Overexpression of *HYOU1* is associated with cisplatin resistance and may depend on m^{6}A modification in patients with cervical cancer

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Abstract. Cervical cancer (CC) is the fourth leading cause of cancer-associated mortalities among women worldwide. The chemotherapeutical platinum-based agent cisplatin (DDP) is the standard therapy for locally advanced or recurrent CC; however, platinum resistance limits its clinical benefit. Therefore, the present study aimed to identify key genes associated with DDP resistance in patients with CC and investigate the underlying molecular mechanisms. Firstly, using the CRISPR-Cas9 dataset of CC cells derived from DepMap portal, 699 genes associated with CC cell survival were identified. Subsequently, using the gene expression profiles of normal and CC samples with a response status to DDP, derived from The Cancer Genome Atlas (TCGA), hypoxia upregulated 1 (*HYOU1*) was further identified as significantly upregulated in CC samples and patients that did not respond to DDP (non-responders) when compared with healthy controls and patients that did respond to DDP (responders), respectively, using unpaired student's t-tests. Additionally, the log-rank test revealed that the high expression of *HYOU1* was significantly associated with the poor survival of patients receiving DDP. The association between the high *HYOU1* expression levels and the poor survival of patients receiving DDP was validated in the remaining TCGA dataset of patients with CC. *HYOU1* expression levels were positively associated with the half-maximal inhibitory concentration value of DDP in CC cells using data derived from the Genomics of Drug Sensitivity in Cancer database. *In vitro*, western blotting experiments revealed high HYOU1 protein expression levels in DDP-resistant HeLa cells compared with their parental HeLa cells. Furthermore, the knockdown of *HYOU1* resulted in an increased sensitivity of HeLa cells to DDP. Finally, using the sequence-based RNA adenosine methylation site predictor program, it was found that N^6-methyladenosine (m^{6}A) was highly enriched in HYOU1. The expression levels of the m^{6}A reader, *EIF3A*, was positively correlated with the expression levels of *HYOU1* and was upregulated in the non-response group compared with the response group in a dataset from TCGA database. Additionally, *EIF3A* had the highest probability of binding to the m^{6}A motifs of *HYOU1* compared with other genes. In GSE56363 obtained from the Gene Expression Omnibus, the non-responders had significantly increased expression levels of *EIF3A* compared with the responders. In conclusion, high expression levels of *HYOU1*, which may be regulated by *EIF3A* due to m^{6}A modifications, was associated with DDP resistance in patients with CC and could potentially be used as an indicator of DDP treatment resistance.

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Abbreviations: CC, cervical cancer; DDP, cisplatin; *HYOU1*, hypoxia-upregulated 1 gene; ER, endoplasmic reticulum; m^{6}A, N^6-methyladenosine; IC_{50}, half-maximal inhibitory concentration; TCGA, The Cancer Genome Atlas; OS, overall survival; GDSC, Genomics of Drug Sensitivity in Cancer; HRs, hazard ratios; CIs, confidence intervals; GSEA, gene set enrichment analysis; WebGestalt, web-based gene set analysis toolkit; FDR, false discovery rates; *SMG5*, nonsense-mediated mRNA decay factor; SRAMP, sequence-based RNA adenosine methylation site predictor; WB, western blotting; RI, resistance index

Key words: cervical cancer, chemotherapy, *HYOU1*, m^{6}A reader, DDP resistance

Introduction

Cervical cancer (CC) is the fourth leading cause of cancer-associated mortalities among women worldwide despite

advancements in diagnosis, prevention and treatment (1,2). The prognosis of patients with advanced or recurrent CC is poor, with a 1-year survival rate of only 10-20% (3). Chemotherapy is the standard treatment for patients with advanced or recurrent CC. Although the chemotherapeutic agent cisplatin (DDP) is the most effective drug for treating CC (4), resistance to DDP-based treatment limits the survival of patients with partial CC, leading to poor prognosis (4).

The mechanisms underlying DDP resistance in CC have been examined and strategies have been proposed to overcome the resistance (5-8). Previous studies show that reduced accumulation of intracellular platinum compounds (5), increased DNA damage repair (6), inactivation of apoptosis (7) and activation of the epithelial-mesenchymal transition (8) are associated with DDP resistance. In the previous number of decades, an increasing number of studies have shown that tumor cells hijack the unfolded protein response to induce chemotherapy resistance by activating the unfolded response sensors activated transcription factor 6, inositol-requiring transmembrane kinase/endoribonuclease 1α and protein kinase R-like endoplasmic reticulum kinase as well as their master regulator glucose regulated protein 78 (9-12). The *hypoxia-upregulated 1* (*HYOU1*) gene encodes a chaperone protein in the endoplasmic reticulum (ER). Various stimuli, including hypoxia, impaired ubiquitination, proteasomal degradation and energy deficiency induce an unfolded protein response in the presence of ER stress, accompanied by the expression of ER molecular chaperones such as protein kinase-like ER kinase, inositol-requiring enzyme 1, and activating transcription factor 6α (13).

