A Novel Immune-Related LncRNA Pair Model to Predict the Prognosis of Triple-Negative Breast Cancer

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Abstract

Background

Breast cancer (BC) is the most prevalent cancer type and is the principal cause of cancer-related death in women. Anti-PD-1/PD-L1 immunotherapy has shown promising activity in metastatic TNBC, but the potential factors affecting its efficacy have not been elucidated. Immune-related long noncoding RNAs (lncRNAs) have been reported to be involved in immune escape to influence the carcinogenic process through the PD-1/PD-L1 signaling pathway. Therefore, exploring the potential regulatory mechanism of lncRNAs in PD-1/PD-L1 immunotherapy in TNBC is of great importance.

Methods

In the current study, we retrieved transcriptome profiling data from The Cancer Genome Atlas (TCGA) and identified differentially expressed lncRNA (DElncRNA) pairs. Least absolute shrinkage and selection operator (LASSO) regression analysis was performed to construct a risk assessment model.

Results

Receiver operating characteristic (ROC) curve analysis indicated that the risk model may serve as a potential prediction factor in TNBC patients. Clinical stage and risk score proved to be independent prognostic predictors by univariate and multivariate Cox regression analyses. Subsequently, we investigated the correlation between the risk model and tumor-infiltrating immune cells and immune checkpoints. Finally, we identified USP30-AS1 through the StarBase and MEM databases, predicted the potential target genes of USP30-AS1, and then discovered that these target genes were closely associated with immune responses.

Conclusion

Although the current study did not investigate the specific mechanism by in vivo and in vitro experiments, we constructed a risk assessment model by immune-related IncRNA pairs regardless of expression levels, which has the potential to predict the efficacy of anti-PD-1/PD-L1 immunotherapy and provide a novel strategy for TNBC treatment.

1. Introduction

Breast cancer (BC) is the most commonly diagnosed cancer in females worldwide and is classified into different molecular subtypes, including luminal A, luminal B, human epidermal growth factor receptor-2 (HER-2) overexpression and triple-negative breast cancer (TNBC). TNBC is characterized by a lack of
estrogen receptor (ER), progesterone receptor (PR), and HER2 expression, representing approximately 15–20% of breast cancer cases\textsuperscript{3,4}. At present, comprehensive mode therapy combining local therapy (surgery, radiotherapy) with systemic therapy (endocrine therapy, chemotherapy, etc.) is a relatively mature treatment method for BC\textsuperscript{4,5}. However, chemotherapy promotes cancer heterogeneity of tumor cells, which could result in chemoresistance and cancer development\textsuperscript{4,5,7}. Progression of chemoresistance has been specified as a key obstacle in increasing the survival of cancerous cases. Over the past few years, the contribution of immune checkpoints to the progression of cancer chemoresistance has been demonstrated\textsuperscript{8}.

Immune checkpoint molecules are composed of ligands on malignant cells and respective receptors on tumor-infiltrating immune cells and can cause the elimination of cytotoxic T lymphocytes (CTLs) and inhibit anticancer immune responses\textsuperscript{2}. The three most important immune checkpoint molecules for drug development include PD-1, PD-L1, and cytotoxic T-lymphocyte-associated protein 4 (CTLA-4)\textsuperscript{2}. ICIs are currently available to induce durable immune responses and potential long-term benefits in different tumor types, including lung cancer, hepatocellular carcinoma, melanoma, and renal cell carcinoma\textsuperscript{9–12}. Breast cancer, as a “cold” cancer, was found to overexpress PD-L1 and CD47, but neither showed a satisfactory response to its corresponding immune checkpoint blockade (ICB) in the clinic\textsuperscript{13}. ICBs have only a modest benefit in advanced BC because BC often establishes an immunosuppressive tumor microenvironment (TME). However, TNBC is associated with tumor-infiltrating lymphocyte (TIL) infiltration and may exhibit clinical responses to ICBs\textsuperscript{1,14}. PD-L1 expression has been found in approximately 20% of TNBCs, and several clinical trials have shown that antibodies against the PD-1/PD-L1 pathway could induce immune responses and improve clinical outcomes in TNBC patients\textsuperscript{2,15,16}. However, not all TNBC patients are sensitive to immunotherapy. Given the heterogeneity of TNBC, it is imperative to explore potential mechanisms for regulating the efficacy of immunotherapy in TNBC patients.

