Prognostic value of Holliday junction-recognizing protein and its correlation with immune infiltrates in lung adenocarcinoma

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Abstract. Lung adenocarcinoma (LUAD) is a disease with high morbidity and mortality rates globally. Holliday junction-recognizing protein (HJURP) has recently been shown to be a potentially useful biomarker for diagnosing and determining the progression and prognosis of different cancer types. The present study assessed the prognostic value of HJURP expression in LUAD and investigated the biological pathways related to HJURP that are involved in LUAD pathogenesis. It was found that high HJURP expression was significantly associated with stage (P=0.001), T grade (P=0.012) and N grade (P=0.012). Overall survival analysis demonstrated that patients with LUAD and high HJURP expression had a worse prognosis compared with those patients with low HJURP expression (P<0.001). Multivariate analysis using the Cox proportional hazards model indicated that the expression of HJURP [hazard ratio (HR), 1.32; 95% confidence interval (CI), 1.09-1.60; P=0.004] and stage (HR, 1.90; 95% CI, 1.19-3.03; P=0.007) were independent prognostic factors for patients with LUAD. Gene set enrichment analysis results showed that genes involved with 'basal transcription factors', the 'cell cycle', 'homologous recombination', 'non-small cell lung cancer' (NSCLC), 'oocyte meiosis', 'p53 signaling pathway', 'pathways in cancer', 'RNA degradation' and 'spliceosome' were differentially enriched in the high HJURP expression phenotype. Significant correlations were also found between HJURP and several tumor-infiltrating immune cells, immunomodulators and immune subtypes. Furthermore, western blotting and qPCR analyses confirmed that HJURP was significantly increased in cell lines of NSCLC. In summary, HJURP may be a potentially useful prognostic molecular biomarker of a poor prognosis in LUAD cases. Further experiments are needed to demonstrate the biological effects of HJURP.

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Abbreviations: CENP-A, centromere protein-A; GSEA, gene set enrichment analysis; HCC, hepatocellular carcinoma; HJURP, Holliday junction-recognizing protein; LUAD, lung adenocarcinoma; NES, normalized enrichment score; NSCLC, non-small cell lung cancer; OS, overall survival; TIL, tumor-infiltrating lymphocyte

Key words: lung adenocarcinoma, HJURP, immune infiltration, prognosis

Introduction

Lung cancer is the first and second most common cause of cancer morbidity among males and females in China, respectively, and is also a leading cause of cancer-related mortality worldwide (1,2). Lung adenocarcinoma (LUAD) is the most frequent subtype of lung cancer, and its incidence has been increasing in recent years (3). Current treatments for LUAD include surgical resection, radiotherapy, chemotherapy, targeted therapy and immunotherapy (4). Although multimodal therapies have been used to treat LUAD, survival outcomes remain unsatisfactory, ranging from 15 to 20% (2). Surgery and drugs used for chemotherapy can lead to complications, such as infections (*Staphylococcus aureus, Escherichia coli, Streptococcus* spp and *Fusarium* spp), which can become severe, and even fatal infections of the surgical site while in the hospital environment (5-7). Therefore, there is an urgent need to explore potential molecular biomarkers that can help determine patient prognosis and be used to prescribe effective treatments for LUAD.

Holliday junction-recognizing protein (HJURP) is a protein that has recently been shown to be required for centromere protein-A (CENP-A) loading in the centromeric chromatin and for the assembly of functional kinetochores (8-10). In humans, HJURP has been demonstrated to be a critical regulator of DNA binding and phosphorylation, and is involved in the regulation of chromosomal segregation and cell division (11,12). Emerging evidence has revealed that HJURP expression is significantly upregulated following DNA damage, that it collaborates with components of the DNA repair machinery and that it plays a role in homologous recombination (10,13). In addition, the upregulation of HJURP, which has now been reported in hepatocellular carcinoma, glioblastoma, breast cancer and ovarian carcinoma, has been correlated with a poor prognosis (14-17). However, there remains limited understanding as to whether HJURP expression can act as a

prognostic biomarker for LUAD, despite continuing reports of the role HJURP plays in carcinogenesis.

Thus, the objective of the current study was to evaluate the prognostic value of HJURP expression in cases of human LUAD, based on data obtained from The Cancer Genome Atlas (TCGA). To gain further insights into the biological pathways involved in LUAD pathogenesis related to the HJURP regulatory network, gene set enrichment analysis (GSEA) was also performed.

Materials and methods

Cell lines. The human LUAD cell line, H1299, and the normal bronchial epithelial BEAS-2B cell line, were purchased from the Cell Bank of the Chinese Academy of Sciences. H1299 cells were maintained in RPMI-1640 medium supplemented with 10% fetal bovine serum (FBS; Gibco; Thermo Fisher Scientific, Inc.) and maintained at 37˚C in an incubator containing 5% CO_2, with 100 U/ml penicillin and 100 mg/ml streptomycin. BEAS-2B cells were maintained in Dulbecco's modified Eagle's medium (DMEM) (Gibco; Thermo Fisher Scientific, Inc.) supplemented with 10% FBS and maintained at 37˚C in an incubator containing 5% CO_2, with 100 U/ml penicillin and 100 mg/ml streptomycin.

Western blotting. H1299 and BEAS-2B cells were lysed in RIPA buffer (Beyotime Institute of Biotechnology). Protein concentrations were then determined using a BCA Protein assay kit (Beyotime Institute of Biotechnology). The protein samples (25 μg/sample) were subjected to SDS-polyacrylamide gel electrophoresis (SDS-PAGE) on a 12% gel before being transferred to polyvinylidene difluoride membranes. To block non-specific protein binding, the membranes were incubated in 5% BSA for 1.5 h at room temperature with gentle agitation. Next, the membranes were incubated with GAPDH (1:1,000; cat. no. ab8245; Abcam) and HJURP (1:2,000; cat. no. ab233541; Abcam) antibodies at 4˚C for 15-18 h, washed three times with TBST (Tris-buffered saline containing 0.1% Tween-20), and incubated with HRP-conjugated secondary antibodies [1:1,000; cat. nos. A0208 (goat anti-rabbit IgG) and A0216 (goat anti-mouse IgG); Beyotime Institute of Biotechnology] for 1 h at room temperature. Finally, the membranes were washed again with TBST and BeyoECL Plus [Ultra Sensitive ECL Chemiluminescence kit (cat. no. P0018S; Beyotime Institute of Biotechnolog]) was used to visualize the protein bands on a ChemiDoc Touch (Bio-Rad Laboratories, Inc.). The bands were quantified using Quantity One 1-D analysis software version 4.6.8 (Bio-Rad Laboratories, Inc.) (18). GAPDH immunoreactivity was used as the loading control for each protein.

