

# The effect of NLRP3 on tumors based on pan-cancer research

## Desheng Tang

Department of Pancreatic and Biliary Surgery, The First Affiliated Hospital of Harbin Medical University, Harbin, Heilongjiang Province, China. Central Laboratory, The First Affiliated Hospital of Harbin Medical University, Harbin, China

## Wei Li

Department of Surgery, the Second Hospital, University of South China, Hengyang, Hunan, 421001, China. Clinical Research Center for Prevention and Treatment of Breast & Thyroid Disease in Hunan Province, Hengyang, Hunan, 421001, China

## Zhengjie Xu

Department of Surgery, Yinchuan women and children's Hospital, Yinchuan, Ningxia, 750001, China

## Suxiao Jiang

Department of Surgery, Yinchuan women and children's Hospital, Yinchuan, Ningxia, 750001, China

## Kun Fang (✉ [k99ftl@163.com](mailto:k99ftl@163.com))

YinChuan women and children hospital <https://orcid.org/0000-0003-3922-4434>

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## Research

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# Abstract

NLRP3 is a multi-protein complex in cells, which can directly or indirectly affect the tumor microenvironment and participate in tumor growth, invasion and metastasis. Tumor and normal tissue gene sequencing was downloaded, clinical and mutation-related data was obtained from the TCGA website, and Kaplan-Meier and cox regression analysis was used to analyze the relationship between NLRP3 and overall survival (OS) as well as Hazard ratio (HR). The correlation between NLRP3 and tumor microenvironment score, immune cell invasion, and immune resistance indicators (tumor mutation burden and microsatellite instability) was performed. Finally, the function of NLRP3 in tumors was analyzed by GSEA.

Inflammation is one of the important factors that cause cancer.

## Background

When chronic inflammation occurs, the local area is in an inflammatory environment for a long time, and it is likely to cause gene mutations and induce cancer 1–3 under the action of various inflammatory mediators. Recently, inflammatory cancer transformation has been a hot topic. NLRP3, a multi-protein complex in cells, plays an important role in the production of IL-1 $\beta$  and IL-18 by activating caspase-1, which is an important part of innate immune response 4–6. Current research showed that NLRP3 was involved in the progression of a variety of cancers. In addition, it was reported that NLRP3 participated in tumor proliferation, invasion and metastasis via affecting the tumor microenvironment, and the level of NLRP3 was closely related to clinical information such as the survival in patients with tumor 7. Therefore, it is helpful to better understand the relationship between inflammation and cancer by investigating the relationship between NLRP3 and tumors. Moreover, the development of second-generation sequencing technology has facilitated in-depth research of cancer. With the continuous improvement of various databases, the commonality between tumors can be discovered when studying the pathways and factors that cause cancer. Furthermore, differences make the systematically abnormal results to provide new thinking directions 8–12.

Therefore, abnormal expression of NLRP3 in tumor tissues was observed in this study. To verify whether NLRP3 played a role in tumor progression, the information from TCGA database was applied to systematically analyze the role of NLRP3 in 33 tumor samples.

## Method

### Data download and analysis

Gene expression data, gene mutation data and patient clinical data of 33 tumors were obtained on TCGA from UCSC Xena (<https://xena.ucsc.edu/>). The relationship between NLRP3 and the survival period, tumor microenvironment, and gene mutations of patients in pan-cancer were analyzed through Gene Set Enrichment Analysis (GSEA) (<https://www.gsea-msigdb.org/gsea/index.jsp>) the role of NLRP3 in tumors.

The abbreviation format of cancer follows the abbreviation format adopted in TCGA (see the abbreviation table for details).

### **Differential expression of NLRP3 in tumor and normal tissues**

The expression of NLRP3 was determined from 33 tumor tissues and normal samples through version R3.6.3, and then R "ggpubr" package was applied to make box plots of the difference between NLRP3 in 33 tumor tissues and normal tissues.

### **The relationship between NLRP3 and clinical information**

Cox regression analysis was carried out to measure the effect of NLRP3 on the OS and calculate the corresponding HR value, and the "forestplot" and "survival" packages in R were used to draw forest plots. According to the clinical stage, the patients are divided into stage I, stage II, stage III, and stage IV. Patients in stage I/II can usually undergo surgery directly, which can be defined as early stage patients, while stage III/IV patients who are often require neoadjuvant chemotherapy or cannot undergo surgery are defined as late stage. Among 33 tumor samples, 12 samples were removed for lacking clinical staging data.

### **Relationship between NLRP3 and tumor microenvironment and immune cell invasion**

The ESTIMATE algorithm was used to calculate the immune score and stromal score to reflect the degree of invasion of non-tumor cells such as immune cells and stromal cells according to the study conducted by Jia et al.. The higher score of immune cells or stromal cells, the higher proportion of immune cells or stromal cells. The ESTIMATE score is the sum of the above two scores. The higher the score, the lower purity of tumor cells 13. CIBERSORT is a gene-based deconvolution algorithm that uses the characteristics of specific marker genes to quantify the relative score of each cell type, and can infer 22 human immune cell types. After removing the data with  $P > 0.05$ , the standardized gene expression data set was uploaded to the CIBERSORT website (<https://cibersort.stanford.edu/index.php>), which used 1000 aligned default signature matrices to run. Finally the proportion of infiltrating immune cells in tumor tissue was obtained, and the relationship between NLRP3 and the proportion of immune cells 14 was calculated.