Long noncoding RNAs (lncRNAs) play a prominent role in carcinogenesis, such as regulating gene expression, cell differentiation, aggressiveness of cancer cells and their ability to metastasize\textsuperscript{17,18,20}. LncRNAs can be transcribed from both protein-coding and noncoding regions of DNA\textsuperscript{19}. Several lncRNAs have been reported to influence the carcinogenic process and have particular applications in the prediction and diagnosis of different cancers\textsuperscript{21}. A metabolism-related lncRNA signature was identified that can predict the RFS of BC patients, and a prognostic nomogram was established that helps guide the individualized treatment of patients at different risks\textsuperscript{22}. Four-lncRNA immune prognostic signatures were prognostic biomarkers and could be possible therapeutic targets in TNBC\textsuperscript{23}.

Immune system disorders play a critical role in the development of various types of cancers\textsuperscript{24,25}. LncRNAs play crucial roles in the regulation of the immune system. Over the past few years, lncRNAs have emerged as key players in epigenetic regulation in the inhibitory tumor microenvironment because of their potential role in regulating tumor immunity by directly regulating genes involved in immune
activation or suppression\textsuperscript{26,27}. For example, IncRNA KCNQ1OT1 secreted by tumor cell-derived exosomes regulates PD-L1 ubiquitination via miR-30a-5p/USP22 to promote immune escape in colorectal cancer\textsuperscript{28}. However, the role of immune-related IncRNAs in regulating malignant progression and the efficacy of immunotherapy in TNBC has rarely been investigated. Exploring the potential mechanism by which IncRNAs regulate PD-L1 expression in TNBC patients is of great significance to identify novel therapeutic targets for TNBC.

In the current study, we identified DEirlncRNAs in TNBC samples according to public transcriptome profiling data and corresponding clinical data of TNBC patients. Then, we utilized a novel modelling method to construct a risk assessment model, which applied IncRNA pairs and did not require precise expression levels. We further investigated the correlation between the risk model and the tumor immune microenvironment. Finally, we predicted the target genes and performed functional annotation to explore the potential mechanisms.

2. Materials and Methods

2.1 Gene expression profiles and clinical data

The independent data included in this study were downloaded from a public database. The data of 146 TNBC samples and corresponding clinical information were obtained from The Cancer Genome Atlas (TCGA, https://portal.gdc.cancer.gov/) database.

2.2 Identification of differentially expressed irlncRNAs

Gene transfer format (GTF) files were retrieved from Ensembl (http://asia.ensembl.org) for annotation. A list of identified immune-related genes was obtained from the ImmPort database (http://www.immport.org) and was used to distinguish irlncRNAs by coexpression analysis. Correlation analysis was performed between immune-related genes and IncRNAs. LncRNAs with immune gene correlation coefficients greater than 0.4 and \( p \) values less than 0.001 were selected as irlncRNAs. The “Limma” package was used for differentially expressed analysis among irlncRNAs to identify DEirlncRNAs. False discovery rate (FDR) < 0.05 and log fold change (FC) > 1 were set as the standard to select DEirlncRNAs for further analysis.

2.3 Pairing DEirlncRNAs

The matching process of DEirlncRNA pairs was as follows: One IncRNA pair included two genes (A and B); if the expression level of A was larger than that of B, the score was 1; otherwise, the score was 0. Next, DEirlncRNA pairs with constant values (0 or 1) were removed from all individual datasets included in the meta-dataset, and the remaining DEirlncRNA pairs were considered candidate DEirlncRNA pairs. Additionally, only samples with a 0.2–0.8 pair ratio, defined as the total pair value, were included.

2.4 Construction of a prognostic risk model
Univariate Cox regression analysis was performed to identify prognostic lncRNA pairs. Least absolute shrinkage and selection operator (LASSO) regression analysis was performed with 10-fold cross validation and a $p$ value of 0.01. Then, multivariate Cox regression analysis was utilized to calculate the coefficients ($\beta_i$) of selected lncRNA pairs. A risk assessment model was constructed using $\beta_i$ and lncRNA expression levels to calculate the risk score of each patient. The risk score was calculated as follows: 
$\text{RiskScore} = \sum \beta_i \times \text{Expi}$. The area under the curve (AUC) of the 5-year ROC curve was evaluated to identify the cut-off point for distinguishing the high-risk or low-risk group among patients. The 2-, 3-, and 5-year ROC curves were plotted to estimate the predictive efficacy of the model. The R packages used in these steps included “survival”, “survminer”, “glmnet”, and “survivalROC”.

