NLRP3 is a Novel Prognostic Biomarker and Correlates With Immune Infiltrates in Lung Adenocarcinoma and Skin Cutaneous Melanoma

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NLRP3 is a novel prognostic biomarker and correlates with immune infiltrates in lung adenocarcinoma and skin cutaneous melanoma

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Abstract

Background: Lung adenocarcinoma (LUAD) and skin cutaneous melanoma (SKCM) are common tumors around the world. However, the prognosis in advanced patients is poor. Because NLRP3 was not extensively studied in cancers, so that we aimed to identify the impact of NLRP3 on LUAD and SKCM through bioinformatics analyses.

Methods: TCGA and TIMER database were utilized in this study. We compared the expression of NLRP3 in different cancers and evaluated its influence on survival of LUAD and SKCM patients. The correlations between clinical information and NLRP3 expression were analyzed using logistic regression. Clinicopathologic characteristics associated with overall survival in were analyzed by Cox regression. In addition, we explored the correlation between NLRP3 and immune infiltrates. GSEA and co-expressed gene with NLRP3 were also done in this study.

Results: NLRP3 expressed disparately in tumor tissues and normal tissues. Cox regression analysis indicated that up-regulated NLRP3 was an independent prognostic factor for good prognosis in LUAD and SKCM. Logistic regression analysis showed increased NLRP3 expression was significantly correlated with favorable clinicopathologic parameters such as no lymph node invasion and no distant metastasis. Specifically, a positive correlation between increased NLRP3 expression and immune infiltrating level of various immune cells was observed.

Conclusion: Together with all these findings, increased NLRP3 expression correlates with favorable prognosis and increased proportion of immune cells in LUAD and SKCM. These conclusions indicate that NLRP3 can serve as a potential biomarker for evaluating prognosis and immune infiltration level.

Keywords: Lung adenocarcinoma, Skin cutaneous melanoma, NLRP3, Tumor infiltrating lymphocytes, Bioinformatics analysis.

INTRODUCTION

Lung cancer is the malignant tumor with characteristic of uncontrolled cell growth in lung and bronchus, the 5-year related survival is 15.5% and 20.3% in men and women respectively (1). Lung adenocarcinoma (LUAD) is the predominant subtype of non-small cell lung cancer (NSCLC), accounting for about 40%-50% of all lung cancers (2). Besides, it is regarded as one of the most leading causes of cancer-related mortality worldwide. LUAD is often diagnosed at an advanced stage III or IV, accounting for its disappointing prognosis (3,4). Therefore, further researches are...
necessary for the development of effective lung cancer treatment, including novel therapeutic targets for lung cancer patients. Melanoma is a common type of cancer in the Western world, and its incidence continues to increase in both male and female. Sun exposure is considered as a major risk factor. The skin cutaneous melanoma (SKCM) is a common form in the Western world and causes the majority (75%) of deaths associated with skin cancer. Transformation of melanocytes into malignant melanoma is a complex process involving both exogenous and endogenous elements. Although tremendous progress has been made in unravelling the carcinogenesis and treatment regimens of melanoma, melanoma particularly metastatic melanoma, is still deemed as a difficult-to-treat disease. Hence, we should increase our knowledge of the molecular pathways responsible for melanoma pathogenesis and progression.

NLR family, pyrin domain containing 3 (NLRP3) is one of the most extensively investigated nucleotide-binding and oligomerization domain (NOD)-like receptors (NLRs). It has the ability to react to a wide range of extrinsic and intrinsic ligands such as pathogen associated molecular patterns (PAMPs) and/or damage associated molecular patterns (DAMPs), which can interact with pattern recognition receptors (PRRs) expressed on immune cells, then initiate a series of events leading to antitumor immunity. NLRP3 together with apoptosis-associated speck-like protein (ASC) and caspase-1 form as NLRP3 inflammasome, which is responsible for the maturation and secretion of proinflammatory cytokines. Therefore, NLRP3 is considered as a crucial innate immune component that orchestrate host immune homeostasis. Although there exist some researchers investigated the role of NLRP3 in cancers, the results are still quite preliminary and seem uncertain. For instance, Ershaid N illustrated that NLRP3 could facilitate tumor growth by recruiting myeloid-derived suppressor cells (MDSCs), and alter the tumor microenvironment toward an immune-suppressive milieu. On the contrary, study of Dupaul-Chicoine showed that NLRP3 could suppress the metastasis of colorectal cancer (CRC) in the liver by promoting tumoricidal activity of natural killer (NK) cells. In light of the conflicting effect of NLRP3 in respect of tumors, we focused on LUAD and SKCM for further research. We aimed to unravel whether NLRP3 posed a positive or negative effect on tumor prognosis and tumor microenvironment (TME).

In this study, we comprehensively analyzed the expression of NLRP3 in tumor tissues and matched normal tissues, its correlation with prognosis, clinical parameters, the status of different tumor-infiltrating immune cells and chemokines in LUAD and SKCM. Our results confirmed the importance of NLRP3 in LUAD and SKCM prognoses as well as the close relationship between NLRP3 and immune system. The workflow of our study is presented in Fig. 1.