Reverse transcription-quantitative PCR (RT-qPCR). Total RNA was extracted from cells using TRIzol® (Thermo Fisher Scientific, Inc.) according to the manufacturer's instructions. Using the PrimeScript RT reagent kit (Takara Bio, Inc.), RNA was reverse-transcribed to synthesize first-strand cDNA, which was then quantified using an SYBR Premix Ex Taq kit (Takara Bio, Inc.), according to the manufacturer's instructions. Primers used in this study were as follows: HJURP forward, 5'-AGTGCCTTTATGTATTGGAG-3' and reverse, 5'-AAGTGAGGGTCTGGATTTA-3'; and GAPDH forward, 5'-GAACATCATCCCTGCCTCTACT-3' and reverse, 5'-ATTTGGCAGGTTTTTCTAGACG-3'. qPCR was performed with the following thermocycling conditions: 95˚C for 15 min, followed by 40 cycles of 95˚C for 10 sec, 60˚C for 20 sec and 72˚C for 10 sec. Fluorescence was detected using a Corbett Research RG-6000 Real-Time PCR Machine (Corbett Life Science, Sydney, NSW, Australia). Each sample was run in triplicate and was compared with GAPDH as the internal control. Results were obtained using the $2^{-\Delta\Delta Cq}$ method (19).

Collection of publicly available data from TCGA and Gene Expression Omnibus (GEO) databases. Gene expression (HJURP) profiling data of 519 LUAD samples and 54 normal tissue samples were downloaded from the publicly available TCGA database (https://gdc.cancer.gov/). Another transcriptome profiling dataset, GSE116959 (20), of 57 LUAD samples and 11 peritumoral normal lung tissue samples was obtained from the NCBI GEO database (https://www.ncbi.nlm.nih.gov/geo/) to verify the expression level of HJURP in LUAD cases. $Log_2FC>2$ indicates that gene expression in tumor samples is upregulated 4 times compared with that of adjacent samples, $log_2FC<-2$ indicates that gene expression in tumor samples is downregulated 4 times compared with that of adjacent samples. HJURP protein expression profiling data were obtained from the UALCAN database (http://ualcan.path.uab.edu/index.html). Relevant clinicopathological information, including age, sex, T stage, N status, M grade, stage (21) and overall survival (OS) time were also extracted from the TCGA database. A total of 480 patients with LUAD with complete follow-up data were included, whose details were recorded prior to November 1, 2019. The clinical end point was OS, defined as the time from surgery to death. In addition, patients who were alive at the last follow-up were considered to be censored observations.

GSEA. GSEA is a computational method that determines whether an *a priori* defined set of genes shows statistically significant, concordant differences between two biological states (22). In the present study, the GSEA first generated an ordered list of all genes according to their correlation with HJURP expression; tumor samples were divided into high expression group and low expression group according to the median value (4.3) of HJURP expression level. GSEA was then performed to elucidate the significant survival differences observed between the high and low expression HJURP groups. Gene set permutations were performed 1,000 times for each analysis. The level of HJURP expression was used as a phenotype label. The nominal P-value and normalized enrichment score (NES) were used to sort the pathways enriched in each phenotype.

Immune infiltration analysis. It is well known that interactions between a tumor and the immune system play a crucial role in cancer initiation, progression and response to treatment. The integrated repository portal for tumor-immune system interactions (http://cis.hku.hk/TISIDB/index.php) (22) was used to examine tumor and immune system interactions in 28 types of tumor-infiltrating lymphocytes (TILs) seen across different human cancer types. The relative abundance of TILs was inferred by using gene set variation analysis based on the HJURP expression profile. Spearman's test was applied to

measure correlations between HJURP and TILs; P<0.05 was considered to indicate a significant difference for all tests.

Statistical analyses. Scatter plots and paired plots were used to show differences in HJURP expression between normal and tumor samples. The cut-off value of HJURP expression was determined by the optimal cutoff values determined by X-tile software (https://medicine.yale.edu/lab/rimm/research/software/). The Wilcoxon rank-sum test or Kruskal-Wallis test was used to assess the association between expression levels and clinicopathological characteristics. The Kaplan-Meier method and log-rank test were used to estimate associations between HJURP expression and OS. Univariate and multivariable Cox proportional hazards regression models were used to evaluate the impact of HJURP expression on OS in the presence of other known risk factors. Two-sided P<0.05 was considered to indicate a statistically significant difference. All analyses were performed using R (v.3.5.2; https://cran.r-project.org/bin/windows/base/old/3.5.2/).

Results

Patient characteristics. As shown in Table 1, 480 primary tumors with both clinical and gene expression data were downloaded from the TCGA database during November 2019. The study cohort included 221 (46.04%) males, with average patient age being 66 years. In the cohort, the T-stage distribution of LUAD was as follows: T1, 164 patients (34.17%); T2, 254 patients (52.92%); T3, 40 patients (8.33%); and T4, 19 patients (3.96%). The N status distribution of LUAD was as follows: N0, 310 patients (64.58%); N1, 87 patients (18.13%); N2, 69 patients (14.38%); and N3, 2 patients (0.42%). The cancer type distribution was as follows: M0, 316 patients (65.83%) and M1, 25 patients (5.21%). Stage I disease was found in 260 patients (54.16%), stage II in 107 patients (22.29%); stage III in 79 patients (16.46%); and stage IV in 26 patients (5.42%). The median follow-up time for patients alive at their last contact was 18.42 months (range, 0-227.07 months).

Table I. Characteristics of lung adenocarcinoma patients from The Cancer Genome Atlas.

