American Journal of BioMedicine
AJBM 2022;**10** (4): 193-205
Research Article
doi:10.18081/2333-5106/2022.10/193

Chemotherapy resistance in non-small lung cancer regulate by holliday junction-recognizing protein through YAP1/NDRG1 transcriptional axis

Chen Luo [1*], Xinxin Liu [1], W.-J. Huang [2], Y.-J. Fang [2], Pingan Luo [1], Zunyou Zheng [2]

Abstract

Lung cancer remains the leading cause of cancer death and often diagnosed at an advanced stage because of its speedy growth and early tendency to spread to other organs and tissues The objective of this study focuses on exploring the role of the HJURP/YAP1/NDRG1 transcriptional regulation axis in NSCLC. We observed significantly increased upregulation of HJURP expression levels in NSCLC tissues. Loss of function experiments identified that HJURP promotes NSCLC cells proliferation and decreases chemo-sensitivity. HJURP could affect the level of ubiquitination modification of YAP1 protein and then regulate its downstream transcriptional activity. Mechanistically, we found that YAP1 positively regulates NDRG1 transcription by binding the promoter region of the NDRG1 gene, and HJURP/YAP1/NDRG1 axis could affect chemotherapy sensitivity in NSCLC. Taken together, these findings provide insights into the HJURP as a tumor promoter in NSCLC via the activation of YAP1/NDRG1 axis, indicating HJURP may be a promising therapeutic target for NSCLC.

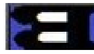
Keywords: NSCLC; HJURP; YAP1/NDRG1 axis

[*]Corresponding author email: Chen81@gmail.com
[1] Department of Basic Research, Sichuan Cancer Center, School of Medicine, Chengdu, China
[2] Department of Oncology, West China Hospital of Sichuan University, China
Received June 12, 2022; revised September 30, 2022; accepted November 09, 2022; published December 26, 2022
Copyright © Xinxin Cao et al., 2022. This is article distributed under the terms of the Creative Commons Attribution 4.0 International License (CC-BY 4.0) (https://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Introduction

Lung cancer is the most frequent malignancy and a major cause of cancer-related death in the world, accounting for 18% (1.4 million) of cancer deaths in 2015 in the light of global cancer statistics [1]. In China, cancer data show that approximately over half a million patients died from lung cancer each year [1-3]. Non-small cell lung cancer (NSCLC) is the most common type of lung cancer [4]. This disease is often diagnosed at an advanced stage because of its speedy growth and early tendency to spread to other organs and tissues [5, 6]. Despite the rapid advancements in chemotherapy, radiation therapy, and lung resection for NSCLC patients, the 5-year survival rate of NSCLC is still below 15% related to recurrence and distant metastasis of this disease [7, 8].

The histone chaperone holiday junction recognition protein (HJURP) functions at the centromere level and has been proved to be required for CENP-A centromeric localization [9]. Recent studies have shown that HJURP plays a dual role in the progression of glioblastoma,


Research Article doi:10.18081/2333-5106/2022.10/193

prostate cancer, and breast cancer [10]. For instance, suppression of HJURP induced cell senescence and abolished cell-cycle dynamics in glioblastoma [11].

HJURP increased the ubiquitination of CDKN1A via the GSK3β/JNK signaling pathway and decreased its stability, thus promoting prostate cancer cell proliferation [12]. In the field of lung cancer research, the expression of HJURP in lung cancer is significantly higher than that in normal tissues [13]. To understand whether and how HJURP involves in the progression of NSCLC, we performed a loss or gain function assay in NSCLC cell lines and found the co-interaction between HJURP and YAP1 protein. More specifically, HJURP affected the ubiquitination level of YAP1 and caused the transcriptional regulation of its target gene NDRG1, thereby affecting cell proliferation and chemo-resistance of NSCLC. This study provides theoretical support for finding relevant therapeutic targeting NSCLC and has certain clinical guiding significance.

Materials and methods

Cell culture

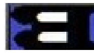
The human lung cancer cell lines (NCI-H322) were maintained in RPMI 1640 medium supplemented with 10% fetal bovine serum and 5% glutamine. NCI-H322 cell lines were maintained in Dulbecco's Modified Eagle's Medium supplemented with 10% fetal bovine serum and 0.01 mg/ml bovine insulin. SK-BR-3 cell line was maintained in McCoy's 5 A Medium supplemented with 10% fetal bovine serum. The NCI-H322 cell line was maintained in Leibovitz's L-15 Medium supplemented with 10% fetal bovine serum and incubated cultures at 37 °C without CO_2. All cell lines except NCI-H322 grow in a humid atmosphere containing 5% CO_2 at 37 °C.