## 2.5 Evaluation of the constructed risk model

To verify the predictive value of the cut-off point, Kaplan–Meier analysis was performed to compare the difference in survival in the high- or low-risk groups. The chi-square test was conducted to investigate the association between the model and clinicopathologic characteristics, and a band diagram was plotted to show the results. Univariate and multivariate Cox regression analyses between the risk score and clinicopathological characteristics were performed to determine whether the ability of the model to predict OS was independent. Box diagrams show the differences in the risk score among different groups of these clinicopathological characteristics by the Wilcoxon signed-rank test. The R packages used in these steps are “survival”, “survminer”, “ggpubr”, and “ComplexHeatmap”.

## 2.6 Investigation of tumor-infiltrating immune cells and immune checkpoint molecules

We further investigated the association between the risk and immune status using XCELL, TIMER, QUANTISEQ, MCPCOUNTER, EPIC, CIBERSORT-ABS, and CIBERSORT. The results are shown in a lollipop diagram generated by the R “ggplot2” package with $p < 0.05$. The correlation between the risk score and the expression levels of immune checkpoint-related genes was exhibited in violin plots using the “ggpubr” package.

## 2.7 Correlation between the risk model and drug sensitivity

We calculated the IC50 of commonly administered chemotherapeutic drugs for TNBC in the TCGA database. The differences in the IC50 of imatinib, erlotinib, bexarotene, methotrexate and camptothecin between the high- and low-risk groups were compared using the R packages “pRRophetic” and “ggplot2”.

## 2.8 Target gene prediction and gene enrichment analysis

We utilized the StarBase and MEM databases to identify potential lncRNAs in the 3 lncRNA pairs, and USP30-AS1 was selected. We performed coexpression analysis to predict the potential target genes of USP30-AS1 and calculated Spearman’s rank correlation coefficients. Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analyses were performed for functional annotation.
2.9 Statistical analysis

The chi-square test was used to compare clinical variables. Kaplan–Meier analysis was performed to compare the difference in survival between different patient groups. The correlation was determined by Spearman correlation analysis. Survival status was analysed by Cox regression analysis. \( p < 0.05 \) was considered statistically significant.

3. Results

3.1 Identification of differentially expressed immune-related lncRNAs (DEirlncRNAs)

We downloaded the transcriptome profiling data of 146 cases of TNBC patients in the TCGA dataset. The expression matrix of immune-related mRNA was obtained from the ImmPort database and was used to identify lncRNAs by coexpression analysis. The patients were divided into a high-expression group and a low-expression group based on the median cut-off value of PD-L1 expression. A total of 58 DEirlncRNAs were identified (Fig. 1A), among which 52 lncRNAs were upregulated in the high-PD-L1 group, and 6 lncRNAs were upregulated in the low-PD-L1 group (\(|\log_2 \text{(fold change)}|>1, \text{FDR} < 0.05\) (Fig. 1B). These results indicated that lncRNAs may be potential regulators of PD-L1 expression in TNBC patients.

3.2 Construction of DEirlncRNA pairs and a risk assessment model

We established 1145 DEirlncRNA pairs and performed univariate Cox regression analysis. Ten DEirlncRNA pairs with prognostic significance were identified (Fig. 1C). LASSO and multivariate Cox regression analyses were applied to identify 3 lncRNA pairs (Table 1) and establish a risk assessment model based on the transcriptome profiling data of the above DEirlncRNAs (Fig. 2A, 2B, 2E). Time-dependent ROC curve analysis was applied to illustrate the sensitivity and specificity of the prediction model, with the area under the curve (AUC) reaching 0.680 at 2 years, 0.795 at 3 years, and 0.795 at 5 years (Fig. 2D). The optimal cut-off value of the 5-year ROC curve was identified as 1.058 (Fig. 2C).

