Pan-Cancer Analysis Based on Gene Mutation and Epigenetic Modification Explains The Value of HJURP in The Tumor Microenvironment

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Research Article

Keywords: HJURP, Pan-cancer, Prognosis, Biomarker, Immune infiltration

Posted Date: January 28th, 2022

DOI: <https://doi.org/10.21203/rs.3.rs-1275234/v1>

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Abstract

Objective: To analyze expression levels, prognostic value and immune infiltration association of Holliday junction protein (HJURP) as well as its feasibility as a pan-cancer biomarker for different cancers.

Methods: The Protter online tool was utilized to obtain the protein structure and localization of HJURP in diverse tumors, then the mutation and methylation of HJURP in tumors were further explored. Thereafter, the mRNA data and clinical characteristics of 33 tumor types from TCGA database were obtained for investigating HJURP levels within different tumor types and different tumor stages and its prognostic relation. Finally, the composition pattern and immune infiltration of HJURP in different tumors were detected in Tumor Immune Estimation Resource.

Results: HJURP was abnormally expressed in most of the cancer types and subtypes in TCGA database. Also, it was related to metastasis, tumor stage, and poor prognosis of different cohorts. Moreover, HJURP was related to tumor immune escape through diverse mechanisms, including T cell rejection and methylation within diverse cancers. Besides, the methylation of HJURP was inversely proportional to mRNA expression levels, which mediated the dysfunctional phenotypes of T cells and poor prognosis of different cancer types. Patients with HJURP mutations had a higher tumor mutation load than those with no HJURP mutations. Alternatively, based on this work, HJURP level was related to immune cell infiltration within diverse cancers.

Conclusions: HJURP may serve as an oncogenic molecule, and its expression and immune infiltration characteristics can be used as the diagnostic, prognostic marker and therapeutic target.

1. Introduction

Early tumorigenesis may be associated with the abnormal function of centromere and the uncertainty in chromosome separation during cell proliferation^[1]. In this process, Holliday junction protein (HJURP) is responsible for the loading and assembling of histone H3 variant-centromere protein A (CENPA) at the centromere in a cellular regulatory manner^[2]. To regulate and affect tumor occurrence and development in the cell cycle and cell cloning in different ways^[3], previous studies have shown that the methylation of HJURP can promote tumor development by reducing G0/G1 phase arrest in the cell cycle^[4]. Meanwhile, HJURP overexpression is also found to regulate tumor cell growth in vivo, while tumor cell invasion in vitro predicts the poor prognosis of the disease^[5, 6]. Tumor occurrence and development are a joint construction of genomic modification at genetic and epigenetic levels^[7]. Mutations in the genome can be driven by epigenetic patterns in terms of the changes of gene function and malignant proliferation of cells, and genetic patterns including DNA methylation and histone modifications can also be disrupted by genomic changes^[8-10]. The tumor microenvironment (TME) is dominated by tumor cells, which can recruit various immune cells, change their anticancer activities, and achieve tumor immune evasion by decreasing immune cell reactivity and pattern, and by promoting their apoptosis^[11-13]. Tumor progression is accompanied by different defense and evasion mechanisms, which may alter the composition pattern

of immune cells in TME^[11, 14, 15]. Therefore, different types of immune cells and different periods can respond more accurately through the extent and nature of immune infiltration. However, there is no sufficient and effective evidence for the pathogenesis of HJURP in a variety of cancers, and it remains unclear about whether HJURP plays a role in TME, cell cycle progression, genetic mutations, tumor treatment and prognosis through a common molecular mechanism in the context of immunocytological action.

Fortunately, thanks to the development and maturity of bioinformatics analysis, researchers can now make systematic explanation of the huge biological genomic information and cell-to-cell interaction. Simultaneously, they can provide objective evidence for cancer diagnosis and prognosis by extracting and analyzing the differences between various cancer clinical information in the database^[16-18]. Therefore, more and more pan-cancer studies have been conducted by using different computational tools and online network platforms. This work aimed to explore the differential expression, tumor somatic mutation, tumor treatment and prognosis of HJURP in different types of tumor cohorts. Also, we also analyzed the association of HJURP with tumor immune infiltration. As a result, HJURP level abnormally elevated in pan-cancer cohort, which was markedly related to a high tumor grade and the poor clinical prognosis. Meanwhile, we found that HJURP knockdown and HJURP mutations were associated with better tumor prognosis. HJURP may participate in the immunogenicity mechanisms of different tumor types, and its immune and mutation characteristics are the possible diagnostic and prognostic biomarkers for tumor stage.