RESULTS
The mRNA Expression Levels of NLRP3 in Different Types of Human Cancers
Initially, we evaluated NLRP3 expression levels in different human tumors and matched normal tissues by analyzing TCGA RNA-seq data (Fig. 2A). As indicated in figure, 1A, NLRP3 was highly expressed in the tissues of bile duct cancer (CHOL), esophagus carcinoma (ESCA) and head and neck squamous cancer (HNSC) and significantly lower in bladder cancer (BCA), thymoma (THYM) and lung squamous cell carcinoma (LUSC) tissues than in their respective normal tissues. These results demonstrated that NLRP3 was abnormally expressed in multiple tumors. In addition, we focused on the NLRP3 expression in lung adenocarcinoma (LUAD) and skin melanoma (SKCM). We found that NLRP3 mRNA expression was remarkably higher
in normal lung tissue compared with adjacent normal tissue (Fig. 2B). In contrast, NLRP3 mRNA expression level was significantly higher in melanoma tissue compared to normal counterparts (Fig. 2C).

**Prognostic significance of NLRP3 in LUAD and SKCM**

Next, we investigated the prognostic value of NLRP3 expression in LUAD and SKCM using the data from TCGA database. As indicated in Fig. 3, high NLRP3 expression was associated with better overall survival (OS) and disease specific survival (DSS) in both LUAD (OS: HR=0.63, 95% CI=0.47 to 0.85, P=0.002; DSS: HR=0.64, 95% CI=0.44 to 0.92, P=0.017; Figure 3A–3B) and SKCM patients (OS: HR=0.62, 95% CI=0.48 to 0.82, P=0.001; DSS: HR=0.57, 95% CI=0.42 to 0.79, P=0.001; Figure. 3D–3E). Moreover, higher expression of NLRP3 was correlated with better progression free survival (PFS) in SKCM (PFS: HR=0.75, 95% CI=0.58 to 0.97, P=0.031; Figure. 3F) but not in LUAD (PFS: HR=0.80, 95% CI=0.61 to 1.05, P=0.103; Figure. 3C).

Univariate analysis using Cox regression indicated that T stage (HR = 1.728,P = 0.002), N stage (HR = 2.601,P < 0.001), M stage(HR = 2.136,P=0.006), pathologic stage (HR = 2.933,P < 0.001), and NLRP3 expression (HR = 0.631,P = 0.002) were significant parameters could affect the survival of LUAD patients(Table 1A). In multivariate analysis, the up-regulated NLRP3 expression (HR = 0.649, P=0.014), T stage (HR = 1.657, P=0.027) and N stage (HR = 1.922, P=0.028) were independent prognostic factors of LUAD prognosis. Results for SKCM was slightly different from LUAD. As shown Table 1B, univariate survival analysis indicated that age (HR = 1.656,P < 0.001), T stage (HR = 2.085, P< 0.001), N stage (HR = 1.752,P < 0.001), M stage(HR = 1.897,P=0.04), TNM stage (HR = 1.846,P < 0.001), and NLRP3 expression level(HR = 0.665,P=0.003)significantly influenced the overall survival in SKCM patients. Multivariate Cox survival analysis revealed that T stage (HR = 2.366, P=0.004), N stage (HR = 2.212, P < 0.001), distant metastasis (HR = 2.436, P = 0.039) together with the expression of NLRP3 (HR = 0.687, P=0.020) were significantly associated with overall survival (Table1B). In summary, we confirmed the prognostic value of NLRP3, and its higher expression was associated with better prognosis in LUAD and SKCM. Furthermore, in order to predict the survival of patients diagnosed with LUAD or SKCM, a nomogram was constructed by incorporating several independent indicators identified by the multivariate analyses (Figure.4A–4B), and the assessment by nomograph clearly supported the positive effect of higher NLRP3 expression in better survival outcome.

**Association between NLRP3 expression and clinicopathologic variables**

The association between NLRP3 mRNA expression and some common clinicopathological parameters including age, sex, race, TNM stage, lymph node metastasis, distant metastasis and vital status of LUAD and SKCM was investigated using TCGA data. Based on the median values of NLRP3 expression level, patients were classified as having high or low expression. As illustrated in Table 2A, univariate analysis using logistic regression with NLRP3 expression as a categorical dependent variable revealed that in LUAD patients, the increased expression of NLRP3 correlated significantly with the no lymph node invasion(p = 0.006), no distant metastasis (p = 0.015),lower TNM stage(p < 0.001), and better outcome of primary therapy (p = 0.002). In SKCM patients, the result of logistic regression analysis demonstrated that higher expression of NLRP3 correlated with lower T stage(p=0.02) and lower pathologic stage (p=0.033) (Table 2B). No significant correlation between NLRP3 expression and other clinicopathological factors, including age, gender and race was observed. In total, our results
verified the significant relationship between higher NLRP3 expression and favorable clinicopathologic characteristics.

**NLRP3-related signaling pathways in LUAD and SKCM identified by GSEA**

In order to investigate the potential molecular function of NLRP3 in carcinogenesis of LUAD and SKCM, gene set enrichment analysis (GSEA) was conducted between samples with low and high NLRP3 expression to predict NLRP3-related signaling pathways. We focused on the top 4 significantly upregulated pathways (Figure. 5A-5B).

In LUAD patients, the significantly upregulated terms enriched in the high NLRP3 group included “Immunoregulatory interactions between a Lymphoid and a non-Lymphoid cell”, “CD22 mediated BCR regulation”, “FCGR activation” and “DNA Damage/Telomere Stress Induced Senescence”. Among these 4 pathways, “DNA Damage/Telomere Stress Induced Senescence” was the only one involved in cellular response to stimuli pathways while the other 3 pathways were all involved in pathways related to adaptive immune system or innate immune system. Slightly differed from LUAD, the top 4 upregulated signaling pathways in SKCM all belonged to adaptive immune system pathways. These 4 pathways were “Immunoregulatory interactions between a Lymphoid and a non-Lymphoid cell”, “Antigen activates B Cell Receptor (BCR) leading to generation of second messengers”, “CD22 mediated BCR regulation”, and “FCGR activation”.