### **The relationship between NLRP3 and TMB and MSI**

The tumor mutation data was attained in TCGA following the previously study conducted by Liu et al.'s 15. Then the TMB and MSI of each tumor tissue were calculated, and the correlation between NLRP3 and tumor TMB and MSI were analyzed, which was drawn by the "fmsb" package of R Radar chart.

### **GSEA enrichment analysis**

The expression of NLRP3 in each tumor was measured and samples were divided it into a high expression group and a low expression group. On the GSEA website, the function of NLRP3 in each tumor

tissue was obtained through single-gene GSEA enrichment analysis, and R software was used to draw the GSEA enrichment analysis graph of NLRP3.

## Statistics

Wilcoxon test was used to verify whether the difference is statistically significant in normal tissues and tumor tissues. Log-rank test was used to determine the differences among survival curves. Spearman correlation coefficient was used to evaluate the correlation between NLRP3 expression and various indicators, including clinical stage, ESTIMATE score, immune infiltration score, TMB and MSI. A P value less than 0.05 was considered as significant difference.

## Result

### NLRP3 is differentially expressed in tumor tissues

Analyzing the differential expression of NLRP3 between normal samples and tumor samples, the expression differences in 11 of 33 cancer types were statistically significant. As shown in **Figure 1**, the expression level of NLRP3 was significantly decreased in lung squamous cell carcinoma (LUSC), pancreatic cancer (PAAD), rectal adenocarcinoma (READ), endometrial cancer (UCEC), while the expression of NLRP3 was elevated in renal clear cell carcinoma (KIRC) and renal papillary cell carcinoma (KIRP) compared to that in normal tissues, bladder urothelial carcinoma (BLCA), breast invasive carcinoma (BRCA), colon cancer (COAD), hepatocellular carcinoma (LIHC), lung adenocarcinoma (LUAD), lung squamous.

### NLRP3 and clinical relevance Univariate Cox regression analysis studies the correlation between NLRP3 and OS.

There were statistical significance differences between NLRP3 and HR in LUAD, skin melanoma (SKCM), testicular cancer (TGCT), and thymic cancer (THYM). As shown in **Figure 2A**, NLRP3 was a low risk factor in LUAD and SKCM, but NLRP3 is a high risk factor in TGCT and THYM. Subsequently, 33 patients with tumor were divided into high expression group and low expression group on the basis of the median value of NLRP3, and then the relationship between NLRP3 and OS was investigated. The results from **Figure 2B** showed that, patients with high expression of NLRP3 had better prognosis in LUAD and SKCM. However, patients with high expression of NLRP3 had a poor prognosis in TGCT and THYM. Next, the relationship between NLRP3 and clinical stage was determined. As shown in **Figure 2C**, the expression of NLRP3 in advanced BLCA patients was significantly increased compared to early stage patients, while, the expression of NLRP3 was significantly reduced in KIRP and LUAD patients. Therefore, NLRP3 was correlated with OS, HR and clinical stage in LUAD.

### Correlation of NLRP3 with TMB and MSI

TMB and MSI are widely used in clinical applications, and they have become important indicators in tumor immunotherapy. These results showed that there was a negative correlation between TMB and

NLRP3 in adrenal cortical carcinoma (ACC), uveal melanoma (UVM), thyroid cancer (THCA), TGCT, gastric cancer (STAD), prostate cancer (PRAD), PAAD, LUSC, LUAD, LIHC, head and neck squamous cell carcinoma (HNSC), diffuse large B-cell lymphoma (DLBC), cholangiocarcinoma (CHOL) and BRCA, while the expression of NLRP3 is positively correlated with TMB in THYM, COAD. In addition, the results revealed that the MSI was related to NLRP3 in 16 tumors, and MSI was negatively correlated with NLRP3 in STAD, SKCM, ovarian serous cystadenocarcinoma (OV), lung squamous cell carcinoma (LUSC), LIHC, head and neck squamous cell carcinoma (HNSC), esophageal cancer (ESCA), DLBC except for COAD (Figure 3).

### **The correlation between NLRP3 and tumor microenvironment**

To assess the relationship between tumors and the immune microenvironment, we selected the three tumors with the highest ESTIMATE scores, DLBC, KIRC, and PAAD, as well as the three tumors with the lowest scores, UVM, low-grade brain glioma (LGG), and ACC. The results showed that the expression of NLRP3 in 33 tumor tissues was positively correlated with stromal cell scores and immune cell scores, which suggested that higher expression of NLRP3 had great influence on tumor microenvironment. Next, the relationship between the expression of NLRP3 in tumors and 22 immune cell subtypes was further analyzed, and the expression of NLRP3 was most closely related to tumor-associated macrophages, especially M2 type macrophages. Figure 5 unveiled that there was a correlation between the content of M2 macrophages in 11 types of tumors and the expression of NLRP3. Among them, NLRP3 was positively correlated with the expression of M2 type macrophages in THYM, UCEC, BLCA, cervical squamous cell carcinoma and adenocarcinoma (CESC), COAD, acute myeloid leukemia (LAML), LUAD, LUSC, OV tumor tissues, while NLRP3 was negatively correlated with the expression of macrophages in THCA and LGG tumor tissues.

