Clinical Signature and Associated Immune Metabolism of NLRP1 in Pan-Cancer

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Abstract

Scattered studies have shown the connection between NLRP1 which is responsible for inflammasome formation and tumor immunity as well as tumor metabolism; however, no research has yet systematically assessed the role and function of NLRP1 on various cancer types. Thus, in this study, data were retrieved and analyzed from public databases, and further showed that NLRP1 was differentially expressed in cancers. High NLRP1 expression was associated with a favorable prognosis for lung adenocarcinoma (LUAD) and pancreatic adenocarcinoma (PAAD). NLRP1 mutation status was also found to be associated with good prognosis. Further, NLRP1 expression was negatively related to tumor stemness, whereas positively related to immune infiltration of tumors. In addition, NLRP1 was found to be significantly related to tumor metabolism. A protein-protein interaction (PPI) network analysis was conducted for NLRP1, as well as pathways and functions of NLRP1. The study aimed to explore the role and function of NLRP1 in tumors by using pan-cancer analysis, and further suggested that NLRP1 may act as a promising therapeutic target for cancer.

1. Introduction

According to the statistics from the World Health Organization (WHO) and the International Agency for Research on Cancer (IRAC), there would be 19.29 million new cancer cases and 9.96 million cancer-related deaths in 2020 [1]. The worse is that the number of cancer patients has been increasing over the past few years. Thus, it is extremely urgent to find new paths to prevent and cure cancer. As an example, pancreatic adenocarcinoma (PAAD) is among the most lethal malignancies. The 1-year survival rate is only 20%, while the 5-year survival rate is less than 10% [2–5]. Since the early stage of pancreatic cancer is asymptomatic, over 80% of patients are diagnosed in the advanced stage (metastatic stage), which makes surgical intervention a dilemma. Besides, the recurrence of PAAD patients with complete surgical resection still remains high within 2 years [6–8]. Since the advantage of surgical treatment for PADD patients is limited, new therapy strategies are needed.

With the deepening of tumor immune research in recent years, it has been found that cell metabolism is a key factor in regulating the tumor immune microenvironment [9]. In addition, nutrient consumption and metabolite production can affect immune response, and the crosstalk among different cell types, including tumor cells and immune cells in a certain microenvironment also has a big influence on the tumor's growth and metastasis [10, 11]. Inflammasomes are at the intersection of innate immune recognition and metabolic control [12].

Pyroptosis, a type of programmed cell death brought on by inflammasomes, is a crucial natural immune response for organisms and aids in the defense against infection [13]. Immunotherapy has temporarily achieved remarkable success using inflammasomes to trigger cell pyroptosis and subsequently kill cancer cells [13, 14]. A small proportion of tumor cells which are undergoing pyroptosis is adequate to change the tumor immune microenvironment and then activate the anti-tumor immune response [15].
Nucleotide-binding oligomerization domain-like receptor protein 1 (NLRP1), the first NOD-like receptor (NLR) protein, is an inflammasome that is vital for both inflammation and innate immunity [16]. NLRP1 interacts with caspase-1, which is necessary to induce pyroptosis and then initiate the immune response [16, 17]. NLRP1 can prevent obesity and metabolic syndrome through IL-18 [18]. NLRP1 can also inhibit the development of intestinal tumors in animal models [19]. It is therefore hypothesized that NLRP1 may play a significant role in tumor immunity and metabolism and serve as an effective clinical biomarker.

However, up until now, there have been only a few studies on the role and function of NLRP1 in cancer studies, most of which are limited to a single type of cancer, and the relationship between NLRP1 and tumor immunity or metabolism is not involved. Therefore, in this study, we discussed the important role of NLRP1 in cancer from the aspects of clinical significance, tumor immune characteristics, tumor metabolic characteristics, tumor dryness, mutation effect, protein interaction network and functional pathway enrichment analysis based on pan-cancer analysis (Fig. 1). It is of great significance to study the role of NLRP1 in tumorigenesis because it can help us better understand the molecular mechanism of tumorigenesis, provide an important scientific basis for the development of effective and safe anti-tumor drugs, and help researchers better understand the process of tumorigenesis, so as to more effectively develop tumor treatment methods.

2. Materials and Methods

2.1 Data collection and analysis

The dataset of uniformly standardized pan-cancer and normal samples from The Cancer Genome Atlas (TCGA, https://portal.gdc.cancer.gov/), Therapeutically Applicable Research to Generate Effective Treatments (TARGET, https://ocg.cancer.gov/programs/target) and Genotype-Tissue Expression (GTEx, https://gtexportal.org/home/) [20] were downloaded from the UCSC database (University of California Santa Cruz Genome Browser, http://genome.ucsc.edu/) [21, 22]. The NLRP1 expression files for each sample along with clinical parameters such as age, gender and survival status were retrieved.