Clinical characteristic	Value
Median age at diagnosis (range), years,	66 (33-88)
Sex, n (%)	
Male	221 (46.04)
Female	259 (53.96)
Clinical stage, n (%)	
I	260 (54.17)
II	107 (22.29)
III	79 (16.46)
IV	26 (5.42)
NA	8 (1.67)
Clinical T grade, n (%)	
T1	164 (34.17)
T2	254 (52.92)
T3	40 (8.33)
T4	19 (3.96)
NA	3 (0.63)
Clinical N grade, n (%)	
N0	310 (64.58)
N1	87 (18.13)
N2	69 (14.38)
N3	2 (0.42)
NA	12 (2.5)
Clinical M grade, n (%)	
M0	316 (65.83)
M1	25 (5.21)
NA	139 (28.96)
Median follow-up time (range), months	18.42 (0-227.07)

HJURP expression and its association with clinicopathological variables. Compared with HJURP expression in normal lung tissues (n=54), HJURP expression was significantly higher in LUAD tissues (n=519) (P<0.05). The scatter plot (Fig. 1A) and paired plot (Fig. 1B) show the differences in HJURP expression between normal and tumor samples. Expression profiling data were also obtained from the GSE116959 dataset (including 57 LUAD samples and 11 normal lung tissue samples), after data preprocessing and quality assessment using R software. According to the cut-off criteria set (P<0.05 and |log2FC|>2.0), a total of 329 differentially expressed genes were obtained, including 85 upregulated genes and 244 downregulated genes. To further investigate HJURP protein expression in patients with LUAD, the levels of HJURP proteomic expression were quantified in normal lung tissues (n=102) and primary LUAD tissues (n=111). The results showed that the expression of HJURP was significantly higher in primary LUAD (median, 0) than in normal lung tissues (median, -1.016) (P<0.01; Fig. 1C). Significant differences were also found in HJURP protein expression with regard to LUAD grades compared with the normal group: Grade 2 (median, -0.28; P<0.01) and grade 3 (median, 0.421; P<0.01). However, the protein expression of HJURP may not be associated with grade 1 LUAD (P=0.202) (Fig. 1D). Significant differences in HJURP expression were observed with regard to the T grade (P=0.012), N grade (P=0.012) and stage (P=0.001) of LUAD (Fig. 2A, B and D). However, the HJURP expression level was not associated with M grade (P=0.101; Fig. 2C).

HJURP expression is associated with the survival rate of patients with LUAD. As presented in Fig. 3, Kaplan-Meier survival analysis showed that LUAD with high expression of HJURP was associated with a worse prognosis compared with LUAD with low expression of HJURP (P<0.001). The univariate analysis revealed that high expression of HJURP was significantly associated with a poor OS [hazard ratio (HR), 1.06; 95% confidence interval (CI), 1.03-1.09; P<0.001]. Other clinicopathological variables associated with poor survival included T grade, N grade and stage (Table II). The multivariate analysis showed that HJURP remained independently associated with OS, with an HR of 1.32 (95% CI, 1.09-1.60; P=0.004), along with stage (HR, 1.90; 95% CI, 1.19-3.03; P=0.007).

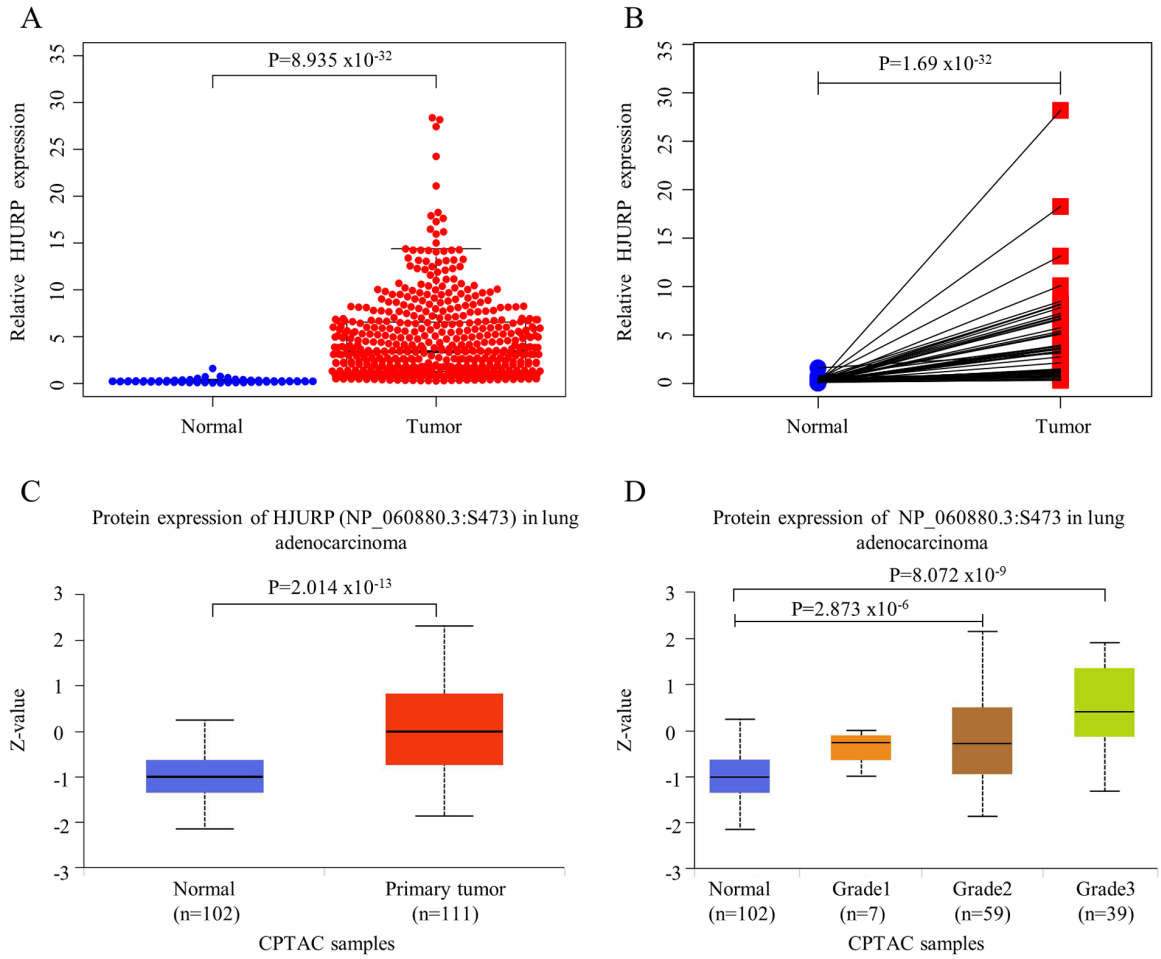

Figure 1. Differential HJURP expression. (A) Scatter plot and (B) paired plot showing the difference in HJURP expression between normal and tumor samples. The HJURP (NP_060880.3:S473) proteomic expression profile based on (C) sample type and (D) tumor grade (data acquired from UALCAN database where n=105 only). HJURP, Holliday junction-recognizing protein; CPTAC, Clinical Proteomic Tumor Analysis Consortium.