**Analysis of genes co-expressed with NLRP3 in LUAD and SKCM**

To further explore the possible effect of NLRP3 in LUAD and SKCM, we tried to identify genes that positively co-expressed with NLRP3 with data downloaded from TCGA. The top 50 genes positively correlated with NLRP3 were selected and displayed as co-expression heatmaps (Fig. 6A-6B). Additionally, we focused on the top 6 genes and investigated their relationship with overall survival outcome in LUAD or SKCM patients. The top 6 genes positively co-expressed with NLRP3 in LUAD included Nck-associated protein 1-like (NCKAP1L), CD84, Src Like Adaptor(SLA), Cytochrome B-245 Beta Chain (CYBB), Dedicator of cytokinesis 2 (DOCK2), and CD86. Based on the Kaplan-Meier plots, higher expression of NCKAP1L, CD84 or DOCK2 was correlated with longer overall survival, while the expression level of SLA, CYBB and CD86 had no impact on survival outcome (Figure. 7A-7F). In SKCM, we found the top 6 genes were Cas scaffold protein family member 4 (CASS4), C-type lectin domain family 4 member A (CLEC4A), Cytochrome B-245 Beta Chain (CYBB), Component 3a Receptor 1 (C3AR1), colony stimulating factor 1 receptor (CSF1R) and Formyl peptide receptor 1 (FPR1). Moreover, high expression of these 6 genes were all associated with better overall survival (Figure. 7G-7L). Therefore, we believe that NLRP3 and its co-expressed genes collectively contribute to the better survival in LUAD and SKCM patients.

**Relationship between NLRP3 and tumor-infiltrating immune cells and chemokines**

Previous analyses suggested tumor-infiltrating lymphocytes as independent predictors for survival in cancer patients (17). Hence, we assessed the correlations between NLRP3 expression and immune infiltration status in LUAD and SKCM. NK cells, macrophages, mast cells, neutrophils, dendritic cells (DCs), B cells and T cells were included in the analysis. In LUAD, NLRP3 expression level had significant positive correlations with infiltrating level of NK cells (P < 0.001), macrophages (P < 0.001), mast cells (P < 0.001), neutrophils (P < 0.001), B cells (P < 0.001), T cells (P < 0.001), T helper cells (P < 0.001), CD8+ T cells (P < 0.001), T helper 1 cells (Th1) (P < 0.001), T regulatory cells (Treg) (P < 0.001), T follicular helper cells (Tfh) (P <
dendritic cells (DCs) (P < 0.001), activated dendritic cells (aDCs) (P < 0.001) and immature dendritic cells (iDCs) (P < 0.001). However, the infiltrating level of T helper 2 cells (Th2) and T helper 17 cells (Th17) had no relationship with NLRP3. As shown in Figure. 8A, NLRP3 expression level was positively correlated with high infiltration of all the above immunocytes in SKCM. Further, we explored the correlations between NLRP3 and immune markers of a series of immune cells via TIMER databases. CD8+ T cells, T cells (general), B cells, neutrophils, dendritic cells, macrophages, monocyte and natural killer cells were included and different functional T cells, such as T helper 1 (Th1) cells, T helper 2 (Th2) cells, Tfh cells, T helper 17 (Th17) cells, and Tregs, as well as exhausted T cells were analyzed. After the correlation adjustment by purity, the results revealed the NLRP3 expression level was significantly correlated with most immune marker sets of various immune cells and different T cells in LUAD and SKCM. Markers of T cells, B cells, dendritic cells, macrophages as well as NK cells all showed significant correlations with NLRP3 expression (Table 3). We also found significant correlations between NLRP3 and marker genes of Treg and T cell exhaustion, such as PD-1, CTLA4, LAG3, and TIM3. In conclusion, these results demonstrated NLRP3 level was strongly correlated with immune infiltration, suggesting that NLRP3 may play a critical role in the lung and skin tumor microenvironment. Further studies need to be done to elucidate the exact role of NLRP3 in the carcinogenesis and tumor progression in LUAD and SKCM. Finally, we explored the associations between NLRP3 expression and chemokines. As shown in Figure. 9, NLRP3 expression was strongly correlated with CCL2, CCL4, CCL13, CCL19, CCL23 and CXCL12 in LUAD. Similarly, CCL2, CCL3, CCL4, CCL5, CCL8 and CXCL16 were significantly correlated with the expression of NLRP3 in SKCM (Figure. 9). We infer it is the positive correlation between NLRP3 expression and chemokines that contributes to the high infiltration of immune cells in LUAD and SKCM.

Discussion

NLRP3 is the sensor component of the NLRP3 inflammasome, and it has the ability to respond to pathogens and other damage-associated signals (18, 19). Based on our study, we find there exist significant differences between tumor tissues and paired normal tissues in various cancers. Further investigations demonstrate that an up-regulated NLRP3 expression is associated with better prognosis and NLRP3 is an independent prognostic factor in LUAD and SKCM. Moreover, the increased level of NLRP3 is correlated with favorable clinicopathological characteristics including lower T stage, N0 stage, M0 stage or lower pathologic stage. It is revealed that diverse immune marker sets and immune infiltration status are correlated with NLRP3 expression in LUAD and SKCM. In conclusion, we hypothesize that NLRP3 has a potential influence on tumor immunology and serves as a promising prognostic biomarker for LUAD and SKCM.

