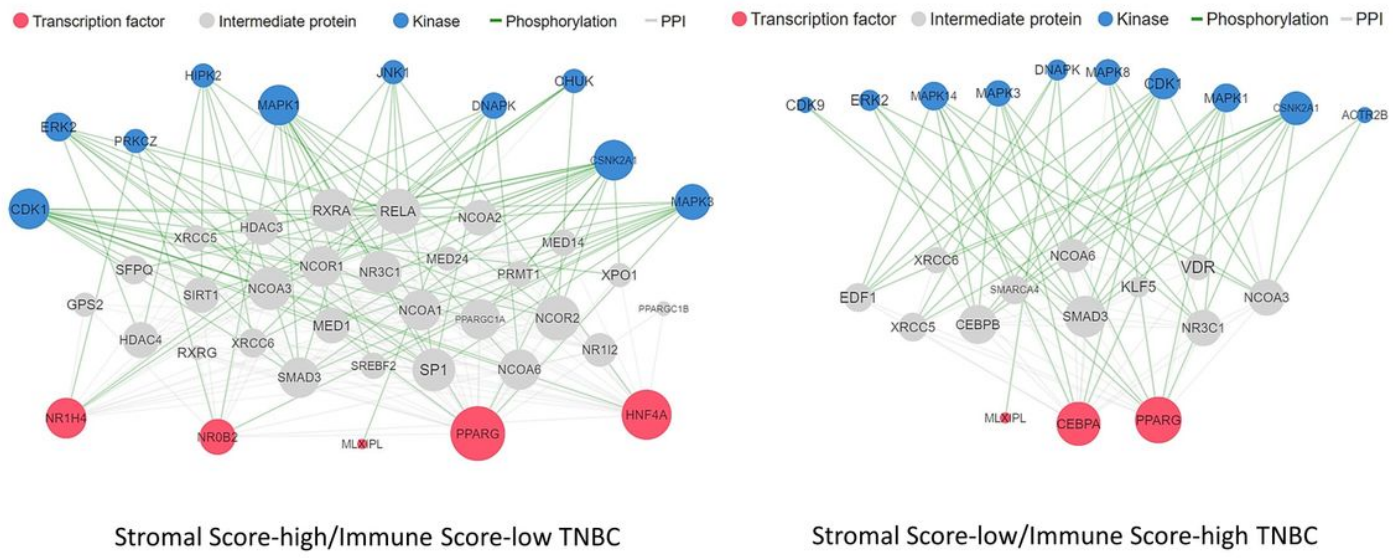


Figure 5

Transcription factors inferred from the X2K in the context of SS-high/IS-low or SS-low/IS-high TNBC patients.