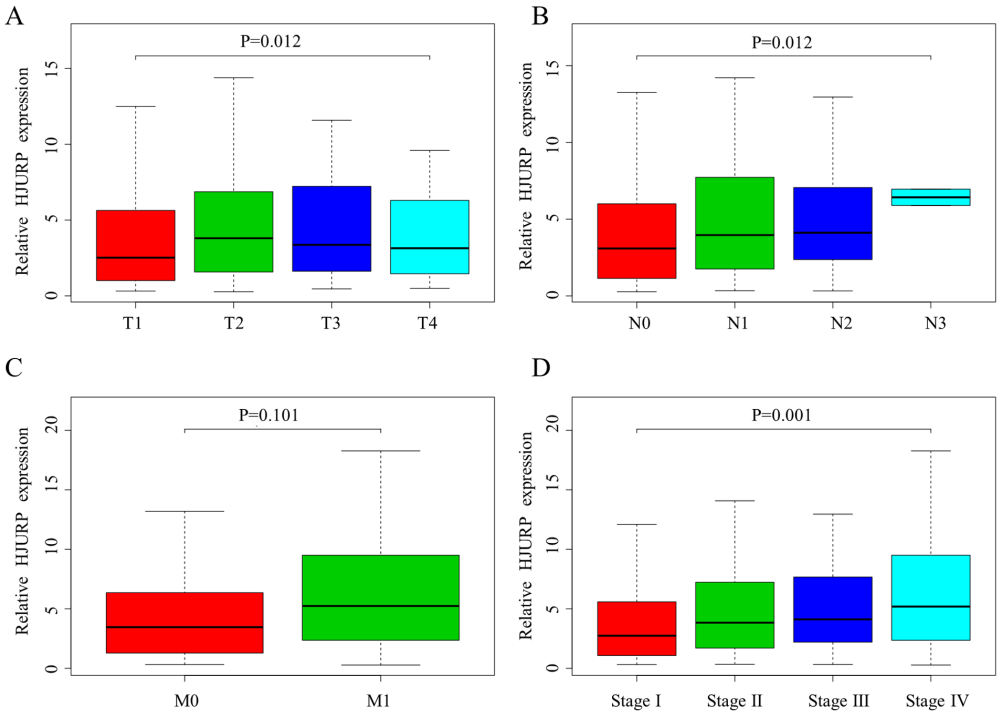

Figure 2. Associations between HJURP expression and clinicopathological characteristics. (A) T grade, (B) N grade, (C) M status and (D) clinical stage. HJURP, Holliday junction-recognizing protein.

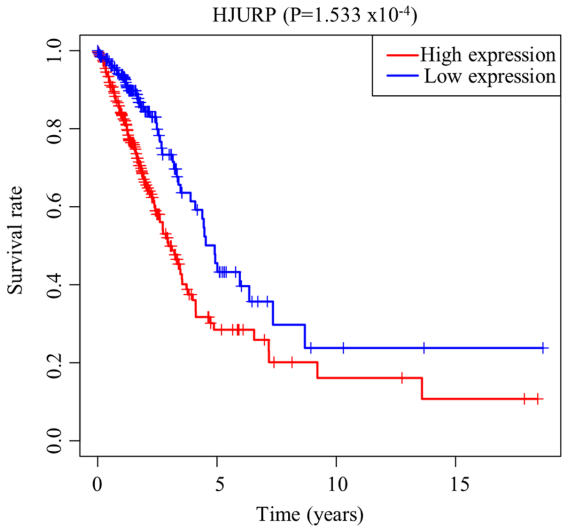

Figure 3. Kaplan-Meier curves showing the effect of high and low HJURP expression on overall survival in patients with lung adenocarcinoma in The Cancer Gene Atlas cohort. HJURP, Holliday junction-recognizing protein.

Molecular mechanisms of HJURP in LUAD. To identify signaling pathways that are differentially activated in LUAD, GSEA was conducted between low and high HJURP expression data sets. GSEA was used to identify significant differences in signaling pathways from the MSigDB Collection (c2.cp.kegg.v7.4.symbols.gmt). False discovery rate <0.05 and nominal P<0.05 were used as thresholds to determine significantly enriched signaling pathways. The most significantly enriched signaling pathways were selected based on their NES (Fig. 4). Fig. 4 shows that 'basal transcription factors', the 'cell cycle', 'homologous recombination', 'non-small cell lung cancer' (NSCLC), 'oocyte meiosis', the 'p53 signaling pathway', 'pathways in cancer', 'RNA degradation' and 'spliceosome' are differentially enriched in the high HJURP expression phenotype.

Correlation of HJURP expression with immune infiltration level. After identifying the HJURP-related signaling pathways, a correlation analysis was performed to explore the relationship between HJURP expression and immune infiltration level in patients with LUAD. Significant correlations were found between HJURP expression and 28 types of TILs across different human cancer types (Fig. 5A). Significant results were found for HJURP expression with the abundance of activated CD4 T cells (ρ=0.591; P<0.001) were notably correlated.

The relationships between three types of immunomodulators, immune-inhibitors (Fig. 6A-E), immunostimulators (Fig. 6F-R) and major histocompatibility complex molecules (Fig. 6S and T), and the expression of HJURP were examined. Significant results were observed using Spearman's correlation test (Fig. 6); however, only HJURP expression and poliovirus receptor (an immunostimulator) exhibited a coefficient indicating that the variables were notably correlated.