Chemicals and antibodies

Chlorhexidine(#HY-B1248), Chloroquine(#HY-17589A), MG-132(#HY-13259), and Cisplatin (#HY-121309) were purchased form Med Chem Express. The primary antibody HJURP (#712465, 1:1000) was purchased from Thermo Fisher Scientific. The primary antibodies YAP1 (#14074, 1:1000), Lamin B1 (#13435, 1:1000), and NDRG1(#9485, 1:1000) were purchased from Cell Signaling Technology. GAPDH (1:1000) antibodies were purchased from Santa Cruz Biotechnology.

Overexpression and knockdown of genes

Overexpressing plasmid or shRNA of indicated genes were transfected into cells using Lipofectamine 3000(Invitrogen, Carlsbad, CA) (Thermo Fisher) according to the manufacturer's instructions which were described previously [14]. Then the efficacy was confirmed 48–72 h later.


Research Article doi:10.18081/2333-5106/2022.10/193

Cell viability assays

Triple-negative breast cancer cells were seeded onto a 96-well culture plate at different times with or without the drug. Then, cell viability was evaluated using the CCK-8 assay. The absorbance was measured at 450 nm by using a BioTek ELx800 absorbance microplate reader.

Cell colony formation assay

Triple-negative breast cancer cells were seeded into a six-well plate and incubated for 14 days. Then, the cells were fixed with 4% polyoxymethylene at room temperature for 10 min. After being washed with PBS three times, the cells were stained with crystal violet solution at room temperature for 30 min. The excess crystal violet was removed and washed with water for three times.

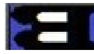
Western blot analysis

Western blots analysis was performed as previously described [15]. Nuclear fractionation used a nuclear protein extraction kit (Beyotime, China). Three independent experiments were performed.

RNA isolation and quantitative real-time PCR

These experiments were performed as described previously [16]. In general, total RNA was extracted from cells and tissues using TRIzol (Invitrogen, Carlsbad, CA) reagent. All samples were amplified thrice in real-time, and the expression was normalized to GAPDH. The following primer sequences were used:

GAPDH forward 5′-TGACTTCAACAGCGACACCCA-3′
GAPDH reverse 5′-CACCCTGTTGCTGTAGCCAAA-3′
HJURP forward 5′-GATTCAAAAAGCGGTGAGGTCG-3′
HJURP reverse 5′-AGTCACACGTACATCCCTTCC-3′
YAP1 forward 5′-TAGCCCTGCGTAGCCAGTTA-3′
YAP1 reverse 5′-TCATGCTTAGTCCACTGTCTGT-3′
Cyr61 forward 5′-GGTCAAAGTTACCGGGCAGT-3′
Cyr61 reverse 5′-GGAGGCATCGAATCCCAGC-3′
CTGF forward 5′-ACCGACTGGAAGACACGTTTG-3′
CTGF reverse 5′-CCAGGTCAGCTTCGCAAGG-3′.


Research Article doi:10.18081/2333-5106/2022.10/193

Immunofluorescence (IF) and immunohistochemical staining (IHC)

Immunofluorescence analyses and immunohistochemical staining were performed as previously described [17]. For immunofluorescence assay, cells were incubated with YAP1 antibody (1:1000) at 37 °C for 1 h, followed by Alexa 555-conjugated (red) goat anti-rabbit antibody (1:1000) (Multisciences, Hangzhou, China). And for the immunohistochemical staining assay, the slices were stained with HJURP (1:100), YAP1 (1:500), and NDRG1(1:1000).

Immuno-precipitation assay

Lung cancer cells were harvested and lysed with NP-40 buffer for 30 min on ice. Then cell lysates were centrifuged at high speed while the remaining 10% of the sample was used to prepare inputs. The remaining cell lysates were immunoprecipitated using specific antibodies and then added to 30 μL agarose A + G. And the mixture was co-incubated at 4 °C overnight on a rotary shaker. The samples were washed with PBS three times and visualized by western blot.