### **NLRP3 GSEA enrichment analysis**

Figure 6 takes CHOL, renal clear cell carcinoma (KICH), KIRP, LGG, STAD, PAAD and other tumors as examples. We enriched the functions of NLRP3 with GSEA, suggesting that the main functions of NLRP3 in tumors are: antigen receptor-mediated signaling pathway, immune response regulation cell surface receptor signaling pathway, B cell activation, leukocyte migration, immunity The negative regulation of system processes, the active regulation of cytokine production, T cell activation, and the regulation of immune effect processes show that NLRP3 plays an important role in the regulation of tumor immune function.

## **Discussion**

NLRP3 is the most well-known inflammasome that has been studied in recent years. It is involved in the production of IL-1 and the main function of NLRP3 in tumors is antigen receptor-mediated signaling pathway 17. Compelling studies have revealed that NLRP3 had direct or indirect impacts on a variety of tumors, especially on the tumor microenvironment. Recently, studies have proved, NLRP3 could regulate tumor growth, invasion and immune resistance through affecting the tumor microenvironment in diverse

tumors 9,18,19. In the current study, due to the many influencing factors in the tumor microenvironment, immune cells and stromal cells were used to represent the two main components of the tumor microenvironment. The results showed that NLRP3 was positively correlated with the ratio of stromal cells and immune cells. The study of shipra et al. showed that IL-1 $\beta$  released by the activation of inflammasomes in pancreatic cancer promotes the activation of resting pancreatic stellate cells, and induces an immunosuppressive environment dominated by M2 macrophages, which is a cancer The transfer created the conditions<sup>20</sup>. Ershaid et al. confirmed that the activation of NLRP3 in breast cancer fibroblasts promoted the production of IL-1 stellate cells, and induced an immunosuppressive environment dominated by M2 macrophages by up-regulating the expression of adhesion molecules on endothelial cells <sup>9</sup>. Evidence indicated that NLRP3 induced the proliferation of M2 macrophages and T cells, and promoted tumor growth and progression in PAAD <sup>21</sup>. Therefore, NLRP3 induced tumor progression by affecting the tumor microenvironment via positively regulating the differentiation and proliferation of immune cells and stromal cells. For tumor-infiltrated immune cells, macrophages are the most important component, especially M2 type macrophages. Our results showed that the content of NLRP3 was positively correlated with the proportion of M2 type macrophages. Studies have proven that M2 type macrophages promoted tumor progression in a variety of tumor tissues. NLRP3 participated in induction of the polarization in M2 type macrophages and promoted tumor development <sup>22</sup>. Deng et al. confirmed that the activation of NLRP3 in macrophages enhanced the migration ability of colorectal cancer cells <sup>19</sup>. In breast cancer, the NLRP3 inflammasome promoted tumor metastasis to lymphatic vessels by mediating the activation of macrophage S1PR1 signaling.

Knockout of NLRP3 macrophages will inhibit tumor invasion and migration <sup>24</sup>. Therefore, NLRP3 could promote tumor invasion and metastasis in tumors by inducing M2 polarization of macrophages and changing the microenvironment, which played a key role in the development of cancer. Thus, the level of NLRP3 could reflect the immune microenvironment of tumor, and then affect tumor progression by regulating the tumor microenvironment.

Recently, with the in-depth understanding of cancer, the promising advances in anti-cancer drugs has been made, is also especially immunotherapy such as PD-1, PD-L1 and other immune checkpoints. The tumor showed good therapeutic effect <sup>10,25,26</sup>. Because the key to immunotherapy is to recognize the surface antigen of the tumor and avoid tumor immune escape, and the efficacy of the drug is closely related to the degree of tumor gene mutation, two important indicators of MSI and TMB are selected to reflect the degree of tumor mutation. In the NCCN guidelines, TMB is listed as a recommended test item for immunotherapy for patients with non-small cell lung cancer. MSI testing for colon cancer patients has also been included in the NCCN guidelines. In addition, MSI also has a suggestive effect on the chemotherapy regimen for colon cancer patients <sup>27,28</sup>. It was reported that the prognosis of nodal cancer patients with high expression of MSI and TMB was poor, but the prognosis was better after receiving immunotherapy. In the present study, the correlation between NLRP3 and MSI and TMB was analyzed, and the results showed that NLRP3 was negatively correlated with MSI and TMB in most tumors, and NLRP3 was positively correlated with MSI and TMB in COAD. This opposite correlation of NLRP3 in COAD may be related to the production of IL-18 downstream of NLRP3. Because IL-18 was involved in the

function of intestinal mucosal epithelial cell damage, repair and intestinal homeostasis, and it had an impact on the development of colorectal tumors, in which NLRP3 might have a special effect on colon cancer 29. In view of and other shortcomings, we can borrow The correlation between TMB and MSI and NLRP3, as well as the level of NLRP3 can be used to indirectly reflect the level of TMB and MSI in tumors due to the high clinical cost and technical difficulties. The results from this study demonstrated NLRP3 was still a factor that induces tumors to produce immunosuppressive microenvironment and immune resistance. Therefore, inhibition of NLRP3 could provide a potential clinical treatment for patients with tumor, similar MCC950 can be used as a selective inhibitor of NLRP3 in animal experiments, and the effect is relatively ideal. However, there is still no clinical drug for NLRP3, but the downstream caspase-1 or IL-1 $\beta$  drugs have made good progress. Thus, it is need further research 30–32.