The distribution of HJURP expression across immune and molecular subtypes was also explored. Fig. 7A shows the associations between HJURP expression and immune subtypes across different human cancer types. The violin plot shows the LUAD distribution across the following subtypes: C1, wound healing; C2, IFN-γ dominant; C3, inflammatory; C4, lymphocyte-depleted; C5, immunologically quiet; and C6, TGF-β dominant (Fig. 7B). In addition, western blotting and qPCR results from a LUAD cell line (H1299) and a normal bronchial epithelial cell line (BEAS-2B) confirmed that HJURP was significantly increased in NSCLC (Fig. 8A-C). The research design of the study is shown in Fig. 9.

Discussion

In the present study, an RNA sequencing dataset of HJURP and relevant clinical parameters of 480 patients with LUAD from the TCGA database were analyzed. The study found that the high expression of HJURP could be considered to be an independent prognostic factor in patients with LUAD, regardless of other clinicopathological variables. HJURP may be a potentially useful prognostic molecular biomarker of poor survival in LUAD cases. Further experiments should be performed to elucidate the biological effects of HJURP.

An increasing number of studies have found that HJURP may be exploited as a potentially effective biomarker in the diagnosis of and determination of progression and prognosis of cancer (14-17). Hu *et al* (14) identified that high levels of HJURP expression could predict a poorer prognosis in hepatocellular carcinoma (HCC) and may promote HCC progression by accelerating HCC cell proliferation. A study by Valente *et al* (15) found that HJURP plays an important role in the maintenance of extremely proliferative cells of high-grade gliomas and pointed to HJURP as a potential therapeutic target for the development of novel treatments for patients with glioma. Montes de Oca *et al* (16) identified HJURP as the first biomarker that can be used to differentiate good and poor prognoses in patients with luminal A breast cancer; the study also noted that HJURP can support the integration of selected chromatin regulators in the clinical setting to help guide treatment plans and improve the overall management of patients with breast cancer. Recently, Li *et al* (17) revealed that increased expression of HJURP could act as an independent negative prognostic biomarker for patients with advanced serous ovarian cancer. These studies suggested that HJURP has potentially useful clinical implications in improving prognostic predictions for cancer. However, there remains a limited understanding on whether HJURP expression is a prognostic biomarker in LUAD.

In the present study, bioinformatics analysis using high-throughput RNA-sequencing data from TCGA demonstrated that the upregulation of HJURP in LUAD was associated with advanced clinicopathological characteristics (T grade, N grade and stage), survival time and a poor prognosis. To further investigate the functions of HJURP in LUAD, GSEA was performed using TCGA data. This GSEA showed that 'basal transcription factors', the 'cell cycle', 'homologous recombination', 'non-small cell lung cancer', 'oocyte meiosis', the 'p53 signaling pathway', 'pathways in cancer', 'RNA degradation' and 'spliceosome' are enriched in the high HJURP expression phenotype. This suggests that HJURP may serve as a potential biomarker of prognosis and a therapeutic target in LUAD.

Table II. Associations between overall survival time and clinicopathological characteristics in patients from The Cancer Genome Atlas according to univariate and multivariate Cox regression analysis.

	Univariate analysis			Multivariate analysis		
Parameter	HR	95% CI	P-value	HR	95% CI	P-value
Age (years)	1.00	0.99-1.02	0.686	1.02	0.10-1.04	0.128
Sex (male vs. female)	1.03	0.72-1.48	0.866	0.89	0.62-1.29	0.554
Stage (III-IV vs. I-II)	1.64	1.40-1.94	≤0.001	1.90	1.19-3.03	0.007
T grade (T3-T4 vs. T1-T2)	1.65	1.33-2.04	≤0.001	1.22	0.96-1.55	0.100
M grade (M1 vs. M0)	1.67	0.92-3.05	0.092	0.41	0.12-1.39	0.152
N grade (N1+N2+N3 vs. N0)	1.79	1.47-2.20	≤0.001	0.97	0.65-1.44	0.864
HJURP expression (high vs. low)	1.06	1.03-1.09	≤0.001	1.32	1.09-1.60	0.004

HR, hazard ratio; CI, confidence interval.