In this study, solid normal tissues, primary blood samples derived from cancer patients, and primary tumor tissues were screened. Log2 (x + 0.001) was used to transform each expression value. The final step was to exclude the cancer types that had fewer than three samples per type. This study’s data acquisition and analysis date were January 29, 2022. \( P \text{ value} < 0.05 \) was statistically significant, \( P \text{ value} < 0.05 \), \( P \text{ value} < 0.01 \), \( P \text{ value} < 0.005 \). The guidelines of the open database have been followed in the conduct of this study.

2.2 Differential expression analysis of NLRP1 in pan-cancer

The Human Protein Atlas (HPA, https://www.proteinatlas.org) database [23], was used to show the expression of NLRP1 in various tissues and cell lines. Meanwhile, the tumor data from the TCGA database were compared with the normal tissue data from the GTEx database and the differential expression between normal samples and tumor samples in different tissues was calculated using
To determine the significance of the difference, the unmatched Wilcoxon rank sum and signed rank tests were used, with a P value of 0.05 considered statistically significant.

### 2.3 Correlation of NLRP1 expression with the prognosis of tumor

The Kaplan Meier Plotter (http://kmplot.com/) [25] was utilized to determine the significance between NLRP1 gene expression and pan-cancer prognosis. The auto-select best cutoff was utilized to categorize patients (n = 7,462) into high-expression and low-expression groups. To further determine the best performer, all possible cutoff values between the lowest and highest quartiles were calculated. Patients surviving over the selected threshold are censored. Next, median survival was calculated and visualized by Kaplan–Meier survival plots [25].

Using Sangerbox 3.0 (http://vip.sangerbox.com) [43], the Cox proportional hazards regression model was used to analyze the correlation between gene expression and the overall survival (OS) and disease-specific survival (DSS) of each tumor [24]. The distribution of OS and DSS was assessed using the log-rank test.

### 2.4 NLRP1 expression in different single cells of different tissues

Using the deconvolution tool EPIC, gene expression analysis was performed in the Gene Expression Profiling Interactive Analysis GEPIA2021 database (http://gepia2021.cancer-pku.cn/) [26] to examine the difference in NLRP1 levels between tumor and normal tissues from the TCGA and GTEx databases in various single cells [26].

### 2.5 Protein-protein interaction network and functional enrichment analysis for NLRP1

STRING (https://cn.string-db.org/), a protein-protein interaction (PPI) database, was employed to conduct the PPI network analysis (minimum required interaction score:0.400) for NLRP1 [27]. Subsequently, functional enrichment was analyzed based on the interaction genes of the PPI network. For gene functional enrichment analysis, KEGG was utilized to obtain the latest gene annotation of the Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway [28]. Gene ontology analyses were performed with the Gene Ontology (GO) in the R software package org.Hs.eg.db (version 3.1.0) [29]. Consequently, the selected gene was mapped to the background set, and the R software package cluster profile (version 3.14.3) was used for enrichment analysis with the minimum gene setting of 5 and the maximum gene setting of 5,000 [30].

### 2.5 Correlation between NLRP1 expression and tumor stemness
mRNA expression-based stemness scores (RNAss) can be used as a positive correlation indicator of tumor stemness [31]. The Pearson correlation was calculated between RNAss stemness index and NLRP1 expression in each tumor sample.

2.6 Analysis of NLRP1 abnormal expression in tumor immune microenvironment

An integrated repository portal for tumor-immune system interactions (TISIDB) database (http://cis.hku.hk/TISIDB/index.php) [32], was utilized to investigate the interaction between NLRP1 and the 28 different types of tumor-infiltrating lymphocytes (TILs) in pan-cancer. On the basis of the NRP1 expression profile, an analysis of gene-set variation was performed to determine the relative abundance of TIL. TISIDB was also used to investigate the relationship between NLRP1 and the expression of three immune pathway marker genes, immune stimulant, MHC molecule, and chemokine receptor protein. The correlation was estimated by the Spearman test.

2.7 Association of NLRP1 abnormal expression and tumor metabolism

Gene sets of glycolysis metabolism, fatty acid metabolism, and amino acid metabolism were downloaded from the Molecular Signatures Database (MSigDB) [33]. In the PAAD and normal tissues, GEPIA2 (Gene Expression Profiling Interactive Analysis2, http://gepia2.cancer-pku.cn/) was used to analyze the relationship between NLRP1 and glycolysis, fatty acid metabolism, and amino acid metabolism [34]. The non-log scale was used for calculation and the log-scale was used for visualization. Pearson's correlation analysis was employed to assess the correlations between NLRP1 expressions and these molecular metabolisms in PAAD.