2. Results

2.1 Mutants, localization, single-cell mutations, functional chaperones and expression profiles of HJURP protein

The topology of HJURP protein was analyzed (Figure 1A). As shown by single-cell RNA sequencing data from fluorescence-based cell cycle index (FUCCI) U-2OS cells, the elevated HJURP mRNA level was associated with cell cycle progression. HJURP showed changes in its protein expression levels temporally associated with cell cycle progression in G1, S, and G2 phases (Figure 1B). In addition, HJURP mRNA expression was detected within diverse healthy human tissues, such as immune, nervous, endocrine, reproductive and muscle tissues (Figure 1C). For characterizing HJURP localization in cells, distribution of HJURP and its microtubules in PC-3, U-2 osteosarcoma (OS) and MCF-7 cells was analyzed by indirect immunofluorescence. As a result, HJURP was co-localized with nuclear, ER and microtubule markers within PC-3, U-2OS and MCF-7 cells, indicating HJURP's subcellular localization (Figure 1D-1J). In addition, according to gene and disease interaction network, HJURP had multiple gene functional partners (Figure 1K).

2.2 Somatic mutation rate of HJURP

The prevalence of HJURP somatic mutations in TCGA pan-cancer cohort was examined by the CIB online website. In all the 10,953 patients, 102 (0.93%) harbored HJURP mutations (Figure 2A), and HJURP mutations occurred in a fraction of tumors in most of the tumor types. The mutation frequencies varied significantly across different tumors ($P < 0.001$). Meanwhile, a total of 133 HJURP mutations were detected, including 116 (87.2%) missense mutations, 15 (11.3%) truncated mutations and 2 (1.5%) homologous mutations (Figure 2B). These mutations occurred in a dispersed manner throughout the sequence. Also, the HJURP 3D protein structure was analyzed (Figure 2C-2D). As discovered in our research, patients with HJURP mutated cancer had significantly higher MSLsensor scores than those with HJURP non-mutated cancer. To further validate the relationship between HJURP mutation and the microsatellite (MSI) status, the MSI MANTIS scores were determined for patients with HJURP mutated cancers and those with HJURP non-mutated cancers. Noteworthily, there were significant differences in the scores between different subtypes of MJURP mutation (Figures 2E-2G). Thereafter, the relationship between OS, PFS, DPS, and DF was compared between HJURP mutated cancers and HJURP non-mutated cancers. The results showed that TCGA cancer survival was related to the HJURP mutant status (Figure 2H-2K).

2.3 Epigenetic modifications of HJURP protein mediate a dysregulated T cell phenotype and the poor prognosis of multiple cancer species

According to previous experimental reports, HJURP hypomethylation is frequently detected within cancers, which promotes the progression and migration of tumor cells. As revealed by the promoter methylation status chart, HJURP was hypomethylated in bladder urothelial carcinoma (Figure 3A, $P=1.02E-3$), renal papillary cell carcinoma (Figure 3E, $P=2.28E-3$), hepatocellular carcinoma (Figure 3F, $P=6.82E-4$), prostate cancer (Figure 3H, $P=1.62E-12$), and testicular germ cell carcinoma (Figure 3I, $P=1.62E-12$), but was hypermethylated in cervical cancer (Figure 3B, $P=2.64E-3$), esophageal carcinoma (Figure 3C, $P=2.73E-3$), renal transparent cell carcinoma (Figure 3D, $P=1.66E-6$), and pancreatic carcinoma (Figure 3G, $P=6.74E-3$). Therefore, the changes caused by the methylation status of HJURP in different cancers were assessed (Figure 3J). Interestingly, HJURP showed positive relation to dysfunctional T cells, but negatively correlated with the shorter survival time in different tumors including glioma, metastatic and non-metastatic melanoma, renal papillary carcinoma, renal clear cell carcinoma, breast cancer and cholangiocarcinoma (Figure 3K, 3L, 3M, 3N, 3Q). And metastatic melanomas (Figure 3P) also exhibited a stronger correlation in T cell disorder state compared with non-metastatic ones (Figure 3O), accompanying with the worse prognosis. As a result, HJURP methylation status was related to tumor stage, and that hypomethylation of HJURP was more pronounced in advanced and metastatic tumors, which predicted poor tumor prognosis.