<table>
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<th>DEirlncRNAs</th>
<th>Coef</th>
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<td>4.205038</td>
<td>1.383095</td>
<td>12.78462</td>
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</tbody>
</table>

3.3 Evaluation of independent prognostic value and correlation between clinical factors and the risk model
To further verify the prognostic ability of the risk model, we calculated the risk scores of the 140 patients with TNBC in the TCGA cohort. The patients were divided into a high-risk and a low-risk group based on the identified cut-off point, of which 55 cases were in the high-risk group, and 91 cases were in the low-risk group. The Kaplan–Meier survival curve indicated that patients in the high-risk group were more likely to exhibit a worse prognosis than those in the low-risk group \( (p = 0.029) \) (Fig. 3A). Univariate and multivariate Cox regression analyses of the risk model were performed to determine whether the prognostic ability of the model was independent. The results of univariate Cox regression analysis showed that clinical stage \( (HR = 11.268, 95\% CI = 4.680-27.127, p < 0.001) \), T stage \( (HR = 3.413, 95\% CI = 1.839–6.332, p < 0.001) \), N stage \( (HR = 3.249, 95\% CI = 2.097–5.033, p < 0.001) \), and risk score \( (HR = 2.031, 95\% CI = 1.255–3.287, p = 0.004) \) were independent predictive factors (Fig. 3B). In multivariate Cox regression analysis, clinical stage \( (HR = 13.055, 95\% CI = 2.413–70.631, p = 0.003) \) and risk score \( (HR = 2.518, 95\% CI = 1.381–4.592, p = 0.003) \) were still independent prognostic predictors (Fig. 3C). A chi-square test was performed to illustrate the relationship between the risk score of TNBC and clinicopathological characteristics (Fig. 3D). We further investigated the correlation between age (Fig. 3E), T stage (Fig. 3F) and risk by the Wilcoxon signed-rank test. The risk score increased as age and T stage increased. These results showed that age and T stage were significantly related to the risk score.

### 3.4 The risk model is highly correlated with tumor-infiltrating immune cells and immune checkpoint molecules

Given that lncRNAs were identified based on the coexpression of immune genes, we investigated whether the prediction model was related to the tumor immune microenvironment. We evaluated the immune infiltration status among the samples using XCELL, TIMER, QUANTISEQ, MCPCOUNTER, EPIC, CIBERSORT-ABS, and CIBERSORT. A detailed Spearman correlation analysis was performed to study the relationship between the risk score and immune infiltrated cells. The correlation coefficients are shown in a lollipop diagram (Fig. 4A) and listed in Table S1. The high-risk group was positively correlated with NK cells, Tregs, and M0 macrophages and negatively correlated with other immune infiltrating cells, which indicated that patients in the high-risk group may be insensitive to immunotherapy. Since checkpoint blockade therapy is extensively used in the clinical treatment of breast cancer, we further investigated the relationship between the risk model and the expression levels of immune checkpoint molecules in the TCGA database. The results of violin plots revealed that high risk scores were positively associated with low expression of PD-1, PD-L1, PD-L2, CTLA4, TIM3, and IDO1 \( (p < 0.05) \) (Fig. 4B).

### 3.5 The risk model may act as a potential predictor of chemotherapeutics

To evaluate the risk model in the clinic for TNBC treatment, we identified the relationship between the risk and the efficacy of commonly administered chemotherapeutic drugs in the TCGA database. The inhibitory concentration (IC50) of chemotherapeutic drugs, including imatinib, erlotinib, bexarotene, methotrexate, and camptothecin, was calculated. The results showed that a high risk score was related to a lower IC50 of imatinib \( (p = 0.043) \), erlotinib \( (p = 0.04) \), and bexarotene \( (p = 0.095) \) (Fig. 5A) and a higher
IC50 of methotrexate ($p = 0.0073$) and camptothecin ($p = 0.039$) (Fig. 5B), indicating that the risk model may act as a potential predictor of chemotherapeutic drug sensitivity.

### 3.6 Target gene prediction and functional analysis of USP30-AS1

Among the selected DEirIncRNAs, USP30-AS1 was identified through StarBase, the MEM database and the expression matrix of irIncRNAs. We predicted the potential target genes of USP30-AS1 in the risk model and identified 941 target genes (correlation coefficients $\geq 0.4$, $p < 0.05$), the top 100 of which are shown in Table S2. The genes encoding PD-1 (PDCD1) and PD-L1 (CD274) were included. Spearman’s rank correlation coefficient revealed that the expression of PD-L1 (Fig. 6A) and PD-1 (Fig. 6B) had a significant positive correlation with the expression of USP30-AS1 ($p < 0.001$). To identify the potential biological functions and pathways of target genes of USP30-AS1, we further performed GO analysis (Fig. 6C) and KEGG pathway analysis (Fig. 6D). Based on GO analysis, the target genes were significantly correlated with cytokine binding, receptor binding, cytokine activity, and several immune-related biological processes. The results of the KEGG pathway analysis revealed that the most relevant pathway for target genes was the cytokine–cytokine receptor interaction. We further generated a KEGG view of the cytokine–cytokine receptor interaction (Fig. 7) and found that most genes in this pathway were positively regulated by USP30-AS1. It can be assumed that USP30-AS1 may play an important role in the immune responses of TNBC.