2.8 Relationship between NLRP1 mutations and the prognosis of tumor

A mutation frequency analysis of NLRP1 in various tumor types utilizing the cBioPortal database (http://www.cbioportal.org/) was conducted [35]. NLRP1 mutations in tumors were also investigated. A possible relationship between genetic alteration in NLRP1 and survival prognosis was investigated.

3. Results

3.1 NLRP1 was differentially expressed in various tumor tissues

Information on 33 types of tumors was obtained from The Cancer Genome Atlas (TCGA, https://portal.gdc.cancer.gov/) and analyzed (Table 1) [36]. Differential expression analysis was conducted to investigate the expression of NLRP1 in tissues and tumors. As Fig. 2A shows, the protein level of NLRP1 was highly expressed in the cerebral cortex, hippocampus adrenal gland, lung, oral
mucosa, stomach, kidney, placenta, skin, spleen, lymph node, tonsil, and so on. NLRP1 is the most highly expressed in the cerebral cortex and hippocampus among these tumors. NLRP1 expression had RNA single-cell type specificity (Fig. 2B). It was highly expressed in five kinds of cells, namely, early spermatids, muller glia cells, horizontal cells, intrinsic endothelial cells, and bipolar cells, among which the expression was highest in Early spermatids. NLRP1 was significantly upregulated in 13 types of tumors including GBM, LGG, KIRP, KIPAN, HNSC, KIRC, WT, PAAD, ALL, LAML, PCPG, CHOL), while downregulated in 20 types of tumors containing (UCEC, BRCA, CESC, LUAD, ESCA, STES, COAD, COADREAD, PRAD, STAD, LUSC, SKCM, BLCA, THCA, READ, OV, TGCT, UCS, ACC, KICH) (Fig. 2C). These results suggest that NLRP1 was differentially expressed in distinct types of tissues and tumors.
Table 1
The abbreviation of cancer type.

<table>
<thead>
<tr>
<th>Cancer Type (Abbreviation)</th>
<th>The Size of Samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>acute myeloid leukemia (LAML)</td>
<td>173</td>
</tr>
<tr>
<td>adrenocortical carcinoma (ACC)</td>
<td>77</td>
</tr>
<tr>
<td>uroepithelial carcinoma of the bladder (BLCA)</td>
<td>407</td>
</tr>
<tr>
<td>invasive breast cancer (BRCA)</td>
<td>1092</td>
</tr>
<tr>
<td>squamous cell carcinoma of the cervix and endocervical adenocarcinoma (CESC)</td>
<td>304</td>
</tr>
<tr>
<td>Cholangiocarcinoma (CHOL)</td>
<td>36</td>
</tr>
<tr>
<td>colonic adenocarcinoma (COAD)</td>
<td>288</td>
</tr>
<tr>
<td>esophageal cancer (ESCA)</td>
<td>181</td>
</tr>
<tr>
<td>glioblastoma multiforme (GBM)</td>
<td>153</td>
</tr>
<tr>
<td>head and neck squamous cell carcinoma (HNSC)</td>
<td>518</td>
</tr>
<tr>
<td>renal suspicious cell carcinoma (KICH)</td>
<td>66</td>
</tr>
<tr>
<td>renal clear cell carcinoma (KIRC)</td>
<td>530</td>
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<tr>
<td>renal papillary cell carcinoma (KIRP)</td>
<td>288</td>
</tr>
<tr>
<td>low grade glioma (LGG)</td>
<td>509</td>
</tr>
<tr>
<td>hepatocellular carcinoma (LIHC)</td>
<td>369</td>
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<tr>
<td>lung adenocarcinoma (LUAD)</td>
<td>513</td>
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<tr>
<td>lung squamous cell carcinoma (LUSC)</td>
<td>498</td>
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<tr>
<td>lymphoid neoplasm spreading large b-cell lymphoma (DLBC)</td>
<td>48</td>
</tr>
<tr>
<td>Mesothelioma (MESO)</td>
<td>87</td>
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<tr>
<td>ovarian plasmacytoid cystic adenocarcinoma (OV)</td>
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<tr>
<td>pancreatic adenocarcinoma (PAAD)</td>
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<tr>
<td>pheochromocytoma and paraganglioma (PCPG)</td>
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<tr>
<td>prostate adenocarcinoma (PRAD)</td>
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<tr>
<td>rectal adenocarcinoma (READ)</td>
<td>105</td>
</tr>
<tr>
<td>sarcoma (SARC)</td>
<td>265</td>
</tr>
<tr>
<td>cutaneous melanoma (SKCM)</td>
<td>474</td>
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<tr>
<td>Cancer Type (Abbreviation)</td>
<td>The Size of Samples</td>
</tr>
<tr>
<td>-----------------------------------------------</td>
<td>---------------------</td>
</tr>
<tr>
<td>gastric adenocarcinoma (STAD)</td>
<td>450</td>
</tr>
<tr>
<td>testicular germ cell tumor (TGCT)</td>
<td>156</td>
</tr>
<tr>
<td>Thymoma (THYM)</td>
<td>122</td>
</tr>
<tr>
<td>thyroid cancer (THCA)</td>
<td>572</td>
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<tr>
<td>uterine carcinosarcoma (UCS)</td>
<td>57</td>
</tr>
<tr>
<td>endometrial cancer of the uterine corpus (UCEC)</td>
<td>201</td>
</tr>
<tr>
<td>uveal melanoma (UVM)</td>
<td>80</td>
</tr>
</tbody>
</table>

