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The expression profiling and clinical significance of a chronic pain-related gene IL1R1

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ABSTRACT

Chronic pain is a disease that existed during cancer treatment for a long time. It has been reported that interleukin (IL)-1 is involved in the inflammatory response during tumor development. IL1R1 and IL1R2 are members of the IL-1 receptor family of cytokine receptors. However, few studies have reported the role of chronic pain-related genes, IL1R1, in pan-cancer. In this study, 8 lumbar disc prolapse (LDP) patients and 8 controls with differentially expressed genes were investigated to find chronic pain-related genes. Then, IL1R1 was analyzed using the TCGA database. The clinical survival data from TCGA were used to analyze the prognostic value of IL1R1. This study further evaluated the relationship between IL1R1 and immune checkpoints, immune-activating genes, immunosuppressive genes, chemokines, and chemokine receptors. IL1R1 was expressed in varying degrees in most TCGA tumor types, indicating a better survival status. The expression of IL1R1 is closely related to T cell infiltration, immune checkpoints, immune-activating genes, immunosuppressive genes, chemokines, and chemokine receptors. The results show that IL1R1 is a kind of potential cancer biomarker. Coordination with other immune checkpoints IL1R1k may adjust the immune microenvironment, immunotherapy can be applied to the development of new targeted drugs.

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Introduction

Chronic pain validation affects patients' quality of life (1), Chronic pain is usually divided into three categories: pain caused by tissue disease or injury (injurious pain, such as osteoarthritis), pain or injury caused by disorders of the somatosensory system (neuropathic pain), and the coexistence of injurious pain and neuropathic pain (mixed pain) (2), Current bioinformatics analyses of pain studies are mainly in mouse models (3, 4), and scholars have revealed that CCl3 and SCI are associated with chronic neuropathic pain (NP) (5). There are few bioinformatics studies on the painfulness surrounding the Homo sapiens model. However, pain is one of the most common symptoms in cancer patients, especially those with advanced metastatic disease (6, 7), Chronic pain is a global public health problem, even though several experimental methods have been used to study pain mechanisms (8, 9), the underlying molecular mechanisms are not fully understood.

In this study, 8 patients with LDP were analyzed with bioinformatics methods, and 8 control groups with differential gene expression were studied to find chronic pain-related genes. Five genes (IL1R1, GRIK1, IL1R2, CTSG, CRHR2) were selected as important DEGs of chronic pain, especially IL1R1. Using the TCGA database to study the pan oncogenesis of IL1R1, In this study, TIMER was used to examine the correlation between the expression of IL1R1 and infiltrating immune cells. The results show that IL1R1 is a potential cancer biomarker. In coordination with other immune checkpoints, IL1R1 could modulate the immune microenvironment and serve for the development of new targeted immunotherapeutic agents.

Materials and Methods

Dataset collection

The GEO (http://www.ncbi.nlm.nih.gov/geo) database was used to screen the gene expression profiles for "chronic pain". Inclusion criteria were as follows : (i) patients with chronic pain or healthy joints in the dataset; (ii) six or more samples in the data set. We selected an eligible dataset including GSE124272 (10) (Expression profiling by array, GPL21185), which analyzed the disease of lumbar disc herniation (LDP).

The Cancer Genome Atlas (TCGA) and Genotype-Tissue Expression (GTEx) RNA expression and clinical data were downloaded from the UCSC Xena database (https://xenabrowser. net/data pages/).

Differentially expressed gene screening

Differentially expressed genes were identified by calculating differential expression p-values and fold changes using the limma package(11) (Version 3.26.9). *P* values were corrected for multiple tests using Benjamini and Hochberg's method. the *P* values <0.05 and |log2FC|>1 were considered thresholds for differentially expressed genes.

Gene enrichment analysis

Names of genes of DEGs were transformed into gene IDs by the R package "org.Hs.eg.db". Gene Ontology

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Analysis (GO) and Kyoto Encyclopedia of Genes and Genomes Analysis (KEGG) were performed with the R "clusterProfiler" (12) package to further investigate the plausible functions of these DEGs. By threshold *P* values < 0.05 and q < 0.05 screen differences obvious GO terms and signaling pathways. Results were visualized by the R packages "enrichplot" and "ggplot2".