### 4. Discussion

Previous studies have revealed that TNBC is critically related to the expression of PD-L1 in the tumor microenvironment (TME). Furthermore, accumulating data have shown that PD-1/PD-L1 inhibitors are able to induce durable clinical responses in some TNBC patients$^{29}$. The KEYNOTE-355 trial suggested that pembrolizumab (an anti-PD-1 antibody)-chemotherapy showed a significant and clinically meaningful improvement in progression-free survival (PFS) versus placebo-chemotherapy among patients with mTNBC$^{30}$. The IMpassion130 trial revealed that atezolizumab (an anti-PD-L1 antibody) plus nab-paclitaxel significantly improved progression-free survival (PFS) and median OS in patients with mTNBC, especially for PD-L1-positive patients$^{31}$. However, the IMpassion 131 trial revealed that the combination of atezolizumab and paclitaxel did not improve PFS or OS compared with paclitaxel alone, suggesting that the specific mechanisms of anti-PD-1/PD-L1 immunotherapies for mTNBC still need to be further explored$^{31,32}$.

In the present study, we used the cyclic single pairing method$^{33}$ and 0 or 1 matrix to verify the signatures of IncRNA pairs to predict the prognosis of TNBC. Therefore, only pairs with high or low expression need to be detected without examining the specific expression value of each IncRNA. Ten pairs with prognostic values were identified by univariate Cox regression analysis. Some IncRNAs of the 10 pairs have been reported to be critically involved in tumorigenesis, such as USP30-AS1$^{34–36}$, HLA-DQB1-AS1$^{37,38}$, and MIR155HG$^{39–41}$. Based on the LASSO regression analysis, we constructed a risk assessment model.
composed of 3 DEIRlncRNA pairs (USP30-AS1|SERPINB9P1, AL731567.1|AC004585.1, AC110995.1|AC007991.2). We further plotted ROC curves for 2, 3 and 5 years, and the model exhibited remarkable prognostic validity. The patients were divided into a high-risk and a low-risk group according to the identified cut-off point. The results of the survival analysis showed that the survival probability of the high-risk group was lower than that of the low-risk group. The results of univariate and multivariate Cox proportional hazard regression analyses showed that clinical stage and risk score were independent predictive factors for TNBC. In addition, age and T stage were significantly related to the risk score. The risk score increased as age and T stage increased. The highest increase in the risk score was found in patients with the T4 stage, which was much higher compared to patients with the T1-T3 stage. The results of the T1, T2 and T3 stages exhibited no significant difference, which may result from the limited samples of patients with low stages.

Immune checkpoint molecules have been identified as key modulators of the immune response, and their expression is closely related to the level of tumor-infiltrating immune cells, response to immunotherapy, and survival of patients. We evaluated the immune infiltration status among the samples using XCELL, TIMER, QUANTISEQ, MCPCOUNTER, EPIC, CIBERSORT-ABS, and CIBERSORT. We further investigated the relationship between the risk score and ICI-related biomarkers in the TCGA database. The results revealed that low risk scores were positively related to high expression of PD-1, PD-L1, PD-L2, CTLA4, TIM3, and IDO1 ($p < 0.05$). Despite progress in understanding the underlying tumor biology, clinical outcomes for TNBC unfortunately remain poor, and chemotherapy is still the mainstay of treatment for TNBC. Thus, we evaluated the association of risk values with the IC50 of some commonly used chemotherapy agents. We further found that this risk model may be beneficial in helping physicians choose more effective chemotherapy agents.