2.4 HJURP is differently expressed within various cancers and affects prognosis of patients with different tumors

The oncogenic effects of HJURP was mined in entire TCGA pan-cancer database, as a result, compared with normal tissues, HJURP was significantly over-expressed in most types of cancers ($P < 0.001$) (Figure

4A). Besides, HJURP expression levels were correlated with multiple tumor stages, like prostate cancer, renal papillary cell carcinoma, adrenal cortical carcinoma, renal cell carcinoma and pancreatic cancer (Figure 4B-4F). In contrast to non-metastatic tumors, HJURP was expressed at higher levels in metastatic tumors, including renal papillary cell carcinoma, adrenal cortical carcinoma, and renal transparent cell carcinoma (Figure 4G-4I). Our results displayed that HJURP up-regulation was significantly related to an advanced tumor stage. Besides, correlation analysis was conducted to analyze relationship of HJURP level with prognosis of different tumors by Kaplan-Meier survival curve analysis. Thereafter, diagnostic efficacy of HJURP was evaluated through the area under the receiver operating characteristic (ROC) curve (AUC) value. Results suggested a clear correlation between HJURP expression and tumors such as lung adenocarcinoma, melanoma, diffuse large B lymphoma, renal cell carcinoma, and thymic carcinoma (Figure 4J-4V), and patients with high HJURP expression had poorer prognosis. Meanwhile, the ROC curves of some tumors showed that HJURP exhibited good diagnostic efficacy (AUC > 0.70) (Figure 4α-4Σ). The above results indicated the potential of HJURP as an early biomarker, prognostic, and follow-up marker for tumors.

2.5 Heatmap showing the correlation between HJURP protein and four immunosuppressive cells/six immune cells in infiltration

In 39 TCGA cancer types and subtypes that assessed tumor immune infiltration, ten (including BLCA, BRCA-Luminal, COAD, HNSC, HNSC-HPVpds, HNSC-HPVneg, KICH, LGG, LIHC, and PRAD) showed markedly positively relation between HJURP level and seven immune cell types (Purity, B cell, CD8 + T cell, CD4 + T cell, Dendritic cell, Neutrophil, Macrophage) infiltration. All the other cancer types did not show any significant positive correlation or any significant association between HJURP expression and tumor infiltration of seven immune cell types (Figure 5A). Moreover, biomarker correlation of HJURP was assessed by comparing it against the HJURP response results to the ICB subcohort and OS predictive ability to standardized biomarkers. Finally, we found AUC > 0.5 in 18 subjects of the 25 ICB subcohort (Figure 5B). As a result, HJURP up-regulation was related to poor programmed death of ACT 1 and ACT in glioblastoma (ICB_Zhao2019_PD1) (Figure 5C-5D). Also, the association of HJURP expression with the infiltration of four immunosuppressive cells contribute to T cell rejection, namely MDSC, CAF, M2-TAM, and Treg cells, was assessed as well. We observed that HJURP expression was associated with ACC, BLCA, BRCA, BRCA-Basai, BRCA-Her2, BRCA-LumA, BRCA-LumB, CESC, CHOL, COAD, ESCA, ESCA, GBM, HNSC, HNSC-HPV-, KICH, KIRC, KIRP, LGG, LIHC, LUAD, LUSC, MESO, OV, PAAD, PCPG, PRAD, READ, SARC, SKCM, SKCM-Metastasis, SKCM-Primary, STAD, THYM, UCEC, and UVMA for MSDC tumor infiltration; CESC, HNSC-HPV-, KIRC, KIRP, LGG, and THCA for CAFs tumor infiltration; BLCA, BRCA, HNSC, HNSC-HPV +, HNSC-HPV-, KIRC, LGG, and THCA for M2-TAM tumor infiltration; and BRCA, HNSC-HPV +, KIRC, PCPG, PRAD, and THCA for Tregs tumor infiltration (Figure 5E-5H).