Prognostic analysis

Kaplan Meier analysis was employed to evaluate the overall survival (OS) of patients in the TCGA cohort. Univariate Cox regression analysis assessed the significance of IL1R1 in predicting pan-cancer OS.

Gene set enrichment analysis

The TCGA data were used to analyze the correlation of IL1R1 with all genes. Pearson's correlation coefficients were calculated. gene set enrichment analysis (GSEA) was performed by selecting genes associated with IL1R1 ($p < 0.05$). This is performed by GSEA using the R package "clusterProfiler" with the following parameters: sperm=1000, minGSSize=10, maxGSSize=1000, *P*-value-Cutoff=0.05. Gene sets were selected from the Reactome pathway database for GSEA.

Immune Cell Infiltration

In this study, the immune cell infiltration score of TCGA was downloaded and analyzed from the TIMER2 database (HTTP:// timer.cistrome.org/), as well as a previously published study For each TCGA tumor type, the patients were divided into two groups (high and low expression according to the median IL1R1 expression level) to compare the degree of immune cell infiltration.

Results

Screening of differentially expressed genes

One microarray raw dataset, including 8 LDP and 8 control, was selected for this study. The data contains 32542 genes. Through differential expression analysis of the dataset, A series of 318 genes differentially expressed (194 upregulated and 124 downregulated) had significant differential expression in the disease group compared with the control group at a threshold of $|\log2FC| \geq 1$, $P < 0.05$. Expression of DEGs being visualized in the volcano plot (Figure 1A) and heat map (Figure 1B).

IL1R1 as pain related genes expression analysis in pan-cancer

This study searched the chronic pain-related genes in the DEGs that were selected as the important DEGs of chronic pain, especially IL1R1. Recent studies have indicated that IL1R1 might participate in the process of cell death and inflammation (13, 14). However, the IL1R1 function is still not clear in pan-cancer.

The mRNA expression level of IL1R1 was analyzed in various cancer types. The results demonstrated that high expression of IL1R1 was found in Stomach adenocarcinoma (STAD). In contrast, low expression of IL1R1 was observed in 16 tumors: Bladder Urothelial Carcinoma (BLCA), Breast invasive carcinoma (BRCA), Cervical squamous cell carcinoma, and endocervical adenocarcinoma (CESC), Colon adenocarcinoma (COAD), Cholangiocarcinoma (CHOL), Kidney Chromophobe (KICH), Kidney renal clear cell carcinoma (KIRC), Kidney renal papillary cell carcinoma (KIRP), Liver hepatocellular carcinoma (LIHC), Lung adenocarcinoma (LUAD), Lung squamous cell carcinoma (LUSC), Pheochromocytoma and Paraganglioma (PCPG), Prostate adenocarcinoma (PRAD), Rectum adenocarcinoma (READ), Thyroid carcinoma (THCA) and Uterine Corpus Endometrial Carcinoma (UCEC) (Figure 1C), Furthermore, the expression of IL1R1 in tumor tissues of TCGA was highest in Uveal Melanoma (UVM), Uterine Carcinosarcoma (UCS), and Uterine Corpus Endometrial Carcinoma (UCEC) (Figure 1D). For the normal human tissues in GTEx, the highest expression of IL1R1 was found in the liver, mammary gland, and thyroid. (Figure 1E).