Ubiquitination assay

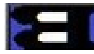
Cells were transfected with the indicated plasmids and treated with 10 μM chloroquine for 12 h or 10 μM MG-132 for 6 h before harvesting. Cells were harvested and lysed with NP-40 buffer for 30 min on ice. Then cell lysates were centrifuged at high speed while the remaining 10% of the sample was used to prepare inputs. The remaining lysates were subjected to immunoprecipitation with IgG or YAP1 antibody at 4 °C overnight. The samples were collected and washed with PBS three times and the ubiquitination of endogenous YAP1 was detected by western blot.

Luciferase assays

The activities of Gaussia luciferase and secreted alkaline phosphatase in a dual-reporter system were measured 48 h post-transfection with an NDRG1 promoter-reporter plasmid using the Secrete-Pair Gaussia Luciferase Assay Kit (LF031, GeneCopoeia) following the manufacturer's instructions. The luminometer was used to acquire the activities. Each experiment was repeated at least three times independently.

Chromatin immunoprecipitation

Chromatin immunoprecipitation was performed using the SimpleChIP® Enzymatic Chromatin IP Kit (Magnetic Beads) (Cell Signaling Technology, 9003) following the instructions of the manufacturer. Chromatin was used for immunoprecipitation with anti-YAP1, anti-histone 3, and a normal rabbit- IgG antibody. ChIP-enriched DNA was measured by using real-time PCR. The primer sequences were used:

American Journal of BioMedicine
AJBM 2022;10(4):193-205

Research Article doi:10.18081/2333-5106/2022.10/193

F1 5′-GAGCCGACCCACAACCC-3′
R1 5′-CACCCCTTCCCCGCTC-3′
F2 5′- AGGGGACTGCAGAGCCGA-3′
R2 5′- CGCGCGGCGGGCGCCCA-3′
F3 5′- CCCTACGACTGCTTGCGCAA-3′
R3 5′- CGCCCACTGGAGCCGCCG-3′.

Tumor xenografts in nude mice

Twenty BALB/c female nude mice (4 weeks old) were purchased from Sichuan Cancer Center Animal Center and housed in a specific pathogen-free environment according to the Ethics Committee for Animal Studies of Sichuan Cancer Center. Sh-NC or Sh-HJURP MDA-MB-231 cells (1 × 10 × 6 per mouse) were injected into the right sub-axillary region. Mice were monitored and were measured twice per week using a slide caliper including tumor length (L), and width (W). The tumor sizes were measured twice a week.

Tumor volume (mm^3) = $\pi/6 \times$ length × $width^2$. When tumors reached a volume of ~150 mm^3, Sh-NC or Sh-HJURP mice were randomly allocated into groups and treated with or without Cisplatin (3 mg/kg every week) via intraperitoneal injection for 24 days. Mice were then sacrificed and some tumor tissues were fixed with 10% paraformaldehyde for immunohistochemical analysis.

Statistical analysis

Data were analyzed in Excel and graphed in GraphPad Prism 7.0 software (San Diego, CA, USA). The comparisons between multiple groups were performed using multiple comparisons by one-way ANOVA. Comparisons between groups were performed using Student's *t*-test and an unpaired two-tailed Student's *t*-test was used to compare mean data. Data were independently obtained from at least three experiments. The results are presented with statistical significance or *P* value (*$p < 0.05$; **$p < 0.01$; NS indicated not statistically significant).

Results

HJURP modulates YAP1 protein level in NSCLC cells

To determine whether HJURP involves in the progression of NSCLC, we firstly examined the expression level of HJURP in lung cancer cells. We revealed the expression of HJURP is significantly higher in NSCLC than in normal lung tissue (Fig. (Fig.1A).1A). Interestingly, the expression of YAP1 protein was also significantly higher in NSCLC cells. Both HJURP and YAP1 were higher in NSCLC compared with normal lung samples (Fig. 1B, C). These results indicated that HJURP may interact with YAP1 in TNBC. To test this hypothesis, we firstly performed Kaplan–Meier analyses using the online Kaplan–Meier–Plotter lung cancer database and the results showed that high expression of YAP1 mRNA level is associated with