### 3.2 NLRP1 was significantly correlated with the prognosis of different types of tumors

Kaplan Meier Plotter analysis was used to determine if differential NLRP1 expression has an impact on tumor prognosis. It was found that high expression of NLRP1 was negatively associated with better OS for KIRC (HR = 1.74, Log-rank P = 6e-04) (Fig. 3A), in contrast, high expression of NLRP1 was positively related to OS in BLCA (HR = 0.67, Log-rank P = 0.034), ESCA (HR = 0.48, Log-rank P = 0.042), HNSC (HR = 0.65, Log rank P = 0.0017), LUAD (HR = 0.58, Log rank P = 0.00019), PAAD (HR = 0.51, Log rank P = 0.0012) (Fig. 3B-F). In the other tumors, there was no significant correlation with OS.

To demonstrate the reliability of these results, a Cox proportional hazards regression model was developed to further analyze the NLRP1’s expression in various cancers. The OS Forest plot (Fig. 4A) showed that high expression of NLRP1 was a high-risk gene in three types of tumors including KIPAN (HR = 1.24, P = 6.0e-5), KIRC (HR = 1.22, P = 0.02), LGG (HR = 1.39, P = 0.06), while it was a low-risk gene in eight types of tumors, including PAAD, LUAD, SKCM, NB, ALL, ALL-R, HNSC, SKCM-M. In addition, the DSS forest plot (Fig. 4B) showed high expression of NLRP1 was associated with increased risk of death in KIPAN (HR = 1.15, P = 0.03) and COAD (HR = 1.30, P = 0.04), however, high expression of NLRP1 was associated with decreased risk of death in PAAD (HR = 0.78, P = 1.7e-3), LUAD (HR = 0.8, P = 7.7e-3), SKCM (HR = 0.9, P = 0.03). Altogether, the NLRP1 expression level was positively associated with prognosis in LUAD and PAAD.

### 3.3 NLRP1 expression in various tissues cells

LUAD and PAAD patients with high levels of NLRP1 were associated with a favorable prognosis, as shown by the data above. However, NLRP1 expression varies between LUAD and PAAD. It was down-expressed in LUAD, whereas it was over-expressed in PAAD (Fig. 3, 4). The expression of NLRP1 in different tissues and cells was examined to better understand the cause of this phenomenon (Fig. 5). In lung tissue, NLRP1 was obviously expressed in immune cells but rarely in endothelial cells (Fig. 5A). NLRP1 expression was decreased more in endothelial cells of LUAD as compared to normal lung tissues; however, NLRP1 in macrophages did not significantly change in LUAD (Fig. 5C). Immune cells such as
macrophages in LUAD may positively correlate with NLRP1 expression. In contrast, NLRP1 was highly expressed in endothelial cells of normal pancreatic tissues but almost undetectable in macrophages and other immune cells (Fig. 5B). Compared with normal pancreatic tissue, NLRP1 was upregulated both in endothelial cells and macrophages in PAAD (Fig. 5D). The significant increase in endothelial cells in PAAD may be the primary cause of the increased NLRP1 expression in this disease. However, a slight rise in immune cells coincides with an increase of NLRP1 expression in PAAD, giving the condition a favorable prognosis of this cancer.