The present study further evaluated IL1R1 expression in different WHO cancer stages and revealed that in the high stages of most tumors, IL1R1 expression was low, including LUAD, and LUSC (Figure 2). On the contrary, IL1R1 expression was higher at higher stages in BLCA, KICH, KIRC, STAD and ESCA (Figure 2). IL1R1 was lowly expressed in BRCA, COAD, KICH, KIRC, LUAD,

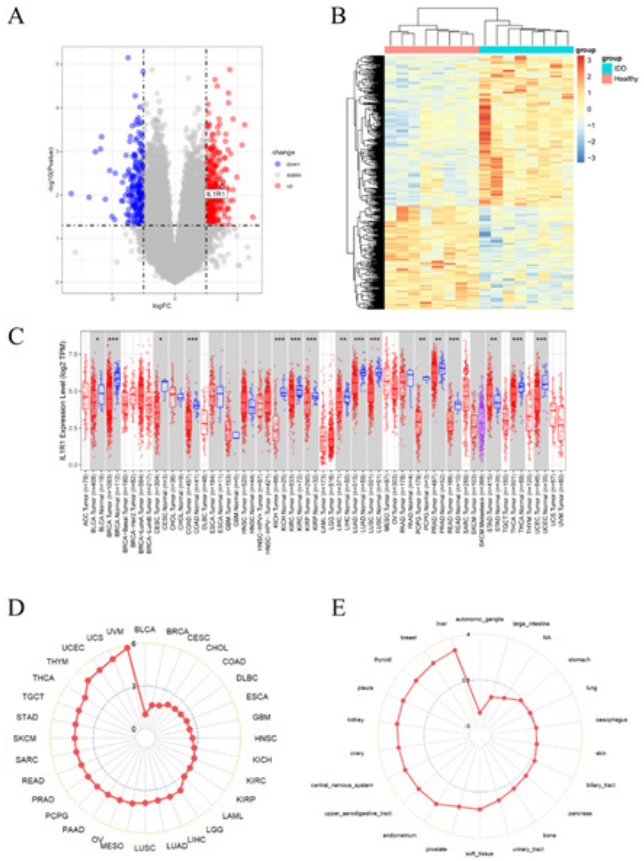

Figure 1. A) Volcano plots displaying significantly differentially expressed genes in LDP and Con. Red dots represent the upregulated genes and blue dots denote the downregulated genes, with thresholds of $|\log2FC| \geq 1$ and adjusted $P < 0.05$. (B) Heatmap displaying the expressions of the 318 DEGs in LDP and Con. Red bricks indicate the more highly expressed DEGs and blue bricks indicate lower expression. (C)Pan-cancer expression of IL1R1 between tumor tissues from TCGA database and normal tissues from TCGA and GTEx database. (D) IL1R1 expression in tumor tissues from TCGA database. The location of the dot represents the mean value of the IL1R1 expression. (E) IL1R1 expression in normal tissues from GTEx database. The location of the dot represents the mean value of IL1R1 expression. *P < 0.05; **P < 0.01; ***P < 0.001; ns, not significant.

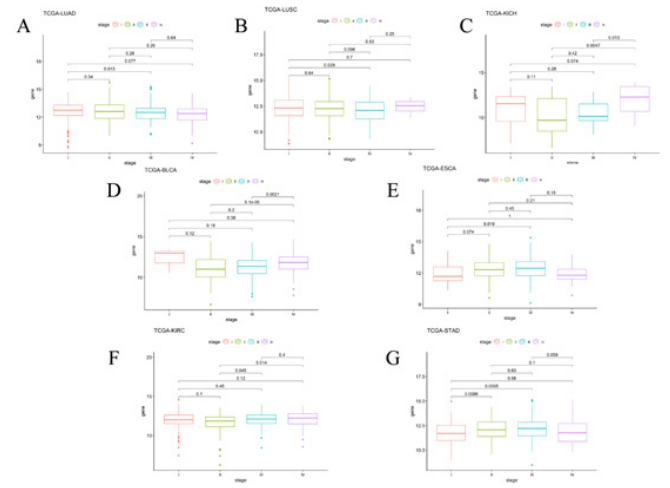

Figure 2. Pan-cancer IL1R1 expression in different WHO stages. (A-J), Pan-cancer differential expression of IL1R1 in WHO stages in indicated tumor types from TCGA database. *P < 0.05; **P < 0.01; ***P < 0.001; ns, not significant.