The whole article mainly focuses on the analysis of clinical data, tumor microenvironment and tumor mutation indicators of NLRP3 and pan-cancer. However, this study mainly uses data in the TCGA database rather than experiments, and the results may produce errors and affect the accuracy of the results. Thus, further experiments warrant further study.

## Conclusions

NLRP3 is related to the survival and pathological stage of certain tumors, and there is a correlation between NLRP3 and tumor suppressive microenvironment and immunological resistance indicators. In summary, NLRP3 can be used to assess tumor microenvironment and immune tolerance.

## Declarations

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### Authors' contributions

Conception and design: Desheng Tang, Wei Li

Collection and assembly of data: Wei Li, Su-xiao Jiang, Zhengjie Xu

Data analysis and interpretation: Desheng Tang, Wei Li, Kun Fang

Manuscript writing: Desheng Tang, Wei Li, Kun Fang

Paper revision: Kun Fang, Zhengjie Xu

Final approval of manuscript: All authors.

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### **Availability of data and materials**

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

### **Ethics approval and consent to participate**

Not applicable.

### **Consent for publication**

Not applicable.

### **Conflict interests**

The authors declare that they have no conflict of interest.

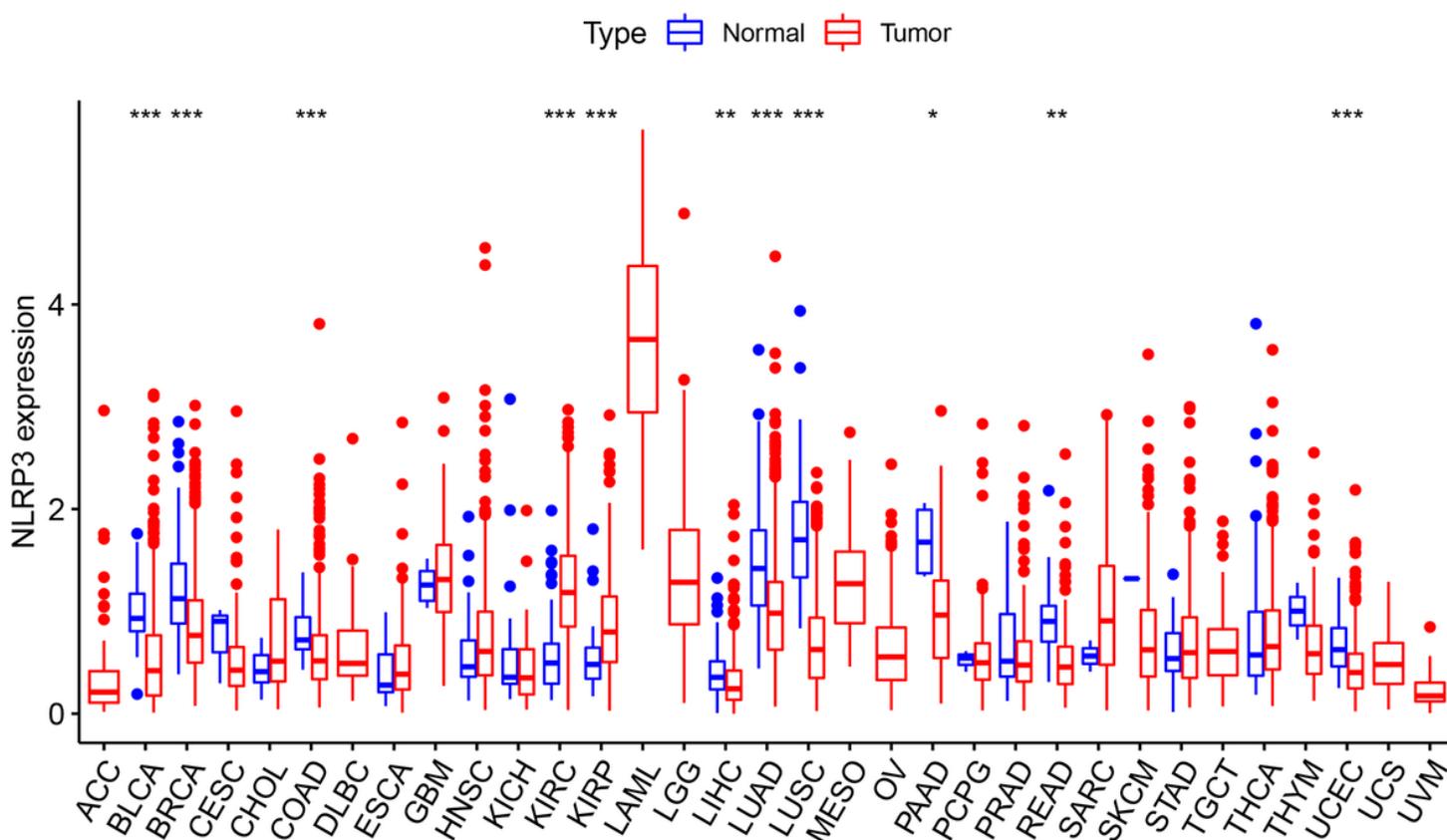
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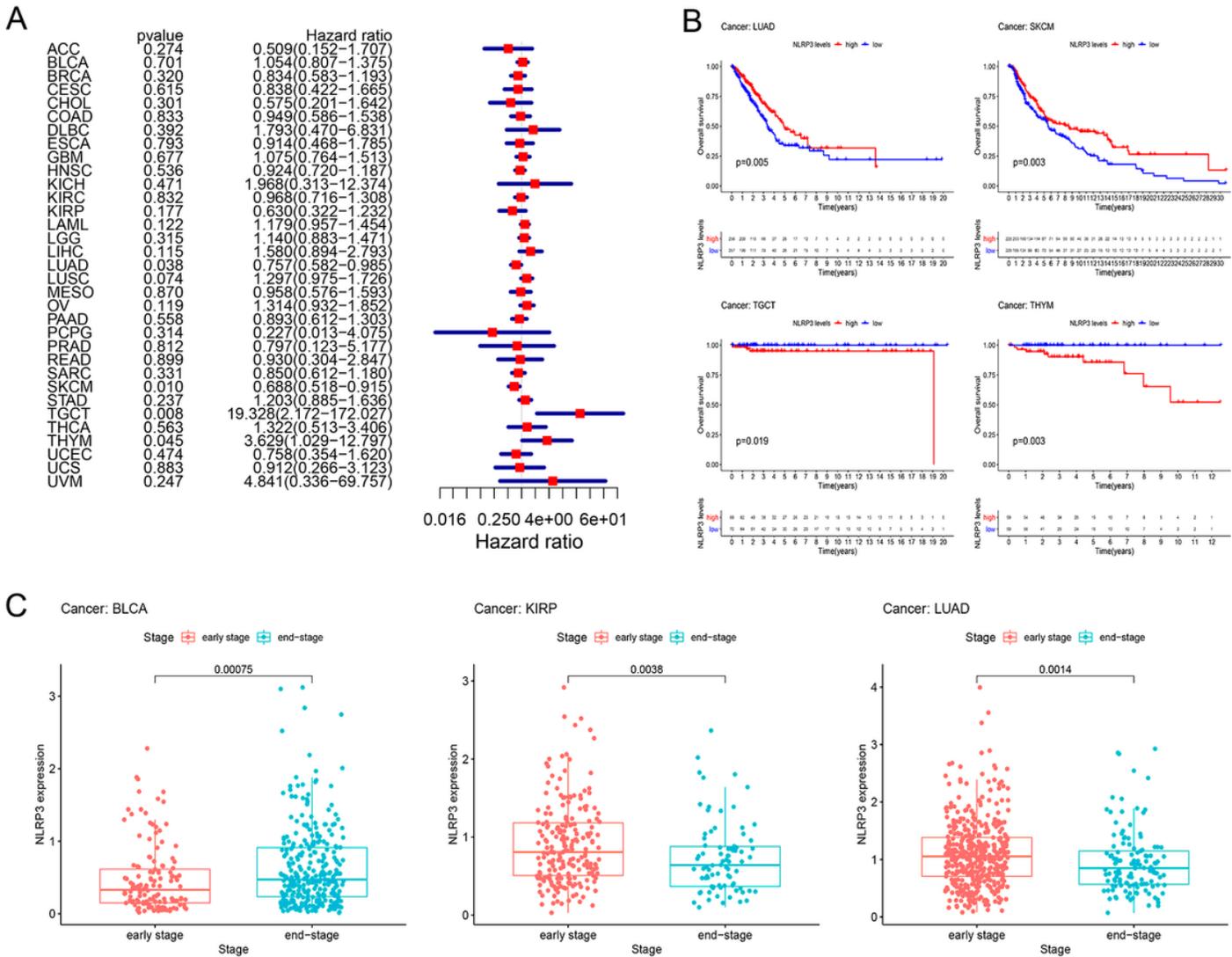
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## Figures