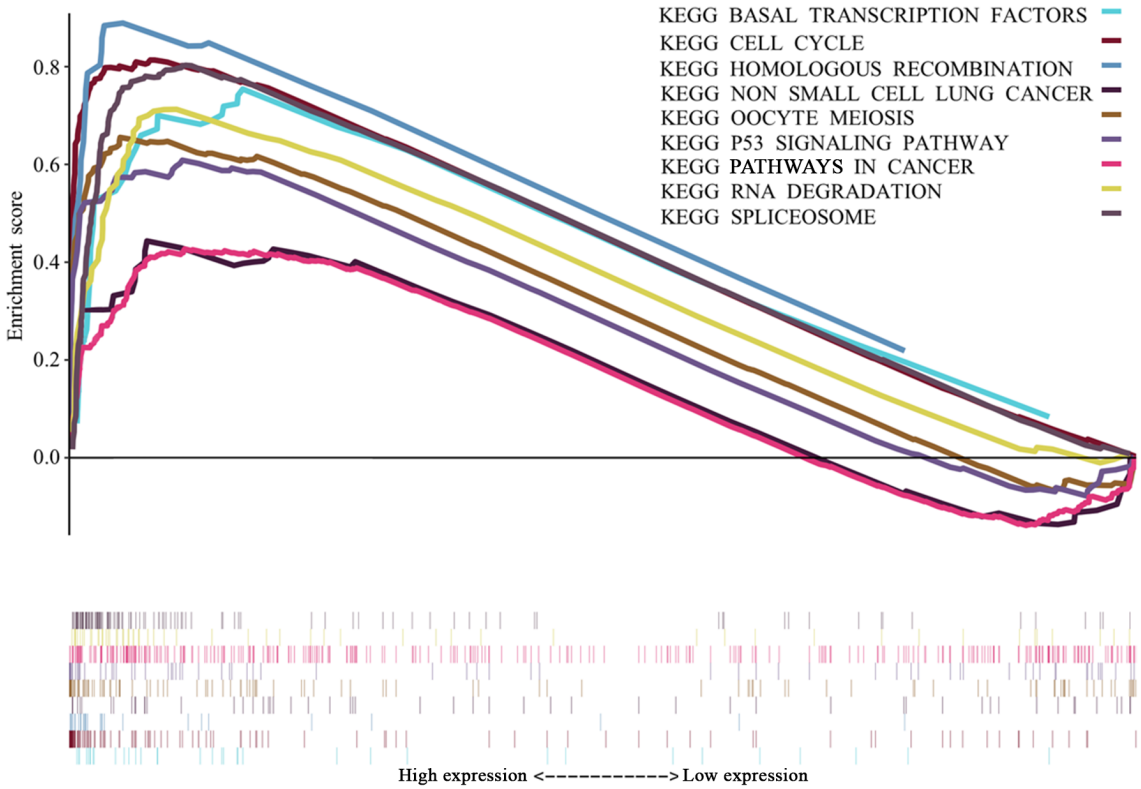

Figure 4. Enrichment plots from the gene set enrichment analysis. Gene set enrichment analysis results showing that 'basal transcription factors', 'cell cycle', 'homologous recombination', 'non-small cell lung cancer', 'oocyte meiosis', 'p53 signaling pathway', 'pathways in cancer', 'RNA degradation' and 'spliceosome' are differentially enriched in Holliday junction-recognizing protein-related lung adenocarcinoma.

The present findings are in agreement with those previously reported for HJURP upregulation in lung tumors compared with HJURP expression in normal lung tissue samples (23), as well as with the increased HJURP levels seen in plasma sediments from patients with lung cancer (24). Zhou *et al* (25) focused on plasma mRNA as a novel non-invasive biomarker for diagnosing lung cancer. Blood specimens were collected from 47 patients with primary lung cancer and 14 healthy individuals. Circulating HJURP and ADAMTS8 mRNAs with superior sensitivity and specificity were revealed, and these molecules were proposed as promising non-invasive biomarkers for the diagnosis of lung cancer. Recently, Wei *et al* (24) confirmed that the increased expression of HJURP was associated with advanced stage and a poor prognosis, based on a small sample size of 74 patients with NSCLC. Additionally, the study provided clues regarding the role of HJURP as a tumor promoter in NSCLC via the activation of the Wnt/β-catenin pathway.

In the present study, using GSEA, it was observed that the HJURP high expression phenotype was associated with 'basal transcription factors', the 'cell cycle', 'homologous recombination', 'non-small cell lung cancer', 'oocyte

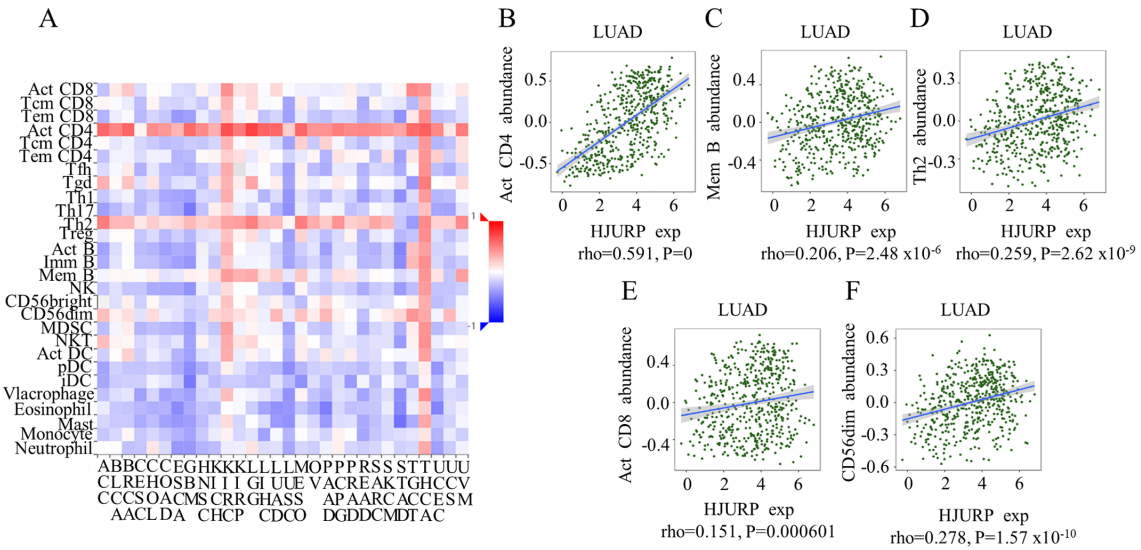

Figure 5. Spearman correlations between the expression of HJURP and TILs across different human cancer types. (A) Relationships between the expression of HJURP and 28 types of TILs across different human cancer types. (B-F) Significant results were found for HJURP expression with regard to the abundance of memory B cells, type 2 T-helper cells, activated CD8 T cells, and CD56(dim) natural killer cells; however, only the abundance of activated CD4 T cells was notably correlated. HJURP, Holliday junction-recognizing protein; TILs, tumor-infiltrating lymphocytes; LUAD, lung adenocarcinoma; Act, activated; Mem B, memory B cells; Th2, type 2 T-helper cells; exp, expression.