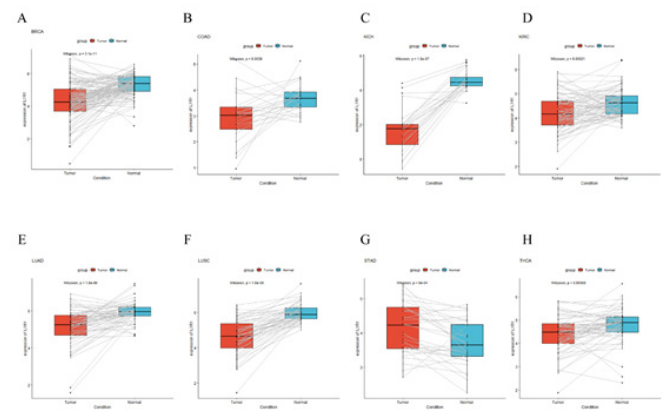

Figure 3. Pan-cancer paired IL1R1 expression. (A-H) Pan-cancer differential expression of IL1R1 in paired tumor and adjacent normal tissues in indicated tumor types from TCGA database. **P < 0.01; ***P < 0.001.

and LUSC and highly expressed in STAD and THCA in TCGA pan-cancer paired tumors and normal tissues (Figure 3)

Prognostic significance of IL1R1

In this study, the prognostic significance of IL1R1 in cancer patients was further evaluated. The findings of the Kaplan-Meier OS analysis demonstrated that IL1R1 was a protective factor for SKCM patients and a risk factor for patients with ACC, OS, KIRP, LGG, STAD, and UVM (Figure 4).

According to the results of univariate Cox regression analysis, results of OS indicated that IL1R1 plays a protective role for SKCM patients, while risk factors for UVM, STAD, LGG, KIRP, KICH, GBM, and BRCA patients. (Figure 5).

GSEA of IL1R1

In the present study, 33 tumor types of TCGA were evaluated using GSEA to assess the possible pathways involved in IL1R1. Results showed a significant association of IL1R1 with cellular life cycle pathways, particularly TNF signaling via NF-kB, β signaling, Kras signaling, inflammatory response, and apoptosis, such as in GBM, LGG, SARC, PCPG, TGCT, and UCS (Figure 6). All these

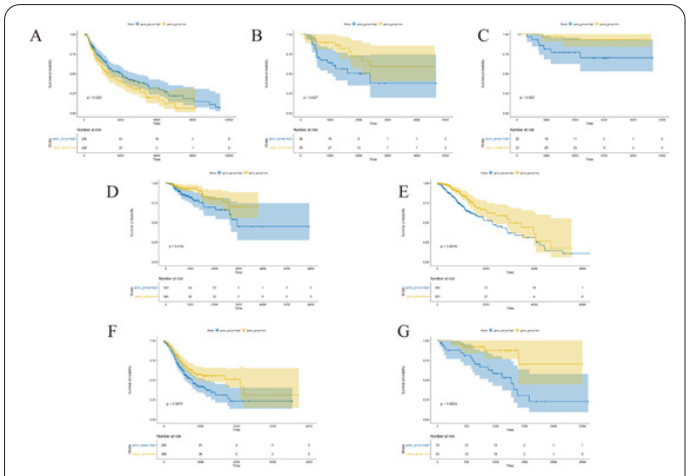

Figure 4. Kaplan-Meier overall survival of IL1R1. (A–I) Pan-cancer Kaplan-Meier overall survival of IL1R1 in indicated tumor types from TCGA database. The median value of IL1R1 in each tumor was taken as the cut-off value.