3.4 Protein-protein interaction network and functional enrichment analysis of NLRP1

The PPI analysis showed that 21 proteins, including AIM2, APAF1, BAD, BCL2L1, BCL2L11, BECN1, BIK, CASP1, CASP5, DHX33, IL1B, MEFV, NEK7, NLRC4, NLRP3, NLRP9, PMAIP1, PSTPIP1, PYCARD, and TP53 formed a protein network with NLRP1 (Fig. 6A). As shown in Fig. 6B, KEGG pathways analysis of the network proteins indicated that NLRP1 is related to the NOD-like receptor signaling pathway, apoptosis, platinum drug resistance, infection, and P53 signaling pathway. Among these pathways, the P53 signaling pathway and platinum drug resistance are tumor-associated pathways [37], NOD-like receptor signaling pathway is an immune-related pathway [38], demonstrating the importance of NLRP1 in tumors and immunity. Go enrichment analysis of these proteins displayed that NLRP1 was significantly associated with cell apoptosis and cysteine-type endopeptidase activity, etc. for biological processes (Fig. 6C), cell membrane and mitochondria, etc. for cell composition (Fig. 6D), and apoptotic process for molecular function (Fig. 6E), which was consistent with KEGG pathways analysis.

3.5 NLRP1 negatively correlates with tumor stemness

As shown in Fig. 6F, NLRP1 was negatively associated with RNAss in 33 tumors with the statistical significance, including PRAD, TGCT, KIRC, COAD, KIPAN, COADREAD, KICH, LAML, READ, UVM, STAD, LUAD, PAAD, SKCM, KIRP, DLBC, BRCA, CHOL, STES, ESCA, THCA, SARC, LUSC, UCS, MESO, CESC, UCEC, BLCA, LIHC, LGG, ACC, HNSC, OV, GBMLGG, indicating NLRP1 was significantly negatively associated with tumor stemness.

3.6 Correlation between NLRP1 and immune microenvironment across pan-cancer

In this section, the analyses showed that NLRP1 expression was positively correlated with 28 different types of TILs with statistical significance (Fig. 7A). In PAAD, NLRP1 was positively correlated with other TILs except for Th17, CD56, IDC, and Monocyte. Immunotherapy has been observed to have a substantial effect on the outcomes of certain cancers. The immune pathway-related marker genes were explored by the Cancer Immunology Atlas (TCIA), which predicted the response of tumors to immunotherapy [39]. In Fig. 6B-D, the expression level of NLRP1 was correlated significantly with three types of immune pathway-related marker genes. In more detail, NLRP1 was positively related to most MHC genes with statistical significance in pan tumors except for CESC,
HNSC, PCPG, and THCA (Fig. 7B). NLRP1 was also significantly associated with most immune-stimulating genes except for CD276, HHLA2, NTSE, PVR, TNFSF18 and UNBP1 in the HNSC-excluded tumors (Fig. 7C). Additionally, NLRP1 was significantly and positively connected to most chemokine receptor genes excluding CCR3, CCR9, and XCR1 with statistical significance in pan tumors (Fig. 7D). These results illustrated that the expression level of NLRP1 was notably correlated with the immune pathway.

Since NLRP1 was associated with immune infiltrating cells and immune pathway genes, further studies were conducted to determine whether it is related to common immune subtypes of tumors. Figure 7E showed that the most of the associations between NLRP1 expression and human cancer immune subtypes were not 0, and some were higher, indicating that NLRP1 expression was significantly different in most tumor immune subtypes. NLRP1 was highest expressed in the C2 subtype of HNSC, C3 subtype of LUAD, the C3 subtype of PAAD, and the C3 subtype of BRCA among subtypes with more than 5 samples (Fig. 7F-I). Several studies had shown that different immune subtypes had diverse influences on tumor prognosis [40]. The different impacts of NLRP1 on the prognosis of various tumors may be explained by the variable expression of NLRP1 in distinct subtypes of tumors.

### 3.7 The association of NLRP1 with tumor metabolic reprogramming

The tumor immune microenvironment and tumor metabolism are closely linked with each other. Possible improvements in tumor immunotherapy by focusing on the tumor metabolic system [41]. For PAAD, for example, NLRP1 expression in normal tissues was negatively correlated with genes controlling glycolytic metabolism compared with tumors (Fig. 8A-B). NLRP1 expression in normal tissues was negatively correlated with genes involved in the fatty acid metabolism compared with tumors (Fig. 8C-D). Moreover, NLRP1 expression in normal tissues was negatively correlated with genes involved in the amino acid metabolism compared with tumor (Fig. 8E-F). These data may explain the relationship between NLRP1 and tumor metabolism.