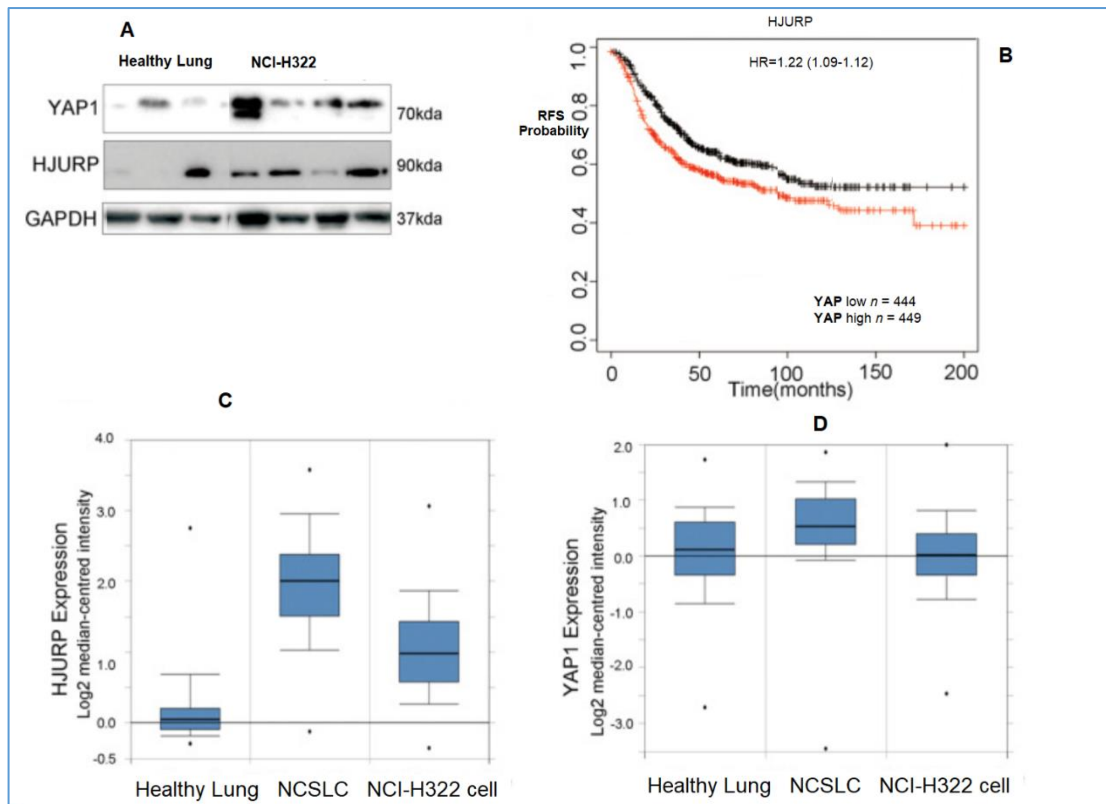
American Journal of BioMedicine
AJBM 2022;10(4):193-205
Research Article doi:10.18081/2333-5106/2022.10/193

poor recurrence-free survival (RFS) in lung cancer patients with high HJURP expression (Fig. (Fig.1D).1D).

To validate the underlying mechanism, we selected MDA-MB-231 and NCI-H322 cell lines for further exploration (Fig. 2). After knocking down HJURP, the protein level of YAP1 expression was significantly decreased (Fig. 2), but the mRNA level of YAP1 did not change (Fig. 2). Next, we tested the cellular localization of YAP1 protein after HJURP depletion. Immunofluorescence localization (Fig. 2) and Cytoplasmic and nuclear fractionation assay (Fig. 2) indicated that the nuclear localization of YAP1 is significantly reduced after knocking down HJURP. Collectively, these results indicated that HJURP can inhibit YAP1 from entering the nucleus, thereby inhibiting its transcriptional activity.

Figure 1.

To determine whether HJURP involves in the progression of NSCLC, we firstly examined the expression level of HJURP in lung cancer cells.