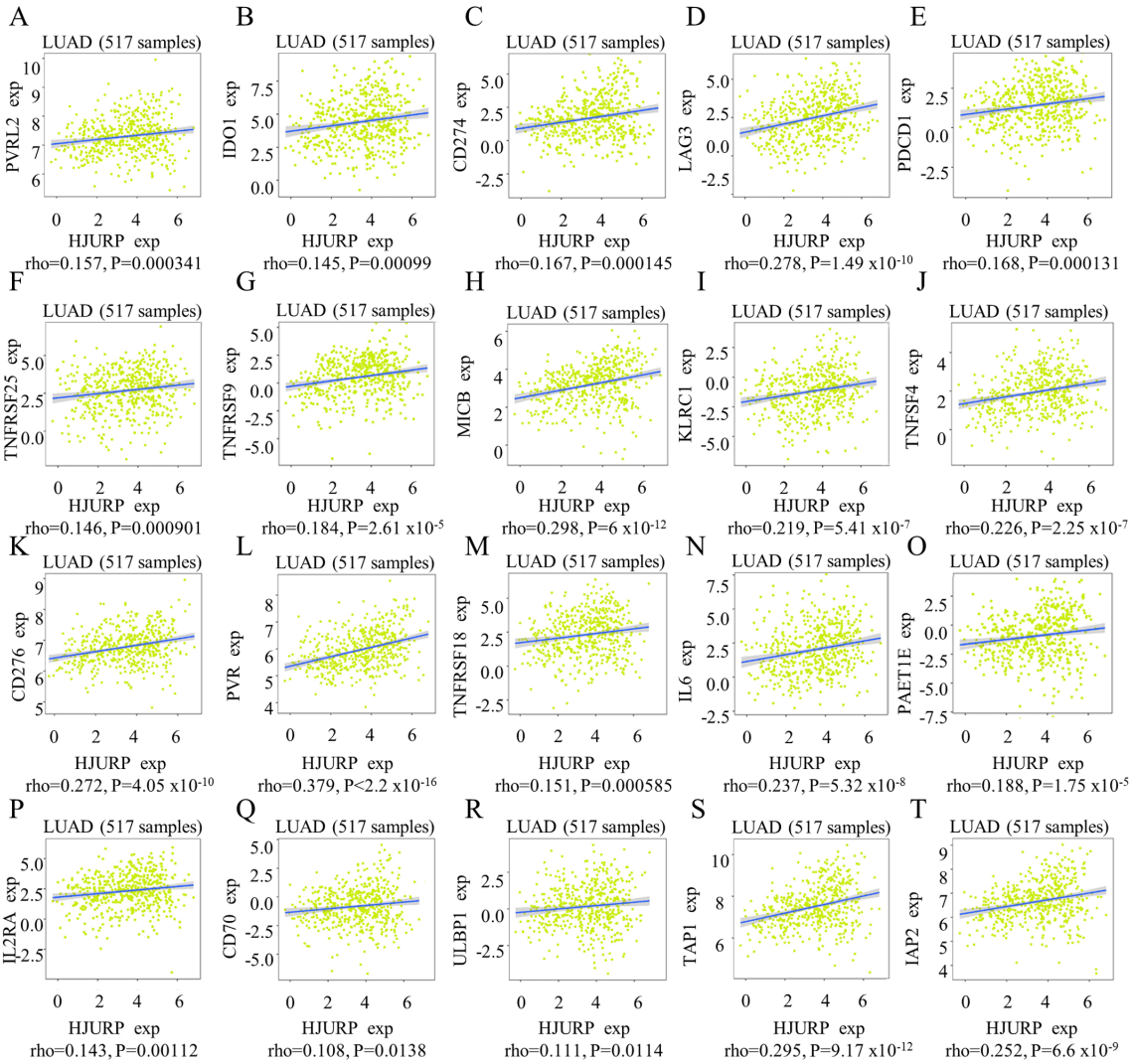

Figure 6. Correlations between three types of immunomodulators and the expression of HJURP. (A-E) Immune-inhibitors, (F-R) immunostimulators and (S and T) major histocompatibility complex molecules. HJURP, Holliday junction-recognizing protein; LUAD, lung adenocarcinoma; exp, expression.

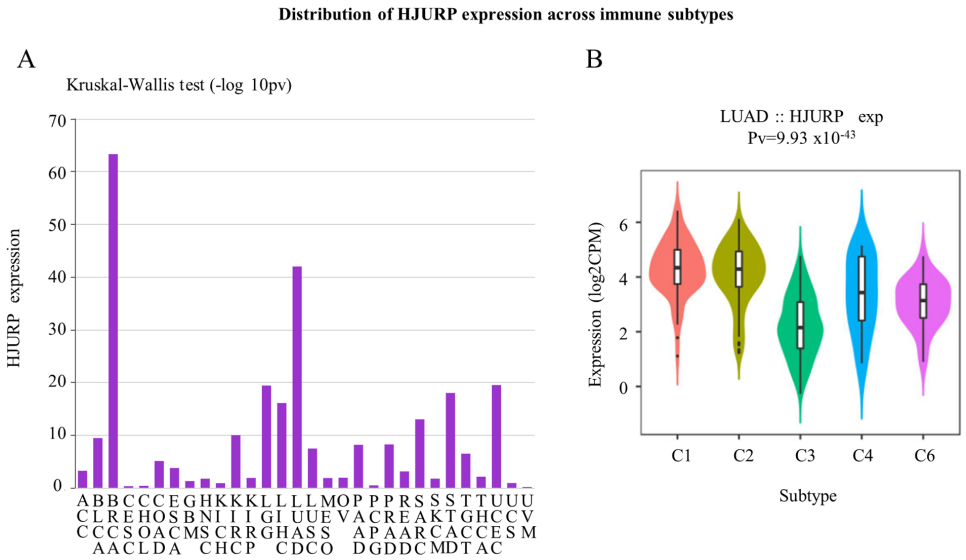

Figure 7. Distribution of HJURP expression across immune subtypes. (A) Associations between HJURP expression and immune subtypes across different human cancer types. (B) Distribution of HJURP expression across immune and molecular subtypes. HJURP, Holliday junction-recognizing protein; LUAD, lung adenocarcinoma; exp, expression.

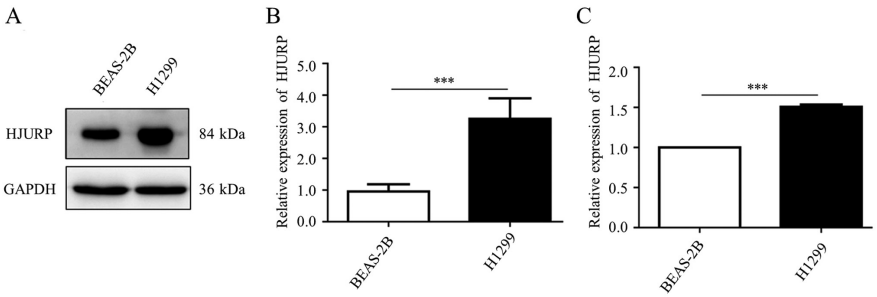

Figure 8. Expression level of HJURP in non-small cell lung cancer. (A and B) HJUPR protein expression was analyzed by western blotting. (C) HJUPR mRNA expression was detected by quantitative PCR. ***P<0.001 vs. control group (BEAS-2B). HJURP, Holliday junction-recognizing protein.