### 3.8 NLRP1 mutations in different tissues

Some gene mutations are common in some malignancies and are strong predictors of tumor prognosis [42]. According to the cBioPortal database, NLRP1 mutations are relatively common in SKCM (15%) and UCEC (10%) (Fig. 9A). Additionally, there were 311 mutation sites in the NLRP1 (243 missense mutations, 44 truncation mutations, 1 in-frame mutation, 15 shear mutations, and 8 fusion mutations), with E739K being the most common mutation (Fig. 9B). In addition, the association analysis between the gene alterations of NLRP1 and the clinical survival prognosis of pan-cancer cases in the TCGA illustrated that the cases with the NLRP1 mutation had a good prognosis in OS (N = 10,804, P = 1.514e-3) and DSS (N = 10,259, P = 9.833e-4) (Fig. 9C, D). Taking together, NLRP1 mutations could act as a predictor of tumor prognosis.

### 4. Discussion
NLRP1, was associated with innate immunity [43]. It played a significant role in tumor immunity as a critical gene of cell pyroptosis [44]. In addition to immunity, NLRP1 may also be related to metabolism. Previous studies had shown that inflammasomes were associated with immune and metabolic diseases [12]. Additionally, the relationship between NLRP1 and tumors has come to the foreground. NLRP1 had various functions in different types of tumors, for example, NLRP1 upregulation reduced the occurrence of colitis-related tumors [19].

It was worthwhile to investigate the effect of NLRP1 on tumor immunology and metabolism since it may have a variety of essential functions in various types of tumors. Available bioinformatics analyses previously reported that several scorch death-related genes, including NLRP1, play a key role in cancer immunity and may be employed as prognostic factors in pancreatic [45], breast [46], lung, and head and neck squamous cell carcinomas [47, 48]. There are also findings confirming that reduced NLRP1 expression is significantly associated with poor prognosis and low immune cell infiltration in patients with LUAD [16].

In previous studies, the research on NLRP1 was restricted to specific tumors and did not consider tumor immunity or immune metabolism. Moreover, a few studies had been conducted on the function of NLRP1 in various tumors. Moreover, the relationship between NLRP1 mutations and expression level with patient prognosis, as well as NLRP1 and tumor immunity, was unclear. According to this study, NLRP1 plays a key role in expression, prognosis, immunity, metabolism, stemness, and mutation in pan-cancer (a summary of the results can be found in Table 2).
Table 2
A comparison of the findings in this study with the earlier related studies in the literature.

<table>
<thead>
<tr>
<th>No.</th>
<th>Interesting Results</th>
<th>Thoroughly mentioned earlier</th>
<th>Partially mentioned earlier</th>
<th>Explored in this study</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>The expression of NLRP1 had cell specificity, and its distribution had tissue specificity.</td>
<td>×</td>
<td>×</td>
<td>√</td>
</tr>
<tr>
<td>2</td>
<td>NLRP1 was significantly upregulated in 13 tumors and significantly downregulated in 20 tumors.</td>
<td>×</td>
<td>[16, 49]</td>
<td>√</td>
</tr>
<tr>
<td>3</td>
<td>NLRP1 expression was significantly related to prognosis in multiple cancers. A significant correlation existed between the elevated NLRP1 expression and the good prognosis of PAAD and LUAD.</td>
<td>[45–48]</td>
<td>\</td>
<td>√</td>
</tr>
<tr>
<td>4</td>
<td>RNAss tumor stemness score and NLRP1 expression were significantly negatively correlated in all 34 tumors.</td>
<td>×</td>
<td>×</td>
<td>√</td>
</tr>
<tr>
<td>5</td>
<td>The expression of NLRP1 was remarkably correlated with ten infiltrating immune cells in most tumors.</td>
<td>×</td>
<td>[16]</td>
<td>√</td>
</tr>
<tr>
<td>6</td>
<td>A robust and significant relationship existed between NLRP1 and expression levels of recognized immune stimulant, MHC molecule, and chemokine receptor protein in most cancers.</td>
<td>×</td>
<td>×</td>
<td>√</td>
</tr>
<tr>
<td>7</td>
<td>NLRP1 expression levels were significantly correlated with immune subtypes in tumors.</td>
<td>×</td>
<td>×</td>
<td>√</td>
</tr>
<tr>
<td>8</td>
<td>NLRP1 had relatively high mutation levels in SKCM (15%) and UCEC (10%). NLRP1 had 311 mutation sites in pan-cancer.</td>
<td>×</td>
<td>×</td>
<td>√</td>
</tr>
<tr>
<td>9</td>
<td>Patients with NLRP1 mutations had a better prognosis in terms of OS and DSS.</td>
<td>×</td>
<td>×</td>
<td>√</td>
</tr>
<tr>
<td>10</td>
<td>PPI network analysis and enrichment analysis showed that NLRP1 is associated with cellular metabolism, inflammatory response, apoptotic processes and the development of some diseases.</td>
<td>×</td>
<td>×</td>
<td>√</td>
</tr>
<tr>
<td>11</td>
<td>NLRP1 expression is metabolically relevant in tumors such as LUAD and PAAD.</td>
<td>×</td>
<td>×</td>
<td>√</td>
</tr>
<tr>
<td>12</td>
<td>The statistically significant increase in both endothelial and macrophage cells of the tumor samples compared with the normal control, may account for the reason why the elevated expressions of NLRP1 in PAAD and LUAD show a favorable effect on the prognosis of these two cancers.</td>
<td>×</td>
<td>×</td>
<td>√</td>
</tr>
</tbody>
</table>