To understand the molecular functions and underlying mechanisms of NLRP3 better, GSEA was performed to investigate the pathways enriched in samples with high expression of NLRP3. We focused on the top 4 upregulated pathways. “Immunoregulatory interactions between a Lymphoid and a non-Lymphoid cell pathway”, “CD22 mediated BCR regulation pathway” and “FCGR activation pathway” are the 3 pathways both enriched in LUAD and SKCM patients with high NLRP3 expression level. Interestingly, all these 3 pathways belong to the category of “immune system pathway”. “Immunoregulatory interactions between a
Lymphoid and a non-Lymphoid cell pathway participates in adaptive immune response, the interactions between lymphoid and non-lymphoid cells are quite complicated, which require cooperation of a number of receptors and cell adhesion molecules (20,21). The common receptor- ligand pairs include TCR-MHC1, CD40L-CD40, CD22- Nectin2, etc. “CD22 mediated BCR regulation pathway” also plays an important part in adaptive immune function. CD22 is a glycoprotein found on the surface of B cells and it is a member of the sialic acid-binding Ig-like lectin (Siglec) family (22). There is convincing evidence showing that CD22 can tightly regulate the activation of B cells (22,23). “Fc gamma receptor activation pathway” functions as an event of phagocytosis, which is a critical innate immune response eliminating the invaded infectious agents. Phagocytosis is an extremely complex process including phagocytic cup formation, particle engulfment, and the release of proinflammatory mediators such as cytokines and reactive oxygen species (24). In addition, another enriched pathway in LUAD is “Antigen activates B Cell Receptor (BCR) leading to generation of second messengers”, indicating that NLRP3 is involved in BCR signaling. In conclusion, the above results confirm that NLRP3 is closely correlated with immune functions.

Next, we explored genes co-expressed with NLRP3 in LUAD and SKCM. In LUAD, NLRP3 is strongly correlated with NCKAP1L, CD84, SLA, CYBB, DOCK2 and CD86, among which high expression of NCKAP1L, CD84 or DOCK2 is significantly correlated with a higher overall survival (OS). In SKCM, the top 6 correlated genes are CASS4, CLEC4A, CYBB, C3AR1, CSF1R and FPR1, moreover, they are associated with a better prognosis. Further study shows these co-expressed genes act a crucial role in innate and adaptive immune system, since they are expressed in various immune cells and mediate different immunomodulatory functions (25-33). For example, FPR1 regulates the process of chemotactic recruitment of DCs and neutrophils (25), and CYBB is significantly correlated with the enrichment of immune pathways related to interferon (IFN)-alpha, IFN-gamma, and NK cell signaling (28). The interactions between the NLRP3-related genes and the immune activities strongly corroborate our inference that NLRP3 has significant impact on the immune system.

We also investigated the impact of NLRP3 expression on the status of tumor infiltrating lymphocytes in LUAD and SKCM. Finally, significantly positive correlation is found between NLRP3 expression and high infiltration of various immune cells, both immunocytes considered as indispensable effectors for anti-tumor responses and those immune-suppressive cells inducing immunological tolerance are involved. NK cells, CD8+ T cells are typical cells closely associated with effective cancer treatment (34), therefore their high infiltration in tumor microenvironment can definitely contribute to the prolonged OS in LUAD and SKCM patients. However, high NLRP3 level is also concomitantly correlated with high infiltration of immunocytes with inhibitory effects such as Tregs and M2 type TAM (35), we consider it may be caused by the suppressive negative feedback loops reported before (36).

As is known to all, the infiltration of immune cells is regulated by a series of chemokines, which have the ability to modulate the biological phenotype of the immunocytes, and influence tumor progression and prognosis (37). We finally explored the relationship between NLRP3 and chemokines. According to our results, high expression of NLRP3 in LUAD and SKCM is significantly correlated with several kinds of chemokines such as CCL2, CCL3, CCL9 and CXCL12, etc. Increased secretion of chemokines contributes to the high infiltration of immune cells.
In summary, our study confirms that increased NLRP3 expression correlates to favorable prognosis in LUAD and SKCM patients. Meanwhile, NLRP3 expression levels are closely associated with tumor-infiltrating immune cells and several chemokines. Therefore, NLRP3 has an essential influence on immune infiltration and has the potential to become a novel predictor to evaluate prognosis and immune infiltration for LUAD and SKCM patients. NLRP3 and its co-expressed genes are collectively involved in the immune response, contributing together to a better prognosis in LUAD and SKCM patients. We think more extensive researches need to be done to explore the possible mechanisms between NLRP3 and tumor-immune interactions especially in immunogenic cancers such as LUAD and SKCM.

**Conclusion**

In this study, we observed NLRP3 mRNA is differentially expressed in tumor tissues and normal tissues. High NLRP3 expression is associated with prolonged overall survival in LUAD and SKCM. Results of GSEA, co-expressed genes and tumor infiltrating lymphocytes prove that NLRP3 has close relationship with the immune system. NLRP3 has great potential to serve as a biologic predictor of prognosis for cancer patients.

**MATERIALS AND METHODS**

**NLRP3 gene expression analysis**

To determine the difference of NLRP3 expression in several tumor and normal tissues, we obtained the mRNA levels (level 3) of NLRP3 from The Cancer Genome Atlas (TCGA) (https://gdc-portal.nci.nih.gov/) (13) and GTEx projects. The clinical information of LUAD and SKCM patients was also acquired from TCGA database. The threshold was determined as follows: fold change of 1.5, P-value of 0.001, and gene ranking of all.