Research Article doi:10.18081/2333-5106/2022.10/193

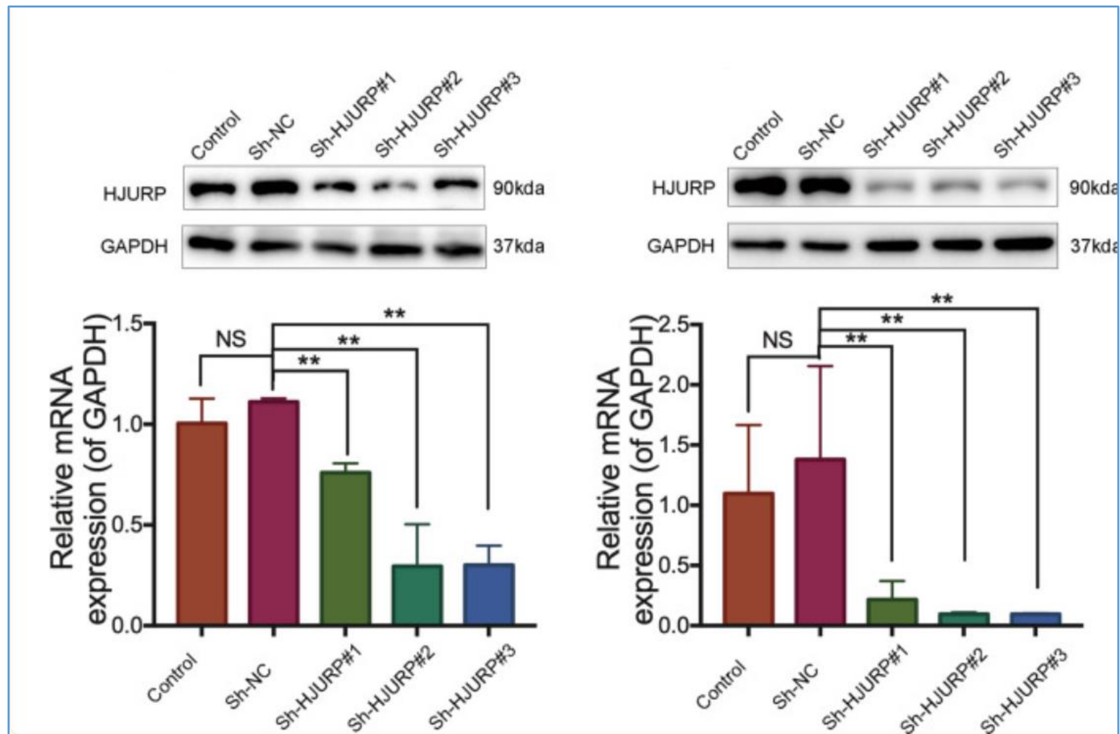


Figure 2.

Validate the underlying mechanism, we selected NCI-H322 cell lines for further exploration

HJURP regulates YAP1 ubiquitination

To further investigate the impact of HJURP on YAP1 activity, we examined the effect of HJURP on YAP1 stability in NCI-H322 cells treated with cycloheximide (CHX), an inhibitor of protein synthesis. We found that YAP1 stability is reduced after depletion of HJURP. As shown in Fig.3 A, HJURP could decrease YAP1 protein level, which could be minimized with the presence of the lysosome inhibitor Chloroquine but not proteasome inhibitor MG-132. Further immunoprecipitation experiments suggested that HJURP can bind with YAP1 protein (Fig. 3B). We then detected the effect of HJURP on the ubiquitination of YAP1 protein. The ubiquitination-based immunoprecipitation showed that HJURP inhibits YAP1 overall poly-ubiquitination Therefore, the above experimental results suggested that HJURP binds to YAP1 protein and then regulates the stability and cellular distribution of YAP1, thereby promoting the ubiquitination level of YAP1.

Research Article doi:10.18081/2333-5106/2022.10/193

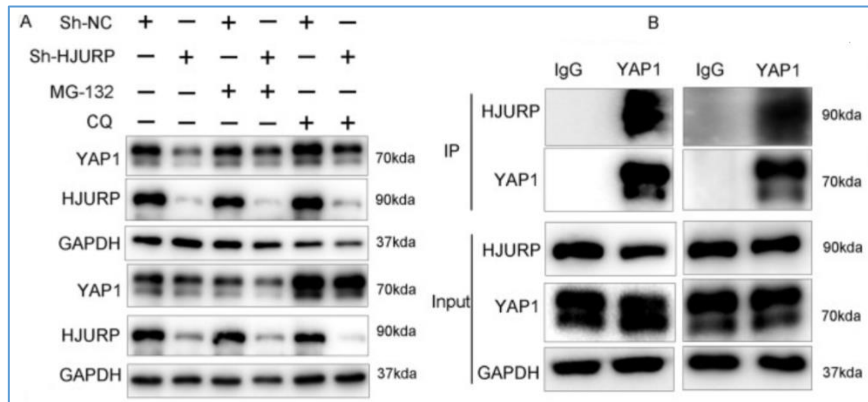


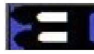
Figure 3.