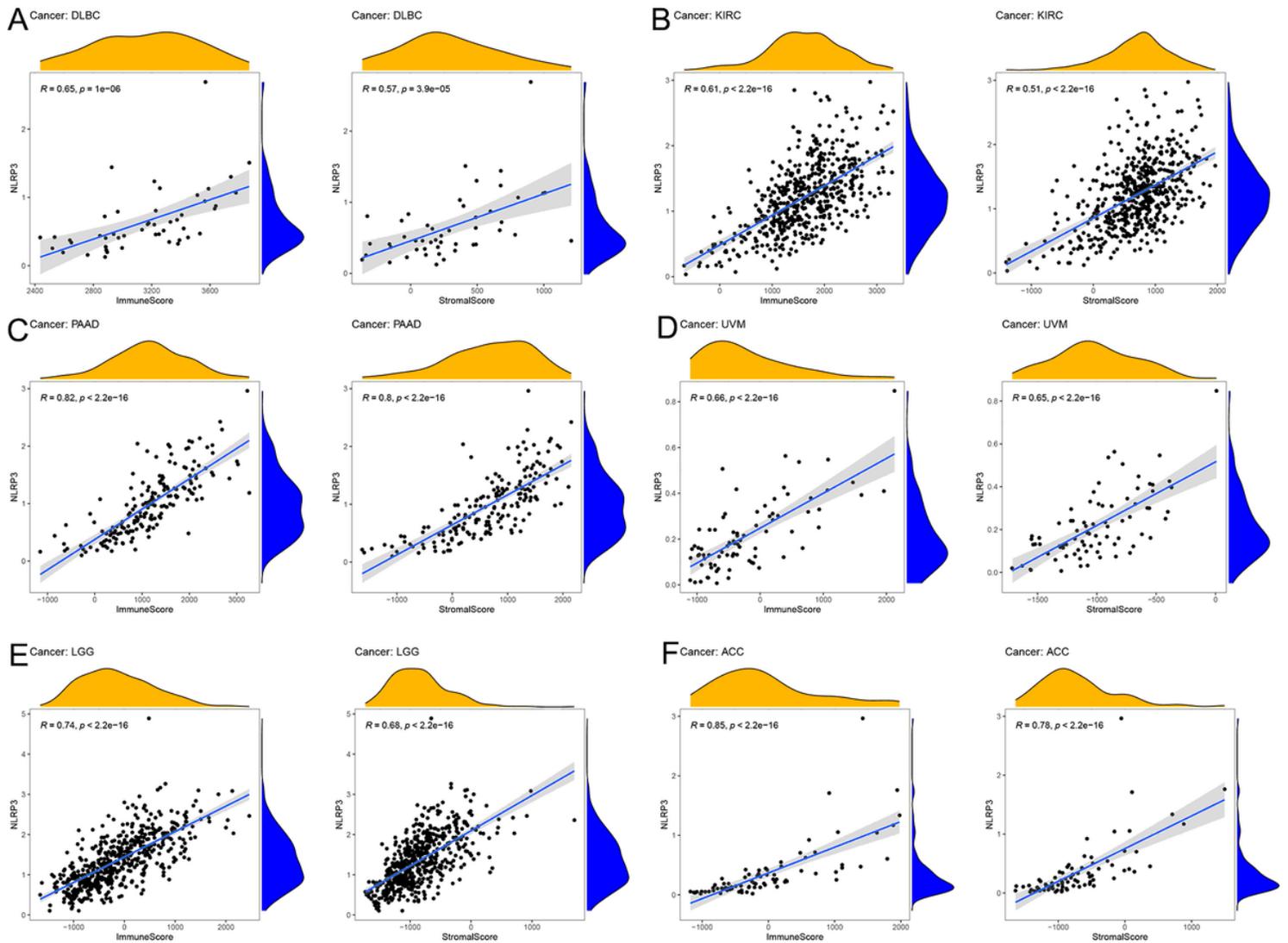
**Figure 1**

The expression level of NLRP3 in 33 tumors and corresponding normal tissues in the TCGA database.