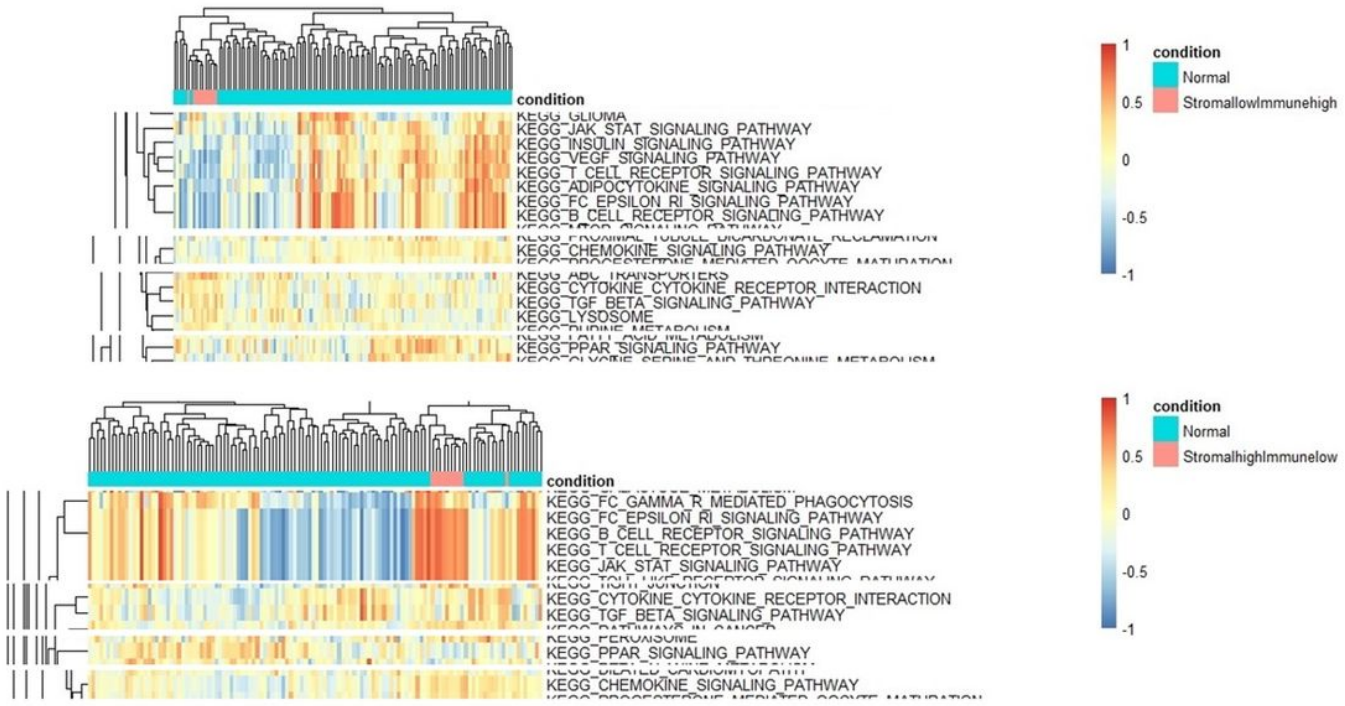
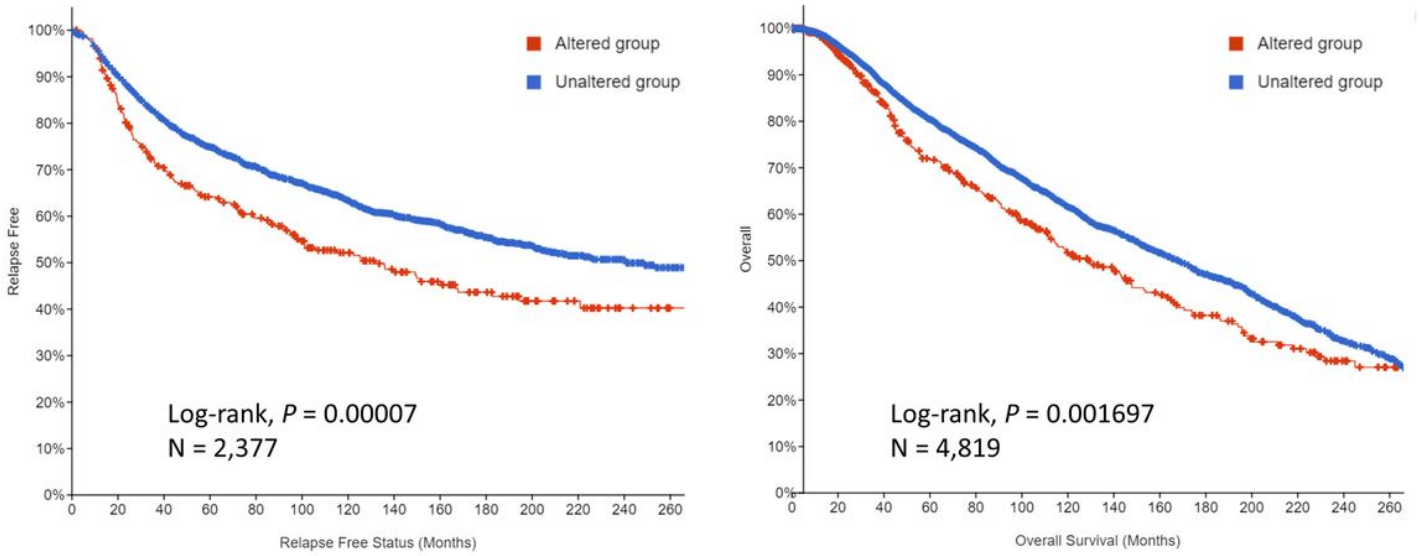


Figure 6

Gene Set Variation Analysis showing the differential pathway activity. The upper panel reveals the GSVA of the 370 DEGs of the SS-high/IS-low subgroup. The lower panel shows the GSVA of the 651 DEGs of

the SS-low/IS-high subgroup. Both demonstrate the gene enrichment in the JAK-STAT signaling, T cell receptor signaling, B cell receptor signaling, cytokine-cytokine receptor interaction, TGF-β signaling, and PPAR signaling pathways.



Survival Type	No. Patients with Data	No. in Altered group	No. in Unaltered group	Median survival in Altered	Median survival in Unaltered	*p*-Value
Relapse- Free	2,377	282	2,095	131.71 mo.	240.95 mo.	0.00007
Overall	4,819	465	4,354	129.6 mo.	168.2 mo.	0.001697

Figure 7

Validation using the cBioPortal demonstrates the relapse-free survival in 2,377 patients and the overall survival in 4,819 patients with invasive breast carcinoma stratified based on the alteration of the transcription factor genes PPARG, CEBPA, MEOX1, CD36, ZNF750, KLF15, EZH2, HNF4A, and NR0B2.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- SuppTable1.docx
- SupplFig1.ImmuneScoreandStromalScorebystages.tif
- SupplFig2.SurvivalbyStromalScoreloworhighNew.jpg
- SupplFig3.jpg
- SupplFig4.tif
- SupplFig5New.tif
- SupplFig6.pdf
- SupplFig7OncoprintforIL17genesaberration.tif