HJURP regulates YAP1 ubiquitination

HJURP/YAP1- axis promotes NDRG1 transcription

In order to further analyze the regulatory network of the HJURP/YAP1 pathway in NSCLC, we performed RNA-sequencing after knocking down YAP1 in NCI-H322 cells (Supplementary Fig. 1A, B). The results showed a total of 326 gene expression changes, of which 136 genes were up-regulated after knocking down the YAP1, and 190 genes were downregulated The Go enrichment was shown in Supplementary Fig. 2C. We found that the expression of NDRG1 transcripts is significantly decreased in the YAP1 knockdown group. Previous research also indicated that mRNA expression of NDRG1 is relatively higher in NSCLC than that in other types of lung cancer cells [12]. Additionally, Kaplan–Meier meta-analyses showed that a high expression of NDRG1 mRNA level is specifically significantly associated with RFS in breast cancer patients with high YAP1 expression. Depletion of YAP1 in NCI-H322 cells decreased the NDRG1 expression.

We then demonstrated that YAP1 can bind to the promoter region of the NDRG1 gene determined by the CHIP results. We constructed the promoter region of the NDRG1 gene to be inserted into the vector. Luciferase reporter system assays also showed that YAP1 can positively regulate NDRG1 transcription. In order to further clarify the binding site, we constructed different truncations of the NDRG1 promoter region and found that only the 0–191 region of the NDRG1 promoter can cause changes in its transcription levels. Taken together, these results indicated that HJURP/YAP1 regulatory axis is important for the transcription activation of NDRG1.


Research Article doi:10.18081/2333-5106/2022.10/193

HJURP/YAP1/NDRG1 pathway relates to the tumor growth of NSCLC in vivo and in vitro

To understand the roles of the HJURP/YAP1/NDRG1 axis underlying the formation of TNBC cells, we knocked down the YAP1 and cell growth was decreased, while this inhibition was reversed by overexpressing NDRG1. The colony number was significantly reduced in the YAP1 knockdown group and increased again after overexpressing NDRG1.

Additionally, NDRG1 overexpression abolished YAP1 knockdown-induced chemotherapy sensitivity both in MDA- NCI-H322 (Fig. 2A). We further investigated the role of this pathway with in vivo assay. Mouse xenograft assays were performed by injecting BALB/c nude mice with NCI-H322 cells stably depleted of HJURP. We found that depletion of HJURP led to decreased tumor size and was more sensitive to Cisplatin suggesting that HJURP activation is important for TNBC tumor growth and chemotherapy response in vivo.

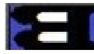
The immunohistochemical images showed HJURP/YAP1/NDRG1 axis promotes NSCLC tumor growth. Taken together, these results supported a crucial role of the HJURP/YAP1/NDRG1 pathway in NSCLC in vivo and in vitro.

Discussion

NSCLC continuously contributes to cancer-related mortality and is a major public health burden worldwide [3]. Growing evidence has shown that altered patterns of several gene expressions correlate with various human diseases, especially various types of cancers and the potential functions of tumor-related genes are very complex [20]. The identification of several critical genes which could be used as biomarkers and therapeutic targets is urgent to improve the prognosis of NSCLC patients.

Many studies reported that increased expression of HJURP in lung cancer with limited evidence [21, 22]. Our results indicated that HJURP was highly expressed in NSCLC than healthy lung tissue. Based on the clinical assays, we found that high HJURP expression was associated with TNM stage and distant metastasis, suggesting the tumor-promoting role of HJURP for clinical progress of NSCLC. Furthermore, we used an online bioinformatics statistics software which can calculate the data from TCGA datasets to explore the association between HJURP and clinical prognosis of NSCLC patients.

Results showed that high expression of HJURP was associated with shorter overall survival and disease-free survival. However, due to the limitation of clinical data, univariate and multivariate analysis were not performed to confirm whether the HJURP expression was an independent prognostic factor for NSCLC patients. Although several studies on HJURP in the tumor have been reported, little is known about the biological function and mechanism of HJURP in tumors [24]. The published studies reported that HJURP inhibition induces cell cycle dysregulation in bladder cancer cells via the PPARγ-SIRT1 feedback loop [25], while other researcher found that HJURP expression was significantly increased in hepatocellular


Research Article doi:10.18081/2333-5106/2022.10/193

carcinoma tissues and correlates with shorter overall survival of hepatocellular carcinoma patients [26]. In vitro experiments indicated that HJURP acted as a tumor promoter in this disease due to its tumor-promoting roles in regulating hepatocellular carcinoma cell proliferation [27].