3. Discussion

The precise diotic inheritance in the mitosis of mammalian nucleated cells is determined by the accurate segregation of chromatin^[19]. This process depends on the unique domain of the chromatin. This locus is

different from other chromatin in its presence of nucleosome constituted by the histone H3 variant, CENP-A (centromeric protein-A). While HJURP participates in CENP-A composition in G1 phase of cell cycle and affects the new CENP-A recruitment during cell replication^[20, 21]. Previous research reports have indicated that the overexpression of CENP-A is accompanied by the overexpression of HJURP, which leads to ectopic CENP-A deposition, thus further leading to mitotic defects, centromeric dysfunction and chromosomal instability^[1, 22]. Eventually, it leads to cancer genesis and development. In the study of Jong-Ik Heo *H et al.*, the cellular senescence regulated by HJURP down-regulation was also found to be mediated via the p53-dependent pathway^[23]. In addition to the genetic involvement in cancer development, HJURP also plays an epigenetic role in tumor progression. In previous studies, Lai *et al.* found that HJURP regulated the cyclin-dependent kinase inhibitor 1 (CDKN1A) through the GSK3/JNK signaling pathway in prostate cancer, and affected the growth of tumor cells^[24]. Chen *et al.* discovered that HJURP disrupted p21 stability via multiple cellular pathways, including AKT/GSK3, thereby enhancing the HJURP-mediated cell growth capacity^[5]. In an in vitro study of breast cancer cell lines by Hu *et al.*, HJURP was reported to adjust the sensitivity of tumor cells to radiotherapy by playing a role in DNA repair^[25]. Epigenetic modifications of abnormal HJURP occur at the very early stages of tumor development, and HJURP has functional effects on different types of tumors through different mechanisms of action, which deserve further investigation.

Thanks to the rapid development of the information age, the functional role of HJURP in various cancers has been gradually revealed. For instance, Wang L *et al.* identified an association of HJURP with poor OS of NSCLC patients by String database analysis, and HJURP was suggested as a key gene for NSCLC development and prognosis^[26]. Fu FQ *et al.* found that HJURP protein expression served as a predictor for lung cancer brain metastasis^[27]. Hu BH *et al.* reported that HJURP up-regulation was markedly related to dismal OS, tumor number, tumor differentiation, TNM staging, and Barcelona clinical liver cancer staging. Moreover, high HJURP expression independently predicted the poor prognosis of liver cancer^[28]. Besides, ChenTC *et al.* discovered that HJURP was important for liver cancer metastasis through up-regulating SPHK1, and its up-regulation might indicate the low DFS rate as well as high microvascular infiltration possibility among HCC cases^[29]. By statistical analysis of the Taylor data set, Chen YF *et al.* found that HJURP up-regulation was significantly related to PSA, high Gleason score, advanced pathological staging, and metastasis^[30]. HJURP exhibits a great potential in pan-cancer research, and the immune-related mechanisms involved have not yet been clarified.

The types and frequencies of HJURP gene mutations in different tumors showed that HJURP gene mutations only occurred in a small part of tumors, with missense mutations being the main mutation type. The hypomethylation of HJURP mediated T cell dysfunction in 7 tumors and predicted the worse clinical prognosis. As revealed by the results of previous studies, the hypomethylation of HJURP can promote tumor development by reducing the stalling of G0/G1 in the cell cycle. Our results further confirmed the oncogenic role of HJURP methylation in tumors. Tumorigenesis is usually dominated by genetic alterations, while the cumulative effect of epigenetic alterations in HJURP may be important to ultimately drive the invasive development and metastases of tumors. The infiltration of immune cells in