Some studies reported that the NLRP1 expression in colorectal tumor cells was lower than that in adjacent normal tissues, which was related to a high tumor metastasis rate and shortened survival,
whereas increased expression of NLRP1 can inhibit tumor growth [19], which was consistent with this study.

There was further investigation into NLRP1 functions, which led to intriguing discoveries.

4.1 NLRP1 is tightly linked to the tumor immune microenvironment.

The identification of immunological checkpoints (ICTs) that control immune response offers a new idea for cancer treatment and increases the likelihood that more patients with metastatic disease may have long-term clinical remission and even be cured, but ICT has little impact on some patient populations. Unfortunately, there are no measures to determine whether ICT is effective. Thus, it is necessary to find new biomarkers or therapeutic strategies for cancer treatment [50].

This study found strong evidence of a link between NLRP1 expression and immune infiltration. Moreover, another study also reported that low levels of immune cell infiltration and poor prognosis were substantially associated with lower expression of NLRP1 [16], which was consistent with this study. This study discovered for the first time that NLRP1 expression was significantly different in various tumors (Fig. 2) and correlated with immune cell infiltration, immune subtypes and immune related pathways (Fig. 7), proving that NLRP1 was both essential to the tumor immune microenvironment and tumor immunotherapy. This study examined the relationship between NLRP1 level and the immune environment in the analyses we performed, which were anticipated to be able to forecast or influence the development of immunotherapy in a range of tumors and give novel guidance for cancer treatment.

4.2 NLRP1 regulates tumor progression by influencing the metabolic environment.

The function of inflammasomes, particularly NLRP1, in tumor metabolism has not yet been investigated. By influencing the metabolic environment, NLRP1 regulates tumor progression. Altered metabolism and metabolic crosstalk of glucose, amino acids and lipids in the pancreatic cancer microenvironment have been reported to lead to unrestricted increases in metabolism and contribute to the unrestricted growth of pancreatic cancer [51]. In the tumor microenvironment, the main metabolites of tumor cells, lactic acid and transforming growth factor-β (TGF-β) can inhibit the activation of inflammasomes to escape immune surveillance [52]. These previous studies revealed that increased metabolism in tumors contributes to growth and immune escape. In this study, NLRP1 was found to be significantly negatively associated with glycolytic metabolism, fatty acid metabolism and amino acid metabolism in adjacent normal tissues, but not in PAAD, where NLRP1 lost its effect on metabolism. These results collectively revealed that NLRP1 was negatively correlated with metabolism. However, metabolic reprogramming during the development of some tumors, including PAAD, breaks the relationship between NLRP1 and tumor metabolism, ensuring the development of tumor metabolism and immune escape [53].
4.3 NLRP1 mutations associated with good tumor prognosis

We revealed that the NLRP1 mutation may be related to a good prognosis for the tumors (Fig. 9C, D). Since this study analyzed the effect of NLRP1 mutations in pan-cancers, the prognosis effect on a single tumor still needed further study. However, this study showed that NLRP1 mutations can be used as a reliable biomarker for the prognosis with good biological characteristics and better prognostic value in pan-cancer.

4.4 NLRP1 expression negatively correlates with tumor stemness

Cancer stem cells (CSCs) are characterized as a kind of self-renewing cell type that have been identified in most cancers, which contribute to the initiation and progression of most cancers. CSCs are identified based on the expression of different cell surface markers and are varied in different tumor types, which may be affected by the signals from CSCs and tumor microenvironment (TME) [54]. To date, the relationship between inflammasomes and tumor stemness has not been studied. In this study, NLRP1 was shown to be inversely connected with the tumor stemness index of RNAss, suggesting that high expression of NLRP1 would be accompanied by a decrease in tumor metastasis, as well as a comparative reduction of the drug resistance and self-renewal ability of tumors.