**Survival analysis**

To evaluate the prognostic value of NLRP3 in LUAD and SKCM patients, Kaplan-Meier survival curve analysis was performed based on the hazard ratios (HR) and log-rank P-values. Kaplan–Meier curve was plotted using the survival (https://cran.r-project.org/web/packages/survival/index.html) in R.

**Correlation analysis of NLRP3 and clinicopathological features**

We downloaded the clinicopathological data of patients with primary lung adenocarcinoma and skin melanoma from TCGA database. The included patients were divided into high and low NLRP3 expression groups according to the median NLRP3 expression value. The collected clinical data involved age, sex, race, grade, TNM stage, lymph node metastasis, distant metastasis and vital status. The association between NLRP3 expression level and the clinicopathological parameters was analyzed using logistic regression.

**Evaluation of tumor-infiltrating immune cells and chemokines**

For reliable immune score evaluation, we used an R software package named immuneconv that integrated six latest algorithms, including TIMER, xCell, MCP-counter, CIBERSORT, EPIC and quanTIseq. We also evaluated the relationship between NLRP3 and crucial markers of several immune cells in TIMER database (https://cistrome.shinyapps.io/timer/) (14). The gene markers included markers of CD8+ T cells, T cells (general), B cells, neutrophils, natural killer (NK) cells, T regulatory (Treg) cells, T-helper 1 (Th1) cells, T-helper 2 (Th2) cells, follicular helper T (Tfh) cells, T-helper 17 (Th17) cells, exhausted T cells and Mast cells etc. The Spearman method was used to determine the correlation coefficient. NLRP3 was used for the X-axis, and other genes of interest are represented on
the Y-axis. The correlation between NLRP3 and chemokines was analyzed by TISIDB database (http://cis.hku.hk/TISIDB/index.php)(15). TISIDB is an online tool that allows users to identify the role of specified gene in tumor-immune interactions through high-throughput data analysis.

**Significant prognostic marker analysis and nomogram establishment**

For statistical analysis, R version 3.6.1 software (survival and survminer packages) was used to handle the data downloaded from TCGA. Independent prognostic factors were identified by univariate and multivariate Cox regression analyses. Furthermore, we utilized the R package “rms” to construct the nomogram which incorporated clinical factors and NLRP3 expression.

**Gene set enrichment analysis**

On expression of NLRP3, patients were divided into high and low groups. The pathways that were enriched by the top ranked genes in the two groups were detected by gene set enrichment analysis (GSEA). For each analysis, the number of gene set permutations was set to 1000. The nominal (NOM) P value, false discovery rate (FDR) and normalized enrichment score (NES) were used to identify the pathways enriched in each phenotype. Gene sets conforming to | NES | > 1 and NOM p < 0.05 were deemed significant.

**Profiling of genes co-expressed with NLRP3**

To further study the NLRP3-related molecular mechanisms, the co-expressed genes of NLRP3 were identified using DESeq2 in R package to screen for proteins interacting with NLRP3. The top 6 significant genes correlated with NLRP3 in LUAD and SKCM patients were selected for further analysis, and their correlation with survival outcome was explored in the Gene Expression Profiling Interactive Analysis (GEPIA) (http://gepia.cancer-pku.cn/index.html) (16).

**Statistical analysis**

SPSS 20.0 and R version 3.6.1 software were used for statistical analyses. The measurement data are presented as mean ± SD. Paired sample t test were used to analyze the differential expression levels of NLRP3 mRNA between the tumor tissues and normal tissues from the TCGA database. The correlations between clinical characteristics and NLRP3 expression were analyzed using logistic regression. Univariate and multivariate analyses were performed using Cox proportional hazards regression model and a predicting nomograph was constructed. The gene expression correlation was accessed by Spearman method. Statistically significant differences were considered when P < 0.05.

**Authors’ contributions**

Conception and design of the work, Chenxi Yuan and Yipeng Song; acquisition and analysis of data, Chenxi Yuan and Xueling Dai; interpretation of data, Chenxi Yuan and Qingwei Wang; writing and preparing the original draft, Chenxi Yuan; supervision, Jinming Yu and Yipeng Song; funding acquisition, Jinming Yu. All authors have read and agreed to the published version of the manuscript and to have agreed to both be personally accountable for the author’s contributions and ensure to answer any questions related to the accuracy or integrity of any part of the work. All authors read and approved the final manuscript.

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Ethics declarations
Competing interests
The authors declare no competing interests.

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Table 1. The results of Cox regression analysis. A. In LUAD, univariate analysis using Cox regression revealed that some factors, including T stage (HR = 1.728, P-value =0.002), N stage (HR = 2.601, P-value < 0.001), M stage (HR = 2.136, P-value < 0.046), pathological stage (HR = 2.933, P-value < 0.001) along with the expression of NLRP3 (HR = 0.631, P-value <0.002) are significantly associated with overall survival. The upregulated TTC21A expression, lower T stage and negative lymph node metastasis are independent prognostic factors of favorable prognosis. B. In SKCM, univariate analysis showed that age (HR = 1.656, P-value < 0.001), T stage (HR = 2.085, P-value < 0.001), N stage (HR = 1.752, P-value < 0.001), M stage (HR = 1.897, P-value < 0.04), pathological stage (HR = 1.846, P-value < 0.001) and the expression of NLRP3 (HR = 0.665, P-value =0.003) are significantly associated with overall survival. The upregulated NLRP3 expression, lower T stage, lower pathologic stage and negative lymph node metastasis are independent prognostic factors of favorable prognosis. *, p< 0.05.