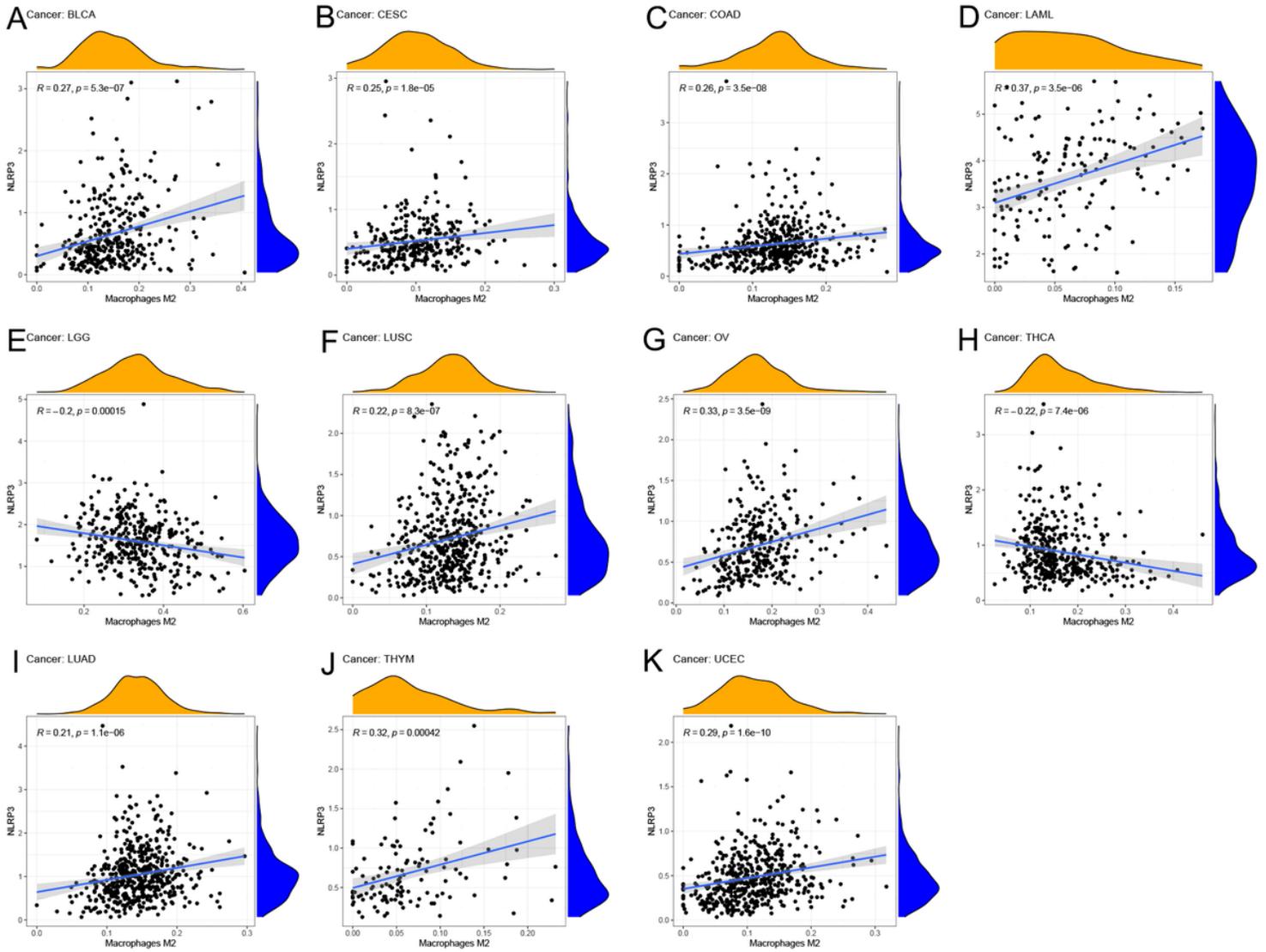
**Figure 2**

2A. Cox regression analysis in the TCGA database to analyze the correlation between the expression level of NLRP3 and OS in 33 tumors. 2B. Tumors LUAD, SKCM, TGCT and YHYM with significant differences in OS between NLRP3 high expression group and low expression group. Figure 2C. Tumor BLCA, KIRP and LUAD with correlation between NLRP3 and clinical grade ( $p < 0.05$  is considered statistically significant).



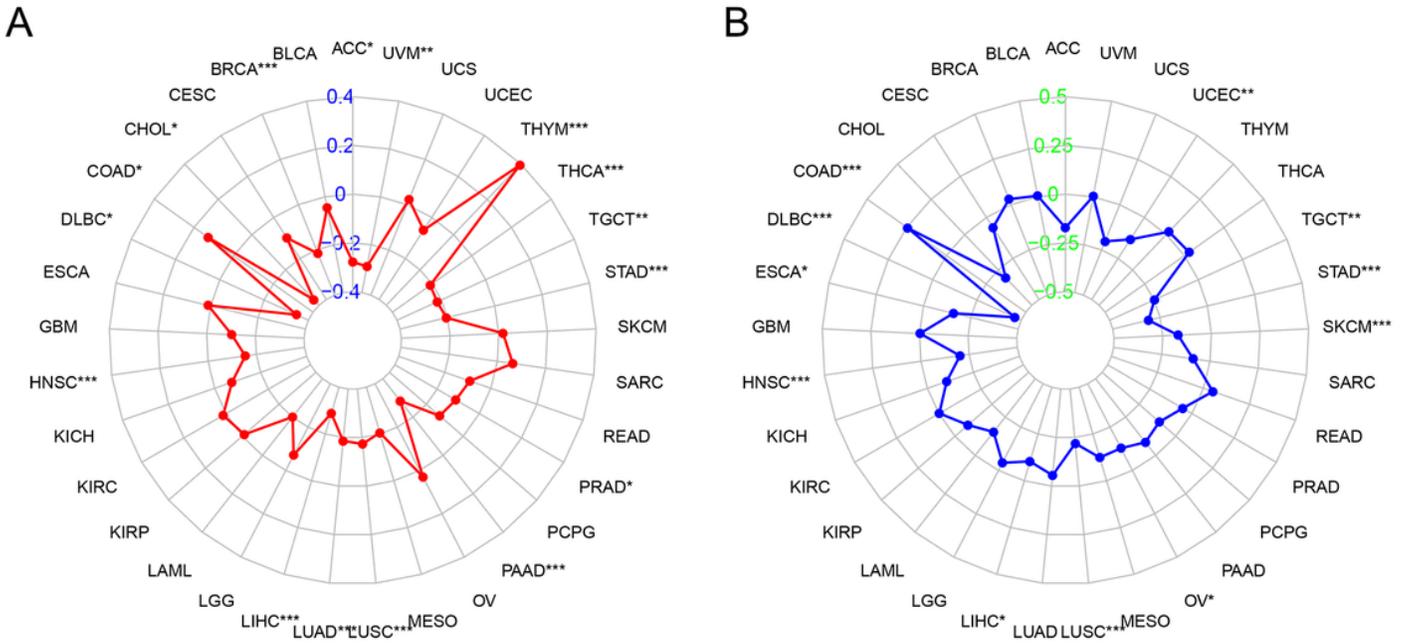
**Figure 3**

The relationship between the expression level of NLRP3 and microsatellite instability (MSI) and tumor mutation burden (TMB) in 33 tumors in the TCGA database. 3A. The correlation between NLRP3 and TMB expression. 3B. The correlation between the expression of MSI and YIF1B (Spearman correlation test,  $p < 0.05$  is considered statistically significant).