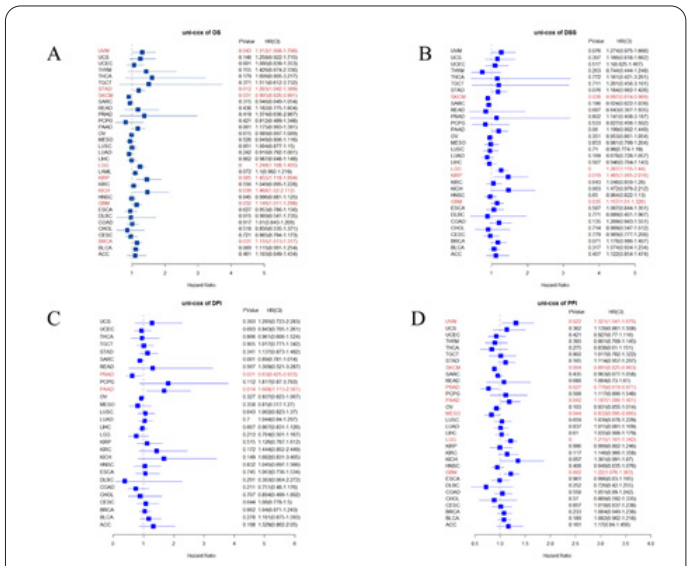

Figure 5. Univariate Cox regression analysis of IL1R1. (A) The Forest map shows the univariate cox regression results of IL1R1 for OS in TCGA pan-cancer. (B) The Forest map shows the univariate cox regression results of IL1R1 for DSS in TCGA pan-cancer. (C) The Forest map shows the univariate cox regression results of IL1R1 for DFI in TCGA pan-cancer. (D) The Forest map shows the univariate cox regression results of IL1R1 for PFI in TCGA pan-cancer. Red colors represent significant results.

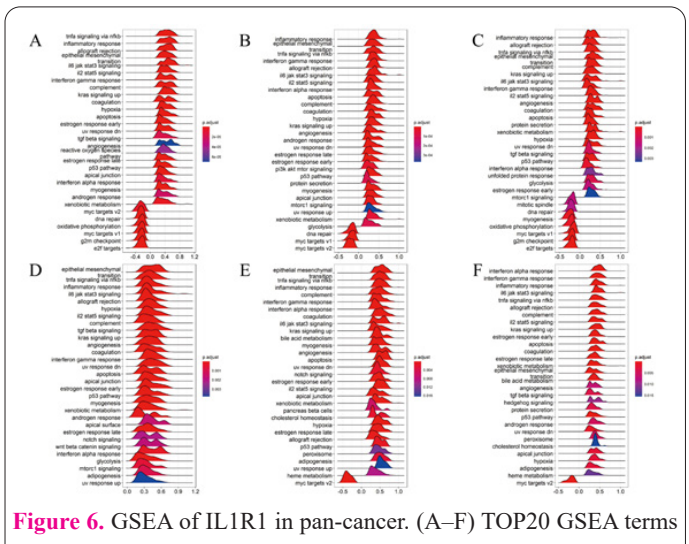

Figure 6. GSEA of IL1R1 in pan-cancer. (A–F) TOP20 GSEA terms in indicated tumor types.

results indicated that IL1R1 is closely associated with the regulation of the cell cycle, cellular inflammation, and immune microenvironment.

Immune cell infiltration analyses

A correlation analysis was performed to explore the relationship between IL1R1 expression and immune cell infiltration using immune cell infiltration data from three different sources in this study. Findings from the TIMER2 database showed no significant correlation between IL1R1 and CD8+ T cells and CD4+ T cells as well as regulatory T cells in TCGA pan-cancer. (Figures7 A-C).

Analyses of correlations using data from published work indicated that IL1R1 was positively correlated with immature dendritic cells, plasmacytoid dendritic cells, central memory CD4 T cells, natural killer cells, and effector memory CD4 T cells. (Figure 7D).