| Characteristics | Total(N) | Univariate analysis | | | Multivariate analysis |
|-----------------|---------|---------------------|-----|---------------------|
|                 |         | Hazard ratio (95% CI) | P value | Hazard ratio (95% CI) | P value |
| Age             | 516     | 1.223 (0.916-1.635) | 0.172 |                     |         |
| Gender          | 526     | 1.070 (0.803-1.426) | 0.642 |                     |         |
| Smoker          | 512     | 0.894 (0.592-1.348) | 0.591 |                     |         |
| T stage         | 523     | 1.728 (1.229-2.431) | **0.002** | 1.657 (1.058-2.596) | **0.027*** |
| N stage         | 510     | 2.601 (1.944-3.480) | <**0.001** | 1.922 (1.075-3.436) | **0.028*** |
| M stage         | 377     | 2.136 (1.248-3.653) | **0.006** | 1.408 (0.762-2.604) | 0.275 |
| Pathologic stage| 518     | 2.933 (2.173-3.958) | <**0.001** | 1.347 (0.718-2.525) | 0.353 |
| NLRP3           | 526     | 0.631 (0.471-0.846) | **0.002** | 0.649 (0.460-0.916) | **0.014*** |
### Table 2. Association between NLRP3 expression and several clinicopathologic variables using logistic regression. (A). In LUAD patients, increased expression of NLRP3 significantly related to the pathological stage, tumor status, lymph node status, distant metastasis status and treatment outcome. (B). In SKCM patients, NLRP3 expression was significantly associated with T stage and pathologic stage. *, p< 0.05.

#### A.

| Characteristics       | Total(N) | Odds Ratio (OR)   | P value |
|-----------------------|----------|-------------------|---------|
| Age (>65 vs. <=65)    | 516      | 1.344 (0.952-1.902) | 0.094   |
| Gender (Male vs. Female) | 535      | 0.769 (0.547-1.080) | 0.130   |
| Race (Black or African American&White vs. Asian) | 468      | 5.871 (0.992-111.346) | 0.103   |
| Smoker (Yes vs. No)   | 521      | 0.751 (0.457-1.227)  | 0.255   |
| T stage (T2&T3&T4 vs. T1) | 532      | 0.898 (0.625-1.289)  | 0.558   |
| N stage (N1&N2&N3 vs. N0) | 519      | 0.597 (0.411-0.863)  | **0.006*** |
| M stage (M1 vs. M0)   | 386      | 0.311 (0.111-0.754)  | **0.015*** |
| Pathologic stage (Stage II&Stage III&Stage IV vs. Stage I) | 527      | 0.524 (0.369-0.741)  | **<0.001*** |
| Primary therapy outcome (SD&PD vs. CR&PR) | 446      | 0.496 (0.317-0.769)  | **0.002*** |

#### B.

| Characteristics       | Total(N) | Odds Ratio (OR)   | P value |
|-----------------------|----------|-------------------|---------|
| Age (>60 vs. <=60)    | 463      | 0.737 (0.510-1.064) | 0.104   |
| Characteristics                                      | Total(N) | Odds Ratio (OR)       | P value |
|-------------------------------------------------------|----------|-----------------------|---------|
| Gender (Male vs. Female)                               | 471      | 0.796 (0.548-1.156)   | 0.231   |
| Race (Black or African American&White vs. Asian)      | 461      | 0.705 (0.206-2.241)   | 0.555   |
| T stage (T3&T4&T2 vs. T1)                             | 364      | 0.444 (0.219-0.865)   | **0.020**|
| N stage (N1&N2&N3 vs. N0)                             | 414      | 1.207 (0.818-1.784)   | 0.344   |
| M stage (M1 vs. M0)                                   | 443      | 1.514 (0.672-3.556)   | 0.323   |
| Pathologic stage (Stage II&Stage III&Stage IV vs. Stage I) | 412      | 0.577 (0.345-0.952)   | **0.033**|

Table3.Correlation analysis between NLRP3 expression and gene markers of CD8+ T cells, T cells (general), B cells, neutrophils, natural killer (NK) cells, T-helper 1 (Th1) cells, T-helper 2 (Th2) cells, follicular helper T (Tfh) cells, T-helper 17 (Th17) cells, exhausted T cells and, Monocytes and Macrophages via TIMER database.