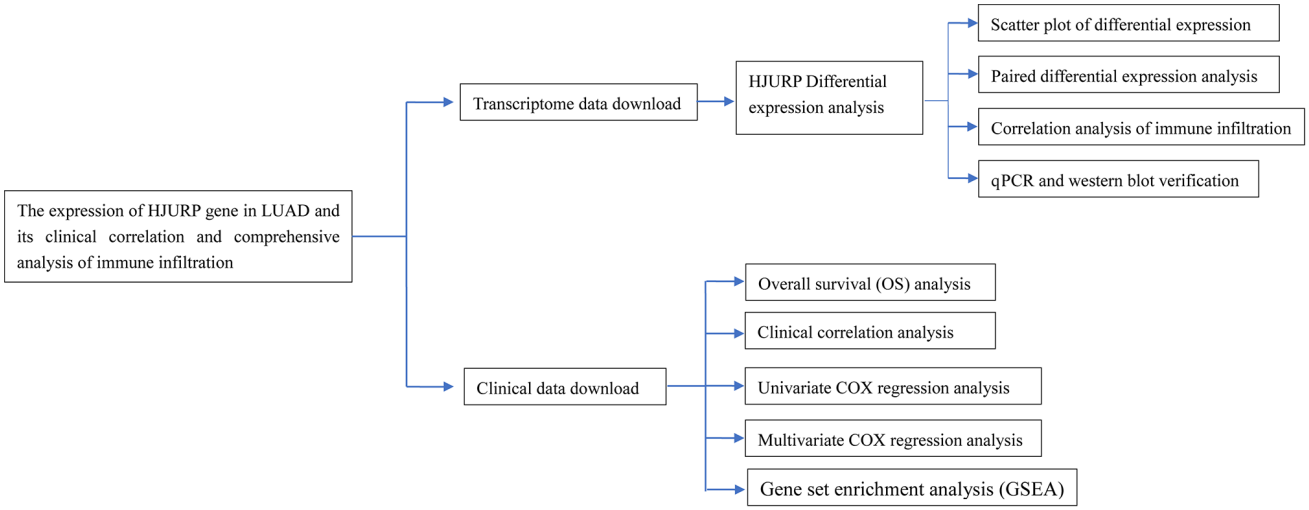

Figure 9. Flow chart of research design showing the use of clinical and transcriptome data, and the assessment processes. HJURP, Holliday junction-recognizing protein; LUAD, lung adenocarcinoma; qPCR, quantitative PCR.

meiosis', the 'p53 signaling pathway', 'pathways in cancer', 'RNA degradation' and 'spliceosome'. Significant correlations were also found between HJURP expression and immunomodulators, immune subtype and several tumor-infiltrating immune cells, such as activated CD4 T cells. There have been many reports on the molecular genetic alterations

of p53 in lung cancer. Dhieb *et al* (26) found that abnormal immunostaining of p53 was detected in 56.16% of patients with LUAD. Abnormal p53 expression was slightly increased in European compared with Asian populations. It has been reported that lung cancer is strongly influenced by mutations of p53 (27). The role played by immune infiltration in LUAD has been highlighted by certain studies. Varn *et al* (28) determined that naive B-cell and $CD8^+$ T-cell infiltration was associated with prolonged prognosis, while myeloid cell infiltration was associated with shorter survival times. Wang *et al* (29) found that increased TTC21A expression was correlated with an increased proportion of immune cells, such as B cells, neutrophils, mast cells and T cells, in patients with LUAD. Previous studies have also found that the expression levels of HJURP mRNA are linked with the regulation of the cell cycle (11,14). Several proteins have been reported to interact with HJURP, including proteins affecting HJURP function and downstream proteins regulated by HJURP. The most well-known molecule regulated by HJURP is the histone H3 variant, centromere-specific protein (CENP)-A. The cooperation between CENP-A and its chaperon HJURP mediates a normal cell cycle, whereas ectopic activation of HJURP is involved in the chromosomal stability and immortality of cancer cells (11). The associations between HJURP expression and 'basal transcription factors', 'homologous recombination', 'oocyte meiosis', 'RNA degradation' and 'spliceosomes' were the first noted in the present study, although the regulatory mechanisms remain to be further elucidated.

The present study found that the expression of HJURP was significantly increased in patients with LUAD and associated with several clinical features and immune infiltrations. HJURP may be a potentially useful prognostic molecular biomarker of poor survival in LUAD cases. The bioinformatics results were confirmed with RT-qPCR and western blotting analyses in the normal bronchial epithelium (BEAS-2B) and human NSCLC (H1299) cell lines. HJURP mRNA and protein levels were significantly increased in the H1299 cells compared with the levels in the BEAS-2B cells. These findings were consistent with those of a previous study (24) and also demonstrated that higher expression of HJURP was associated with advanced stage, distant metastasis and a poor prognosis in cases of NSCLC. Similarly, higher HJURP levels may be associated with early stage lung cancer (11,25), and HJURP activation seems to play a pivotal role in the immortality of cancer cells (11). Therefore, higher HJURP levels promote a poor prognosis in NSCLC; a precise mechanism for this showing that HJURP promotes tumor cell proliferation, migration and invasion through the Wnt/β-catenin signaling pathway has been reported (24), and the present study has provided further support for this mechanism.

In conclusion, the present study demonstrated that high levels of HJURP expression are correlated with a poor prognosis in patients with LUAD. HJURP may be a promising therapeutic target for the development of anticancer drugs and may also act as a biomarker for LUAD diagnosis.

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Availability of data and materials

The dataset analyzed during the present study can be downloaded from TCGA (https://cancergenome.nih.gov/), GEO (https://www.ncbi.nlm.nih.gov/geo/), UALCAN (http://ualcan.path.uab.edu/index.html) and TISIDB (http://cis.hku.hk/TISIDB/index.php) databases. The remaining data (including PCR and WB experiments) are available from the corresponding author on reasonable request.

Authors' contributions

LC and JY designed the experiments and wrote the manuscript. LC and CZe performed the experiments. LY and WL analyzed and interpreted the results. LC performed the statistical analysis. LC, CZe and CZh assembled the data. JY contributed to every process as a supervisor. LC, CZe, WL, LY, CZh and JY confirm the authenticity of all the raw data. All authors read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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