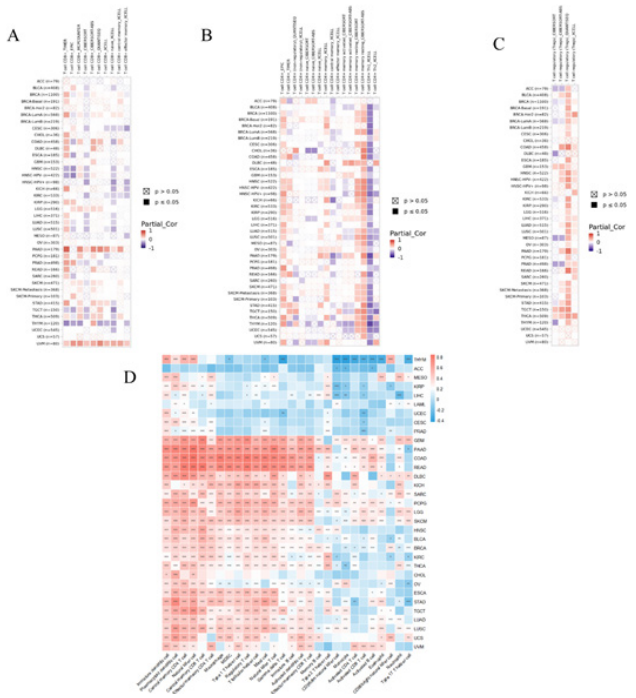

Figure 7. Immune cell infiltration analysis. (A) The correlation between IL1R1 and infiltration level of CD8+ T cells using TIMER2 database. (B) The correlation between IL1R1 and infiltration level of CD4+ T cells using TIMER2 database. (C) The correlation between IL1R1 and infiltration level of Tregs using the TIMER2 database. (D) The correlation between IL1R1 and infiltration level of indicated immune cells using data from published work. *P < 0.05; **P < 0.01; ***P < 0.001.

Discussion

Cancer-related pain is a complex, multidimensional phenomenon. Chronic pain seriously affects the quality of life of cancer patients (15), impairs their physical function, and even leads to more serious cancer-related consequences (16-18). Up to this point, with no effective approach to treating chronic pain, something that makes opioid management more difficult (19, 20), and there is an urgent need for non-pharmacological treatment options. This study first obtained 318 differential genes from GSE124272, including 5 chronic pain-related genes, including IL1R1, GRIK1, IL1R2, CTSG, and CRHR2. we focused on the role of IL1R1 which can regulate multiple immune functions (21), Studies have demonstrated its association with several pain-related inflammatory diseases (22), IL-1β-activated astrocytes can enhance and possibly prolong chronic pain caused by neuroinflammation (23), Mailhot B et al. suggested that the pro-inflammatory cytokine IL-1β could directly induce pain by activating the neuron IL-1R1 (24).

IL1R1 has pleiotropic effects on malignant tumors, IL1R1 regulates gene expression associated with tumor chemotherapy resistance, such as COX2, and the increased expression of COX2 promotes tumor growth and multidrug resistance (25, 26). IL-1 signaling promotes the expression of chemokines and adhesion molecules, resulting in the concentration of immune cells in tumor tissues and promoting tumor erosion and migration (27). In addition, IL-1 signaling contributes to the activation of antigen-presenting cells in lymph nodes, where it induces the activation and development of immune memory of tumor-specific CD4 and CD8 T cells (28).

The tumor microenvironment (TME) consists of a complicated complex structure of tumor cells, blood vessels, extracellular, stroma, and other materials (29, 30). As a result of the interaction with various components of TME, tumor cells promote immune escape, leading to tumor recurrence and metastasis, and also increasing the difficulty of immunotherapy for the treatment of tumors (31, 32). Understanding the immune infiltration of individual patients for individualizing immunotherapy is particularly important. with the function of IL1R1 and its effect on the immune microenvironment of tumors has not been sufficiently investigated.