| Description          | Gene markers | LUAD | SKCM |
|----------------------|--------------|------|------|
| Tell(general)        | CD3D         | 0.488| 3.122SE-32 | 0.348| 1.6503E-15 | 0.577| 3.50E-43  | 0.414| 2.16E-20 |
|                      | CD3E         | 0.584| 1.904E-48 | 0.472| 8.9996E-29 | 0.576| 5.00E-43  | 0.415| 1.72E-20 |
|                      | CD2          | 0.566| 5.8513E-45| 0.446| 1.5199E-25 | 0.606| 1.20E-48  | 0.461| 1.54E-25 |
| CD8+ T cell          | CD8A         | 0.472| 0      | 0.354| 4.87E-16   | 0.574| 8.92E-43  | 0.430| 4.38E-22 |
|                      | CD8B         | 0.343| 1.219E-15| 0.231| 1.90E-07   | 0.530| 1.66E-35  | 0.362| 1.26E-15 |
| Th1                  | T-bet        | 0.505| 0      | 0.397| 4.34E-20   | 0.583| 2.31E-44  | 0.434| 2.00E-22 |
|                      | STAT1        | 0.409| 0      | 0.323| 1.89E-13   | 0.529| 2.21E-35  | 0.433| 2.40E-22 |
|                      | STAT4        | 0.512| 8.45E-36| 0.410| 1.90E-21   | 0.604| 2.61E-48  | 0.473| 6.56E-27 |
|                      | IFN-γ        | 0.273| 2.62E-10| 0.151| 0.00005694 | 0.520| 4.72E-34  | 0.367| 5.04E-16 |
|                      | TNF-α        | 0.376| 0      | 0.250| 1.71E-08   | 0.506| 4.06E-32  | 0.350| 1.12E-14 |
| Th2                  | GATA3        | 0.443| 2.876E-26| 0.334| 2.50E-14   | 0.557| 8.97E-40  | 0.384| 1.43E-17 |
|                      | STAT6        | 0.231| 1.16E-07| 0.275| 5.15E-10   | 0.091| 0.0468967 | 0.130| 0.00334169|
|                      | STAT5A       | 0.676| 4.77E-70 | 0.612| 4.71E-52   | 0.167| 0.0002529 | 0.188| 5.10E-05 |
|                      | IL13         | 0.185| 2.36E-05| 0.111| 0.00055894 | 0.167| 0.0002662 | 0.102| 0.02891906|
| Th17                 | STAT3        | 0.262| 1.36E-09| 0.297| 1.53E-11   | 0.411| 1.25E-20  | 0.407| 1.13E-19 |
|                      | IL17A        | 0.107| 0.0145633| 0.016| 0.71594608 | -0.011| 0.79526261| -0.052| 0.2620235 |
| Treg                 | FOXP3        | 0.593| 0      | 0.501| 9.78E-33   | 0.538| 9.07E-37  | 0.376| 7.58E-17 |
|                      | CCR8         | 0.648| 8.95E-63| 0.575| 9.24E-45   | 0.643| 2.33E-56  | 0.537| 1.43E-35 |
|                      | STAT5B       | 0.434| 3.69E-25| 0.438| 1.54E-24   | 0.369| 1.20E-16  | 0.430| 4.69E-22 |
|                      | TGFβ         | 0.564| 0      | 0.515| 9.60E-35   | 0.540| 3.72E-37  | 0.456| 6.34E-25 |
| T cell exhaustion    | PD-1         | 0.485| 7.35E-32| 0.376| 5.03E-18   | 0.525| 7.75E-35  | 0.359| 2.35E-15 |
|                      | CTLA4        | 0.584| 0      | 0.481| 5.71E-30   | 0.511| 1.06E-32  | 0.385| 1.28E-17 |
|                      | TIM-3        | 0.792| 0      | 0.750| 1.68E-90   | 0.754| 5.62E-88  | 0.680| 2.25E-63 |
**Fig. 1. Workflow of this study.**