tumor can change the function of T cells and further promote tumor escape from the host immune system, leading to tumor progression and metastasis. We explored the immune infiltration patterns of 33 tumors and found that 10 cancer types, including BLCA, BRCA-Luminal, COAD, HNSC, HNSC-HPVpds, HNSC-HPVneg, KICH, LGG, LIHC and PRAD, showed a correlation with immune infiltration of immunosuppressive cells (such as CAF, Tregs) to varying degrees. The infiltration of M2 TAM and MDSC can serve as an objective response to tumor T cell rejection. Surprisingly, we found that the expression level of HJURP was associated with the infiltration of immunosuppressive cells in almost all tumors. These results show that HJURP is most likely to jointly promote tumor metastasis and escape in TME through the infiltration of immune cells and the immune exclusion of T cells, with the immune exclusion of T cells occupying a dominant position. At the same time, the oncogenic role of HJURP was mined in the entire TCGA pan-cancer database. It was found that compared with normal tissues, HJURP expression was significantly higher in most types of tumor tissues. The high expression of HJURP was obviously related to the advanced stage of the tumor. Meanwhile, the ROC curves of some tumors were also shown, which suggested that HJURP had good diagnostic efficacy (AUC > 0.70). The above studies strongly suggest the potential of HJURP as an early biomarker, prognostic, and follow-up marker for tumors.

Certainly, some limitations should be noted in this study. First, only one TCGA database was selected for the study, which might lead to sample bias. To improve the credibility of the results, the sample size should be further expanded. Moreover, our results should be further validated through experiments in vitro and in vivo, so as to reveal the relevant biological functions of HJURP. To sum up, this work first examines the role of differential HJURP expression in various tumor cohorts, tumor somatic mutations, tumor treatment and prognosis. In addition, this study also deeply explores the association of HJURP with tumor immune infiltration, and confirms that HJURP expression and immune infiltration characteristics are the biomarkers for cancer detection and follow-up.

4. Conclusion

HJURP may serve as an oncogenic molecule, and its expression and immune infiltration characteristics can be used as the diagnostic, prognostic marker and therapeutic target.

5. Materials And Methods

5.1 Material collection and data analysis tools

The Protter Online Tools ([www.http://wlab.ethz.ch/protter/start/](http://wlab.ethz.ch/protter/start/)) can intuitively demonstrate the distribution of variants on the protein topology that have different effects on HJURP protein function. cBioPortal (<http://www.cbioportal.org/>) provides exploration, visualization and analysis of multi-dimensional cancer genomic data, where the data types cover DNA copy-number alterations (CNAs), somatic mutations, DNA methylation, and mRNA and microRNA (miRNA) expression levels. On the other hand, Tumor Immune Dysfunction and Exclusion ([www.http://tide.dfci.harvard.edu/login/](http://tide.dfci.harvard.edu/login/)) can

determine the failure degree of T cells in immunothermal tumors and the enrichment of three T cell suppressive cells in immunocold tumor species. Tumor Immune Estimation Resource ([www.http://timer.comp-genomics.org/](http://timer.comp-genomics.org/)) determines the relationship of immune cells with cancer cells based on detection and quantification of RNA-seq expression profile data. TISIDB is based on five large databases to pre-calculate the gene correlation between immune cell infiltration in various tumors, including lymphocytes, immunomodulators, and chemokines. Altogether 113093 mRNA data were obtained for 33 TCGA-derived tumor types, including 730 normal samples and 10363 tumor samples. The results were processed in R language (version 3.6.3), and analyzed by "ggplo2", "survival", "pROCmRNA" package.

5.2 Differential expression analysis of HJURP in normal, tumor, metastatic tissues and tumor stages

To comprehensively analyze the differences in HJURP expression in cancer compared with healthy tissues in diverse TCGA cancers, TIMER2.0, the UALCAN Interactive web and the Gene Expression Spectrum Interactive Analysis (GEPIA2) algorithm were utilized. In addition, the differential expression of HJURP during different tumor stages was analyzed, and the pathological stage of TCGA cancer types was plotted by using the expression DIY module of GEPIA2. Moreover, differential gene expression analysis in healthy and metastatic samples of diverse cancers was also performed by TNMplot module of Kaplan-Meier (KM) plotter. $p < 0.05$, < 0.01 , and < 0.001 indicate statistical significance.