In this study, IL1R1 expression and prognostic significance were evaluated in pan-cancer and it was found to be highly expressed in one tumor STAD. In comparison, low IL1R1 expression was observed in 16 tumors, including BLCA, BRCA, CESC, COAD, CHOL, KICH, KIRC, KIRP, LIHC, LUAD, LUSC, PCPG, PRAD, READ, THCA, and UCEC. The Kaplan-Meier OS has shown that IL1R1 for SKCM patients is a protective factor and for UVM, STAD, LGG, KIRP, KICH, GBM, and BRCA patients is a risk factor. In the case of DSS, the univariate Cox regression analysis showed that IL1R1 of SKCM patients was a protective factor and of LGG, KIRP and GBM patients was a risk factor. Nevertheless, using OS as an endpoint might undermine the viability of clinical studies, and non-cancer causes of death do not always infer tumor biology, aggressiveness, or reaction to treatment. Furthermore, greater follow-up is required with OS or DSS. Thus, using a DFI or PFI can more validly convey the influence of factors on patients in many clinical trials. Considering these reasons, univariate Cox regression analysis was further investigated in this study to evaluate the interaction between IL1R1 and DFI or PFI in tumor patients. DFI analysis showed that IL1R1 was a protective factor in PRAD patients and a risk factor in PAAD patients. In parallel, the detection of PFI analysis revealed that IL1R1 played a protective role in patients with SKCM, PRAD, and MESO, whereas it was a risk factor in patients with UVM and GBM. All these results suggest that IL1R1 expression plays a predominantly protective role in the majority of tumor types.

By GSEA analysis of IL1R1, this study revealed that it is significantly associated with cell cycle-related pathways, including NFA signaling via NF-kB, tgf β signaling,

Kras signaling, inflammatory response, and apoptosis.

In summary, the present study provides a thorough evaluation of IL1R1, which reveals its potential role as a prognostic indicator for patients and its immunomodulatory role. A potential new immune checkpoint, IL1R1 has the potential as a target for tumor immunotherapy.

Ethical approval
Not applicable

Competing interests
The authors have no conflicts of interest to declare

Authors' contributions
All authors made substantial contributions to the conception or design of the work, the acquisition, analysis, or interpretation of data for the work, and drafting the work or revising it critically for important intellectual content. Ling Zhou drafted the work or revised it critically for important intellectual content. Chenglong Wu and Rongchun Li prepared figures. Li Wang and Dongji Han reviewed the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials
Not applicable.