NLRP1 is differentially expressed in different cells in tissues. In this study, the expression of NLRP1 in immune cells was found to be stable and elevated whether in LUAD or normal lung tissue. However, the expression of NLRP1 in PAAD differed dramatically from that in normal pancreatic tissue. NLRP1 is highly expressed in non-immune cells such as endothelial cells, and nearly absent in immune cells such as macrophages in normal pancreatic tissue. While NLRP1 increased significantly in endothelial cells in the development of PAAD, which may be the cause of NLRP1 being highly expressed in PAAD than in normal pancreatic tissue. However, NLRP1 in macrophages and other immune cells also increased in PAAD, which may stimulate the immune response, thereby improving the tumor microenvironment and the prognosis of patients with PAAD. The variability of tumor and normal tissue microenvironment may lead to different expressed patterns in the same parts. The unique environment of normal pancreatic tissue and PAAD influenced the expression characteristics of NLRP1.

In conclusion, NLRP1 had a significant impact on the expression, prognosis, immunity, metabolism, stemness, and other features of cancer. Its mutation also affected patients’ prognosis. The upstream and downstream regulations of NLRP1 were needed to further investigate. This study explored the relationship between NLRP1 and tumor immune microenvironment and tumor metabolism in pan-cancer, especially in PAAD. The different characteristics of NLRP1 in various tumors indicated that the expression and distribution of NLRP1 may affect the metabolism and immune response in the tumor microenvironment, which could further affect prognosis. Furthermore, the clinical significance of NLRP1
expression in various cancers and its associated mechanisms may be complicated and influenced by a variety of circumstances the mechanisms still need to be further investigated.

5. Conclusion

In this study, the first comprehensive pan-cancer analysis of NLRP1 may help to elucidate the role of NLRP1 in the development of tumors, offering new strategies for tumor treatment, and reserving potential useful resources for the development of immunometabolic therapy in the future.

Declarations

Author Contributions

ZH, YL and JW designed the project. YL, PY and JW performed the analysis. YL, PY, and JW wrote the manuscript with the supervision of ZL and ZH. All authors approved the submitted version.

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Data Availability Statement

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

Conflicts of Interest

The authors declare no conflict of interest.

References


Figures
Figure 1

The flow chart of the study.
Figure 2

Differential expression of NLRP1. (A) Expression of NLRP1 in different normal tissues. (B) Expression of NLRP1 in different single cells. (C) Expression of NLRP1 in multiple cancer tissues and the adjacent normal tissues.
Figure 3

Overall survival curves of NLRP1 in multiple cancer samples.
Figure 4

The forest plot for the association of NLRP1 expression with (A) overall survival (OS), and (B) diseasespecific survival (DSS).
Figure 5

Expression of NLRP1 in different cells. (A) Expression of NLRP1 in different cells in the lung. (B) Expression of NLRP1 in different cells in the pancreas. (C) Differential expression of NLRP1 in macrophages and epithelial cells in lung adenocarcinoma and normal controls. (D) Differential expression of NLRP1 in macrophages and epithelial cells in pancreatic cancer and normal controls.
Figure 6

Interaction network, functional enrichment of NLRP1 and correlations between NLRP1 expression and tumor stemness. (A) Protein-protein interaction network by STRING software. (B)-(E): KEGG and GO enrichment analyses of NLRP1, showing the top ten results. (B) KEGG pathway enrichment. (C) Biological process in GO pathway enrichment. (D) Cell composition in GO pathway enrichment (E) Molecular function in GO pathway enrichment (F) Correlations between NLRP1 expression and RNAss.
Figure 7

Correlations between the expression of NLRP1 and those of (A) TILs; (B) MHC markers; (C) immune stimulating makers; (D) chemokine receptors; (E) immune subtypes; (F) immune subtypes in HNSC; (G) immune subtypes in LUAD; (H) immune subtypes in PAAD; (I) immune subtypes in BRCA.
Figure 8

Correlation between the expression of NLRP1 and those of (A) glycolytic genes in normal samples; (B) glycolytic genes in PAAD patients; (C) fatty acid metabolism gene in normal samples; (D) fatty acid metabolism gene in PAAD patients; (E) amino acid metabolism gene expressions in normal samples; (F) amino acid metabolism gene in PAAD patients.
Figure 9

Relationship between NLRP1 mutations and the prognosis of tumor. (A) The highest frequency of PRKDC alteration in the majority of tumors. (B) Exhibition of the type of alterations, mutated sites, and mutation cases. (C) Correlation between NLRP1 mutation and progression OS of pan-cancer. (D) Correlation between NLRP1 mutation and progression DSS of pan-cancer.