In this study we explore the specific effects of HJURP on NSCLC behaviors, via used si-HJURP to down-regulate the levels of HJURP in lung cancer cells. Then, we performed a series of cells experiments and found that the knockdown of HJURP acted as a significant positive regulator in the modulation of proliferation, migration, and invasion of lung cancer cells.

Also, we found that down-regulation of HJURP promoted apoptosis. We found that YAP1/NDRG1 were significantly down-regulated in lung cancer cells after transfected with si-HJURP, indicating that HJURP displayed functions in the metastasis of NSCLC cells via regulating the EMT progress. The YAP1/NDRG1 signaling pathway, a highly conserved molecular mechanism, plays a critical role in the regulation of the development of morphogenesis, gene transcription, differentiation, and proliferation [28].

This pathway has been involved in the modulation of various tumors, including cervical cancer, melanoma, glioblastoma, and NSCLC, via modulating its downstream targets, such as Cyclin D1 [22]. A growing interest toward tumor-related genes in cancer has been sparked and several functional genes have been confirmed to display their oncogenic or anti-oncogenic roles by modulating various critical signaling pathways, including YAP1/NDRG1 signaling pathway [29]. Resulted data showed that the mRNA and protein level of these genes were decreased in HJURP knockdown group, which suggested that HJURP may act as a tumor promoter in NSCLC by promoting the YAP1/NDRG1 pathway.

Conclusion

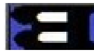
We showed that HJURP was upregulated in NSCLC tissues and NCI-H322 cell lines and provided the first evidence that HJURP promoted. NSCLC cell proliferation and metastasis by inactivating the YAP1/NDRG1 pathway. The data described in this study suggested that HJURP is a potential candidate for further functional research in NSCLC and indicated the clinical value of HJURP as a novel therapeutic target for NSCLC patients.

Competing interests

The authors declare no conflict of interest.

Ethics Statement

This study has been approved by the Ethical Review Committee of the School of Medicine, Chengdu. The publication of any potentially identifiable images or data contained in the article requires personal written informed consent. The research team will provide consultations for all subjects and their families to answer any research questions. Before signing the informed consent form, after the patients and their families fully understand the research process, our


Research Article doi:10.18081/2333-5106/2022.10/193

team members will organize the patients to sign the informed consent form or withdraw from the research. All subjects or their guardians will sign informed consent. Authors tend to submit research results to peer-reviewed journals or academic conferences for publication.

Authors' contributions

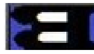
All authors shared in the conception and design and interpretation of data, drafting of the manuscript and critical revision of the case study for intellectual content and final approval of the version to be published. All authors read and approved the final manuscript.

Open access

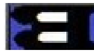
This is an open access article distributed in accordance with the Creative Commons Attribution Non-Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial.
http://creativecommons.org/ licenses/by-nc/4.0/.

References

1. Lanuti M. Surgical management of lung cancer involving the chest wall. Thorac Surg Clin 2017; 27:195-199.
2. Johnson DH, Schiller JH, Bunn PA, Jr. Recent clinical advances in lung cancer management. J Clin Oncol 2014; 32: 973-982.
3. Veronesi G. Lung cancer screening: the European perspective. Thorac Surg Clin 2015; 25:161-174.
4. She J, Yang P, Hong Q, Bai C. Lung cancer in China: challenges and interventions. Chest 2013; 143:1117-1126.
5. Wu QP, Min JX, Jiang L, Li JM, Yao K. Screening of biomarkers for lung cancer with gene expression profiling data. Eur Rev Med Pharmacol Sci 2013; 17: 3221-3228.
6. Perpelescu M, Hori T, Toyoda A, Misu S, Monma N, Ikeo K, Obuse C, Fujiyama A, Fukagawa T. HJURP is involved in the expansion of centromeric chromatin. Mol Biol Cell 2015; 26:2742-2754.
7. Yousif NG. Fibronectin promotes migration and invasion of ovarian cancer cells through up-regulation of FAK–PI 3 K/A kt pathway. Cell biology international 2014; 38(1):85-91.
8. Kato T, Sato N, Hayama S, Yamabuki T, et al. Activation of Holliday junction recognizing protein involved in the chromosomal stability and immortality of cancer cells. Cancer Res 2007; 67:8544–8553.
9. Dunleavy EM, Roche D, Tagami H, Lacoste N, Ray-Gallet D, Nakamura Y, et al. HJURP is a cell-cycle-dependent maintenance and deposition factor of CENP-A at centromeres. Cell 2009; 137:485–97.