5.3 Analysis of tumor immune and immunosuppressive cell infiltration

TIMER2 server was employed for analyzing the correlation of HJURP level with the six immune cell infiltration, namely, B cells, CD4 + T cells, CD8 + T cells, macrophages, dendritic cells (DCs) and neutrophils. Also, the association of HJURP level with tumor infiltration in four immunosuppressive cells promoting T cell rejection, namely, cancer-associated fibroblasts (CAFs), myeloid-derived suppressor cells (MDSCs), regulatory T (Treg) cells, and M2 subtypes of tumor-associated macrophages (M2-TAMs), was analyzed. Association analysis was performed by statistical significance and purity-corrected partial Spearman Rho values ($p < 0.05$). The GraphPadPrism software was utilized for data visualization. Heatmap was used to observe the immune cell infiltration levels in 33 TCGA cancers.

5.4 Analysis of epigenetic methylation

The TCGA methylation module in UALCAN Interactive Network resource was used to analyze the differences in HJURP methylation levels of cancer compared with matched healthy tissues of different TCGA cancers. The promoter methylation level was indicated by b-value, which ranged from 0 (unmethylated) to 1 (fully methylated). Different b-value cutoffs stood for hypomethylation ($b: 0.3-0.25$) and hypermethylation ($b: 0.7-0.5$). Besides, Query module of Tumor Immune Dysfunction and Exclusion (TIDE) algorithm was utilized for evaluating how epigenetic and genetic alterations of HJURP affected the dysfunctional T cell phenotypes.

5.5 Analysis of prognostic correlation

For analyzing prognostic correlations of HJURP, gene alteration, and treatment outcome, overall survival (OS), disease-free survival (DFS) and disease-free progression survival (PFS) in the cohort were analyzed by using KM curves. In the survival analysis of differential HJURP expression between different cancer cohorts, median level was determined to divide cases as high or low HJURP expression group. Finally, hazard ratios (HRs), 95% confidence intervals (CIs) and log-rank test p-values were obtained. The HRs were obtained using Cox proportional hazards regression model according to comparisons between high and low expression groups.

Abbreviations

CAFs cancer-associated fibroblasts

CDKN1A cyclin-dependent kinase inhibitor 1

CENPA variant-centromere protein A

Cis confidence intervals

CNAs copy-number alterations

DCs dendritic cells

DFS disease-free survival

FUCCI fluorescence-based cell cycle index

GEPIA2 Gene Expression Spectrum Interactive Analysis

HJURP Holliday junction protein

HRs hazard ratios

KM Kaplan-Meier

M2-TAMs M2 subtypes of tumor-associated macrophages

MDSCs myeloid-derived suppressor cells

miRNA microRNA

MSI microsatellite

OS osteosarcoma

OS overall survival

PFS disease-free progression survival

ROC receiver operating characteristic

TIDE Tumor Immune Dysfunction and Exclusion

TME tumor microenvironment

Treg regulatory T

Declarations

Author Contributions: Conceptualization: Junwu Li, Jun Zheng, Ronggui Zhang, Weili Zhang, Yuanfeng Zhang; Methodology: Junwu Li, Jun Zheng, Junyong Zhang, Yuanfeng Zhang; Formal Analysis: Junwu Li, Jun Zheng, Yuanfeng Zhang; Writing—Original Draft Preparation: Junwu Li, Jun Zheng; Writing—Review and Editing: Ronggui Zhang, Weili Zhang; Supervision: Junyong Zhang, Yuanfeng Zhang; Project Administration: Junwu Li, Jun Zheng, Yuanfeng Zhang. All authors have read and agreed to the published version of the manuscript.

Data availability statement: The data that support the findings of this study are openly available in online databases such as TCGA database.

Funding: This work was supported by the Research Program of Natural Science Foundation in Chongqing (cstc2021jcyj-msxmX0484); National Natural Science Foundation of China(No.81801507); and Kuanren Talent Program of Second Affiliated Hospital of Chongqing Medical University (KY2019Y004).

Ethics approval: The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in this manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties. Since all information from the online database has been deidentified and no personal identifying information was used in our analysis, informed consent was not required in our study.

Conflicts of Interest: The authors declare no conflict of interest.

Consent for publication: Not applicable.

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Figures

Figure 1

Caption not included with this version.

Figure 2

Caption not included with this version.

Figure 3

Caption not included with this version.