| B cell | CD19 | 0.386 | 8.36E-20 | 0.240 | 6.22E-08 | 0.419 | 1.52E-21 | 0.262 | 1.38E-08 |
|        | CD79A | 0.381 | 0 | 0.247 | 2.57E-08 | 0.421 | 1.09E-21 | 0.237 | 2.68E-07 |
| Natural killer cell | KIR2DL1 | 0.147 | 0.00076012 | 0.090 | 0.0449387 | 0.322 | 7.15E-13 | 0.199 | 1.80E-05 |
|       | KIR2DL3 | 0.218 | 5.68E-07 | 0.132 | 0.00325156 | 0.419 | 1.70E-21 | 0.252 | 4.29E-08 |
|       | KIR2DL4 | 0.197 | 6.56E-06 | 0.111 | 0.01351375 | 0.453 | 3.11E-25 | 0.289 | 2.87E-10 |
|       | KIR3DL1 | 0.198 | 5.36E-06 | 0.128 | 0.00418489 | 0.400 | 1.53E-19 | 0.245 | 1.00E-07 |
|       | KIR3DL2 | 0.249 | 9.85E-09 | 0.168 | 0.00016537 | 0.441 | 6.21E-24 | 0.279 | 1.27E-09 |
|       | KIR3DL3 | 0.053 | 0.22429016 | 0.023 | 0.60501469 | 0.071 | 0.11884452 | 0.0007 | 0.9865355 |
|       | KIR2DS4 | 0.219 | 4.84E-07 | 0.154 | 0.00059487 | 0.286 | 2.35E-10 | 0.156 | 0.00078985 |
| Dendritic cell | HLA-DPB1 | 0.673 | 2.09E-69 | 0.611 | 7.86E-52 | 0.665 | 1.37E-61 | 0.556 | 1.78E-38 |
|       | HLA-DQB1 | 0.503 | 2.05E-34 | 0.417 | 2.91E-22 | 0.614 | 3.24E-50 | 0.487 | 1.04E-28 |
|       | HLA-DRA | 0.667 | 0 | 0.600 | 1.10E-49 | 0.693 | 8.22E-69 | 0.594 | 5.12E-45 |
|       | HLA-DPA1 | 0.680 | 0 | 0.623 | 2.05E-54 | 0.628 | 3.90E-53 | 0.510 | 1.06E-31 |
|       | B2M-1 | 0.525 | 7.46E-38 | 0.471 | 1.17E-28 | 0.536 | 1.94E-36 | 0.400 | 5.40E-19 |
|       | B2M-4 | 0.397 | 0 | 0.389 | 2.36E-19 | 0.598 | 4.47E-47 | 0.566 | 3.90E-40 |
|       | CD11c | 0.718 | 7.03E-83 | 0.666 | 1.42E-64 | 0.570 | 4.53E-42 | 0.449 | 3.63E-24 |
| Neutrophils | CD66b | 0.321 | 7.57E-14 | 0.329 | 6.14E-14 | 0.013 | 0.76485864 | 0.046 | 0.32460064 |
|       | CD11b | 0.791 | 0 | 0.764 | 1.19E-95 | 0.738 | 3.53E-82 | 0.672 | 2.14E-61 |
|       | CCR7 | 0.598 | 0 | 0.497 | 4.03E-32 | 0.536 | 1.67E-36 | 0.370 | 2.38E-16 |
| Monocyte | CD86 | 0.827 | 0 | 0.790 | 9.61E-107 | 0.766 | 2.73E-92 | 0.694 | 4.43E-67 |
|       | CD115 | 0.861 | 4.38E-153 | 0.835 | 1.54E-129 | 0.784 | 2.13E-99 | 0.720 | 2.54E-74 |
| M1 Macrophage | iNOS | 0.255 | 3.97E-09 | 0.187 | 2.72E-05 | 0.105 | 0.02160502 | 0.118 | 0.01103753 |
|       | IRF5 | 0.493 | 0 | 0.428 | 1.76E-23 | 0.601 | 1.96E-47 | 0.469 | 1.81E-26 |
|       | CD21 | 0.064 | 0.14214415 | 0.082 | 0.06884115 | 0.297 | 4.26E-11 | 0.271 | 3.47E-09 |
| M2 Macrophage | CD163 | 0.774 | 0 | 0.741 | 3.35E-87 | 0.748 | 1.47E-85 | 0.678 | 7.41E-63 |
|       | V5K4 | 0.701 | 0 | 0.666 | 1.07E-64 | 0.687 | 4.71E-67 | 0.609 | 9.29E-48 |
|       | MS4A4A | 0.724 | 0 | 0.677 | 1.34E-67 | 0.724 | 1.02E-77 | 0.641 | 2.10E-54 |
| TAM | CCL2 | 0.526 | 0 | 0.459 | 3.86E-27 | 0.654 | 6.48E-59 | 0.555 | 2.07E-38 |
|       | CD68 | 0.663 | 0 | 0.617 | 4.34E-53 | 0.516 | 1.86E-33 | 0.411 | 4.17E-20 |
|       | IL10 | 0.622 | 1.52E-56 | 0.547 | 5.58E-40 | 0.718 | 4.33E-76 | 0.644 | 4.84E-55 |
Fig. 2. The expression level of NLRP3 in different types of human cancer.
(A) Increased or decreased NLRP3 in data sets of different cancers compared with normal tissues.  (B) NLRP3 mRNA levels in LUAD tissues and normal lung tissues in TCGA.  (C) NLRP3 mRNA levels in SKCM tissues and matched normal skin tissues in TCGA.  ns, p≥0.05; *, p< 0.05; **, p<0.01; ***, p<0.001.
Fig. 3. Kaplan-Meier survival curve analysis of the prognostic significance of high and low expression of NLRP3 in LUAD and SKCM(A–F). (A–C) High NLRP3 expression was correlated with better overall survival (OS) (n=526), disease specific survival (DSS) (n=491) but not progression free interval (PFI) in LUAD cohorts (n=526). (D–F) High NLRP3 expression was correlated with better OS, DSS and PFI (n=456, n=450, n=457) in SKCM cohorts.

Figure 4. The nomogram to predict the overall 1-year, 2-year, and 3-year survival rate in (A) LUAD (C-index=0.669) and (B) SKCM (C-index=0.683) based on NLRP3 expression and clinicopathologic parameters.
Figure 5. GSEA pathways enriched in samples with high NLRP3 expression (A–H). The GSEA results showed that the terms “Immunoregulatory interactions between a Lymphoid and a non-Lymphoid cell” (A), “CD22 mediated BCR regulation” (B), “FCGR activation” (C) and “DNA Damage/Telomere Stress Induced Senescence” (D) were enriched in LUAD samples with high NLRP3 expression. Terms that “Immunoregulatory interactions between a Lymphoid and a non-Lymphoid cell” (A), “Antigen activates B Cell Receptor (BCR) leading to generation of second messengers” (B), “CD22 mediated BCR regulation” (C), and “FCGR activation” (D) were enriched in SKCM samples with high expression of NLRP3. NES, normalized enrichment score.

Figure 6. Heatmaps showing genes co-expressed with NLRP3 in LUAD (A) and SKCM (B).
Fig. 7. Kaplan–Meier survival curve analysis of the prognostic significance of genes co-expressed with NLRP3 in LUAD and SKCM (A–L). (A–F). Correlation between NCKAP1L, CD84, SLA, CYBB, DOCK2 and CD86 with OS in LUAD. (G–L). High expression of CASS4, CLEC4A, CYBB, C3AR1, CSF1R and FPR1 was correlated with better OS in SKCM.

Figure 8. Correlation analysis of NLRP3 expression and infiltration levels of immune cells in LUAD(A) and SKCM(B). ns, p ≥ 0.05; *, p < 0.05; **, p < 0.01; ***, p < 0.001.
Figure 9. Chemokines positively correlated with NLRP3 expression in LUAD and SKCM (A-L).

(A-F) CCL2, CCL4, CCL13, CCL19, CCL23 and CXCL12 were significantly associated with expression of NLRP3 in LUAD patients. (G-L) CCL2, CCL3, CCL4, CCL5, CCL8 and CXCL16 were positively associated with high expression of NLRP3 in SKCM.