Research Article doi:10.18081/2333-5106/2022.10/193

10. Serafim RB, Cardoso C, Di Cristofaro LFM, et al. HJURP knockdown disrupts clonogenic capacity and increases radiation-induced cell death of glioblastoma cells. Cancer Gene Ther 2020; 27:319–29.
11. Kang DH, Woo J, Kim H, Kim SY, Ji S, Jaygal G, et al. Prognostic relevance of HJURP expression in patients with surgically resected colorectal cancer. Int J Mol Sci 2020; 21:7928.
12. Mohammed KG, Mohammed SM, Hadi N, et al. Association between Natural Killer Cell Cytotoxicity and the Progression of Non-Small Cell Lung Cancer. Sys Rev Pharm 2020; 11(4):543-551.
13. Lai W, Zhu W, Xiao C, Li X, Wang Y, Han Y, et al. HJURP promotes proliferation in prostate cancer cells through increasing CDKN1A degradation via the GSK3beta/JNK signaling pathway. Cell Death Dis 2021; 12:583.
14. Chen T, Huang H, Zhou Y, Geng L, Shen T, Yin S, et al. HJURP promotes hepatocellular carcinoma proliferation by destabilizing p21 via the MAPK/ERK1/2 and AKT/GSK3beta signaling pathways. J Exp Clin Cancer Res 2018; 37:193.
15. Zhou X, Li Y, Wang W, Wang S, Hou J, Zhang A, et al. Regulation of Hippo/YAP signaling and esophageal squamous carcinoma progression by an E3 ubiquitin ligase PARK2. Theranostics. 2020; 10:9443–57.
16. Zhu H, Yan F, Yuan T, Qian M, Zhou T, Dai X, et al. USP10 promotes proliferation of hepatocellular carcinoma by deubiquitinating and stabilizing YAP/TAZ. Cancer Res 2020; 80:2204–16.
17. Zhang Z, Du J, Wang S, Shao L, Jin K, Li F, et al. OTUB2 promotes cancer metastasis via hippo-independent activation of YAP and TAZ. Mol Cell 2019; 73:7–21.e27.
18. Popovic D, Vucic D, Dikic I. Ubiquitination in disease pathogenesis and treatment. Nat Med 2014; 20:1242–53.
19. Pang D, Wang W, Zhou X, Lu K, Zhang J, Chen Z, et al. RACO-1 modulates Hippo signalling in oesophageal squamous cell carcinoma. J Cell Mol Med 2020; 24:11912–21.
20. Dunleavy EM, Roche D, Tagami H, Lacoste N, Ray-Gallet D., Nakamura Y., Daigo Y., Nakatani Y., et al. Almouzni-Pettinotti G. HJURP is a cell-cycle- dependent maintenance and deposition factor of CENP-A at centromeres. Cell 2009; 137:485–497.
21. Fang BA, Kovacevic Z, Park KC, Kalinowski DS, Jansson PJ, Lane DJ, et al. Molecular functions of the iron-regulated metastasis suppressor, NDRG1, and its potential as a molecular target for cancer therapy. Biochim Biophys Acta 2014; 1845:1–19.
22. Jung EU, Yoon JH, Lee YJ, Lee JH, Kim BH, Yu SJ, et al. Hypoxia and retinoic acid-inducible NDRG1 expression is responsible for doxorubicin and retinoic acid resistance in hepatocellular carcinoma cells. Cancer Lett 2010; 298:9–15.


Research Article doi:10.18081/2333-5106/2022.10/193

23. Yan X, Chua MS, Sun H, So S. N-Myc down-regulated gene 1 mediates proliferation, invasion, and apoptosis of hepatocellular carcinoma cells. Cancer Lett 2008; 262:133–42.
24. Ito H, Watari K, Shibata T, Miyamoto T, Murakami Y, Nakahara Y, et al. Bidirectional regulation between NDRG1 and GSK3β controls tumor growth and is targeted by differentiation inducing factor-1 in glioblastoma. Cancer Res 2020; 80:234–48.

American Journal of BioMedicine
Journal Abbreviation: AJBM
ISSN: 2333-5106 (Online)
DOI: 10.18081/issn.2333-5106
Publisher: BM-Publisher
Email: editor@ajbm.net