We identified the lncRNA USP30-AS1 based on the StarBase and MEM databases. USP30-AS1 is an antisense lncRNA that plays a critical role in regulating gene expression at the replication, transcription, and translation levels. The function of antisense lncRNAs is not related to the position of encoding genes but to the coexpressed protein-encoding genes. We further predicted potential target genes of USP30-AS1 and discovered that genes encoding PD-1 (PDCD1) and PD-L1 (CD274) were also included, and the expression levels of PD-1 and PD-L1 were positively correlated with USP30-AS1 expression. The results indicated that USP30-AS1 may be involved in the regulation of PD-1 expression. Furthermore, the predicted target genes were used to perform GO analysis and KEGG pathway analysis. A KEGG pathway, cytokine-cytokine receptor interaction, was significantly enriched by the predicted target genes. In addition, most genes in this pathway were positively regulated by USP30-AS1, suggesting that USP30-AS1 was closely associated with the tumor immune response. Therefore, we speculate that USP30-AS1 may serve as a potential target molecule to affect the efficacy of immunotherapy, and the potential mechanism is that it activates the transcription and upregulates the expression of PD-L1 to promote the immune escape of patients with TNBC.

However, our study has some limitations. First, our immune-related prognostic features of lncRNA pairs were developed through retrospective studies. Thus, prospective cohort studies are needed to further
validate our results. Second, although functional annotation has revealed that lncRNA USP30-AS1 can regulate the expression of PD-L1, in vivo and in vitro experiments are further needed to explore the specific mechanisms.

5. Conclusion

In conclusion, our study constructed a risk assessment model by immune-related lncRNA pairs regardless of expression levels, which contributed to predicting the efficacy of immunotherapy in TNBC. Furthermore, the lncRNA USP30-AS1 in the model was positively correlated with the expression of PD-L1 and provided a potential therapeutic target for TNBC.

Declarations

8.1 Ethical Approval

Not applicable.

8.2 Competing interests

The authors declare that they have no competing interests.

8.3 Authors’ contributions

All the authors were involved in the study. Study design and administration: JL and EC, Writing original draft: JL, Analysis and interpretation of data: PF and HP, Writing editing: SW and EC. All the authors took part in the discussions of the results and contributed to the manuscript. All authors read and approved the final manuscript.

8.4 Funding

Not applicable.

8.5 Availability of data and materials

The data comes from a public database.

References


**Figures**
Figure 1

Identification of differentially expressed immune-related IncRNAs (DEIncRNAs) in TCGA dataset. (A) Gene expression levels of DEIncRNAs in the TCGA dataset; (B) volcano plot of DEIncRNAs; (C) forest map of 10 DEIncRNA pairs identified by univariate Cox regression analysis.
Figure 2

Construction of a risk assessment model based on DEirIncRNA pairs. (A, B) Three IncRNA pairs for establishing the risk model were identified by LASSO Cox regression; (C) the optimal cut-off value was identified according to the maximum inflection point; (D) the 2-, 3-, and 5-year ROC curves for evaluating the sensitivity and specificity of the prediction model; (E) a 3-pair risk assessment model was established by multivariate Cox regression analysis.
Figure 3

Prognosis prediction and clinical evaluation of the risk assessment model. (A) Kaplan–Meier survival curve showed that patients in the low-risk group experienced a longer OS than patients in the high-risk group; (B) univariate Cox regression analysis of the model; (C) multivariate Cox regression analysis of the model; (D) the strip chart shows the relationship between the risk score and clinicopathological characteristics; (E) age was significantly associated with the risk score; (F) T stage was significantly associated with the risk score.
**Figure 4**

**Estimation of tumor-infiltrating immune cells and immune checkpoint molecules.** (A) The correlation coefficients are shown in a lollipop diagram. The high-risk group was more positively associated with NK cells, Tregs, and M0 macrophages and negatively correlated with other immune infiltrating cells. (B) Violin plots showing the expression levels of immune checkpoint inhibitor (ICI)-related genes in the low- and high-risk groups.
Figure 5

The risk model may act as a potential predictor of chemotherapeutic drug sensitivity. (A) A high-risk score was associated with a lower IC50 of imatinib, erlotinib, and bexarotene; (B) a high-risk score was associated with a higher IC50 of methotrexate and camptothecin.
Figure 6

**Target gene prediction and functional annotation of USP30-AS1.** CD274 (A) and PDCD1 (B) were identified as target genes of USP30-AS1; results of GO (C) and KEGG (D) analyses in the TCGA dataset.
Figure 7

A KEGG view of the cytokine–cytokine receptor interaction. Most genes in the cytokine–cytokine receptor interaction pathway were positively regulated by USP30-AS1.

Supplementary Files

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- SupplementalFiles.docx