References

1. Moisset X, Page MG. Interest of registries in neuropathic pain research. Rev Neurol-France 2021; 177(7): 843-848.
2. Baron R. Mechanisms of disease: neuropathic pain--a clinical perspective. Nat Clin Pract Neurol 2006; 2(2): 95-106.
3. Nassar MA, Stirling LC, Forlani G, et al. Nociceptor-specific gene deletion reveals a major role for Nav1.7 (PN1) in acute and inflammatory pain. P Natl Acad Sci Usa 2004; 101(34): 12706-12711.
4. Priest BT, Murphy BA, Lindia JA, et al. Contribution of the tetrodotoxin-resistant voltage-gated sodium channel NaV1.9 to sensory transmission and nociceptive behavior. P Natl Acad Sci USA 2005; 102(26): 9382-9387.
5. Zhang G, Yang P. Bioinformatics Genes and Pathway Analysis for Chronic Neuropathic Pain after Spinal Cord Injury. Biomed Res Int 2017; 2017(6423021.
6. Ventafridda V, Tamburini M, Caraceni A, De Conno F, Naldi F. A validation study of the WHO method for cancer pain relief. Cancer-Am Cancer Soc 1987; 59(4): 850-856.
7. Afsharimani B, Kindl K, Good P, Hardy J. Pharmacological options for the management of refractory cancer pain-what is the evidence? Support Care Cancer 2015; 23(5): 1473-1481.
8. Foulkes T, Wood JN. Pain genes. Plos Genet 2008; 4(7): e1000086.
9. Young EE, Lariviere WR, Belfer I. Genetic basis of pain variability: recent advances. J Med Genet 2012; 49(1): 1-9.
10. Wang Y, Dai G, Li L, et al. Transcriptome signatures reveal candidate key genes in the whole blood of patients with lumbar disc prolapse. Exp Ther Med 2019; 18(6): 4591-4602.
11. Ritchie ME, Phipson B, Wu D, et al. limma powers differential expression analyses for RNA-sequencing and microarray studies. Nucleic Acids Res 2015; 43(7): e47.
12. Yu G, Wang LG, Han Y, He QY. clusterProfiler: an R package for comparing biological themes among gene clusters. Omics 2012; 16(5): 284-287.
13. Kovtonyuk LV, Caiado F, Garcia-Martin S, et al. IL-1 mediates microbiome-induced inflammaging of hematopoietic stem cells in mice. Blood 2022; 139(1): 44-58.
14. Gehrke N, Hovelmeyer N, Waisman A, et al. Hepatocyte-specific deletion of IL1-RI attenuates liver injury by blocking IL-1 driven autoinflammation. J Hepatol 2018; 68(5): 986-995.
15. Mao JJ, Armstrong K, Bowman MA, Xie SX, Kadakia R, Farrar JT. Symptom burden among cancer survivors: impact of age and comorbidity. J Am Board Fam Med 2007; 20(5): 434-443.
16. Deandrea S, Montanari M, Moja L, Apolone G. Prevalence of undertreatment in cancer pain. A review of published literature. Ann Oncol 2008; 19(12): 1985-1991.
17. van den Beuken-van EM, Hochstenbach LM, Joosten EA, Tjan-Heijnen VC, Janssen DJ. Update on Prevalence of Pain in Patients With Cancer: Systematic Review and Meta-Analysis. J Pain Symptom Manag 2016; 51(6): 1070-1090.
18. Zylla D, Steele G, Gupta P. A systematic review of the impact of pain on overall survival in patients with cancer. Support Care Cancer 2017; 25(5): 1687-1698.
19. Paice JA. Managing Pain in Patients and Survivors: Challenges Within the United States Opioid Crisis. J Natl Compr Canc Ne 2019; 17(5.5): 595-598.
20. Vitzthum LK, Riviere P, Murphy JD. Managing Cancer Pain During the Opioid Epidemic-Balancing Caution and Compassion. Jama Oncol 2020; 6(7): 1103-1104.
21. Sims JE, Smith DE. The IL-1 family: regulators of immunity. Nat Rev Immunol 2010; 10(2): 89-102.
22. Dinarello CA, Simon A, van der Meer JW. Treating inflammation by blocking interleukin-1 in a broad spectrum of diseases. Nat Rev Drug Discov 2012; 11(8): 633-652.
23. Zhang JM, An J. Cytokines, inflammation, and pain. Int Anesthesiol Clin 2007; 45(2): 27-37.
24. Mailhot B, Christin M, Tessandier N, et al. Neuronal interleukin-1 receptors mediate pain in chronic inflammatory diseases. J Exp Med 2020; 217(9):
25. Weber A, Wasiliew P, Kracht M. Interleukin-1beta (IL-1beta) processing pathway. Sci Signal 2010; 3(105): m2.
26. Malik A, Kanneganti TD. Function and regulation of IL-1alpha in inflammatory diseases and cancer. Immunol Rev 2018; 281(1): 124-137.
27. Xu D, Matsuo Y, Ma J, et al. Cancer cell-derived IL-1alpha promotes HGF secretion by stromal cells and enhances metastatic potential in pancreatic cancer cells. J Surg Oncol 2010; 102(5): 469-477.
28. Ben-Sasson SZ, Hogg A, Hu-Li J, et al. IL-1 enhances expansion, effector function, tissue localization, and memory response of antigen-specific CD8 T cells. J Exp Med 2013; 210(3): 491-502.
29. Mlecnik B, Bindea G, Pages F, Galon J. Tumor immunosurveillance in human cancers. Cancer Metast Rev 2011; 30(1): 5-12.
30. Galon J, Angell HK, Bedognetti D, Marincola FM. The continuum of cancer immunosurveillance: prognostic, predictive, and mechanistic signatures. Immunity 2013; 39(1): 11-26.
31. Zhang Y, Zhang Z. The history and advances in cancer immunotherapy: understanding the characteristics of tumor-infiltrating immune cells and their therapeutic implications. Cell Mol Immunol 2020; 17(8): 807-821.
32. Murciano-Goroff YR, Warner AB, Wolchok JD. The future of cancer immunotherapy: microenvironment-targeting combinations. Cell Res 2020; 30(6): 507-519.