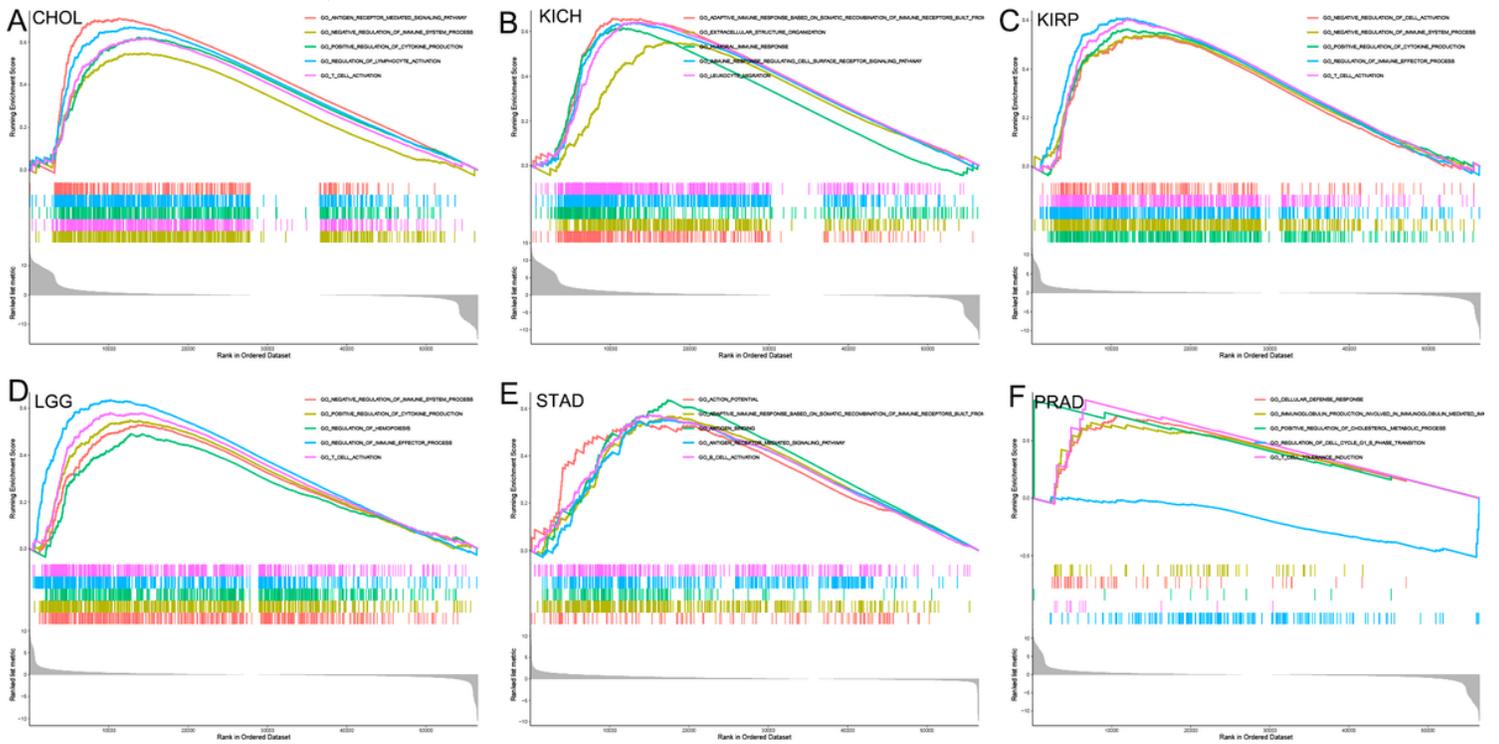
**Figure 4**

The relationship between NLRP3 and the immune microenvironment in the TCGA database. The three tumors with the highest ESTIMATE score, DLBC, KIRC and PAAD, and the three tumors with the lowest ESTIMATE score, UVM, LGG and ACC were selected. Indicates the correlation between NLRP3 and stromal cell score and immune cell score (Spearman correlation test,  $p < 0.05$  is considered statistically significant).



**Figure 5**

M2 macrophages and NLRP3 are most closely expressed in immune cells. BLCA, CESC, COAD, LAML, LGG, LUSC, OV, THCA, LUAD, THYM and UCEC tumors that are correlated between NLRP3 and M2 macrophage invasion (Spearman correlation test,  $p < 0.05$ )



**Figure 6**

takes 6 tumors including CHOL, KICH, KIRP, LGG, STAD, and PRAD as examples to show the GSEA enrichment analysis of the function of NLRP3 in tumors. are considered statistical Learning meaning).