N^6-methyladenosine (m^{6}A), which is among the most prevalent and reversible internal RNA modifications in eukaryotic RNAs (14), occurs at the consensus motif RRACH (R is G, A or U; H is U, A or C) and regulates RNA transcription, splicing, degradation and translation (15). m^{6}A modification of RNA is catalyzed by the m^{6}A methyltransferase enzyme complexes (writers), removed by m^{6}A demethylase enzymes (erasers) and recognized by specific proteins (readers) (16-20). Previous studies demonstrate that the m^{6}A modification is involved in promoting the tumorigenesis, metastasis and drug resistance of different types of cancer (21-23). However, whether the m^{6}A modification is involved in regulating DDP resistance in CC remains unclear.

The present study aimed to utilize bioinformatic methods to identify genes associated with DDP resistance in CC using various public databases. Using CRISPR data of CC cell lines and the gene expression profiles of CC samples, key genes associated with the survival and DDP resistance of CC were investigated. Furthermore, the association of key genes with the survival of patients with CC treated with DDP were also investigated using public datasets and *in vitro* experiments. Additionally, the m^{6}A-associated genes involved in regulating dysregulated genes were investigated.

Materials and methods

Gene expression data of CC samples. The dataset associated with CC [The Cancer Genome Atlas (TCGA)-CC] was obtained by searching for the keywords 'cervical cancer' in TCGA (https://portal.gdc.cancer.gov/) database. The dataset (accession no. GSE56363) was obtained from the Gene Expression Omnibus (GEO; www.ncbi.nlm.nih.gov/geo/) database. The inclusion criteria were as follows: i) Patients with CC who received DDP; and ii) survival information or response status to DDP were recorded.

The expression profile of CC and clinical data were obtained by searching for 'cervical cancer' in the TCGA database from the Genomic Data Commons (GDC) Data Portal (https://portal.gdc.cancer.gov/), and consisted of 178 CC tissues and three adjacent non-tumor tissues. Based on the clinical information of the patients in the TCGA-CC dataset, there were 43 patients with both the response status to DDP and overall survival (OS) status recorded. These patients were selected as the discovery set (TCGA-CC$_1$ set; Table I) to identify the genes associated with DDP resistance. The 40 samples, which only recorded the OS of patients receiving DDP were used as the validation set (TCGA-CC$_2$ set; Table I) to support the association of genes with DDP resistance. To exclude the prognostic association of the genes, the 95 patients that did not receive treatment were selected as the control set (TCGA-CC$_3$ set; Table I) for survival analysis (24). GSE56363 consisted of 12 CC samples with complete response to DDP and 9 CC samples with non-complete response to DDP.

RNA-sequencing data were downloaded from TCGA via the GDC Data Portal (https://portal.gdc.cancer.gov/), which had been detected using the Illumina HiSeq 2000 platform. The fragments per kilobase of transcript per million mapped read values were log2-scaled plus 1 for gene expression level measurements.

Database. To identify key genes associated with CC cell survival, the CRISPR-Cas9 screening data of CC cell lines were downloaded from the DepMap portal (https://depmap.org/portal/) by selecting 'Version: DepMap Public 21Q2' and 'CRISPR_gene_effect' sections. The database recorded the gene essentiality scores [CRISPR-Cas9 gene knockout scores (CERES)] of genes in CC cell lines, which indicated the influence of knockout genes on the proliferation in CC cell lines (25,26). The lower the CERES score, the greater the effect after the gene knockout.

To validate the association of genes with DDP resistance, the gene expression profiles of CC cell lines and their half-maximal inhibitory concentration (IC$_{50}$) values for DDP drugs were acquired from the Genomics of Drug Sensitivity in Cancer (GDSC; https://www.cancerrxgene.org; release-8.2) database (27) by selecting the 'Cell Line Gene Expression Data' and 'Drug Sensitivity Data' sections.

Relevant literature was used to identify 30 m^{6}A-associated genes (28-31), including 11 methyltransferases, two demethylases and 17 reader proteins (Table II).

Cell culture. HeLa, a human CC cell line, was purchased from Macgene Biotechnology (https://www.macgene.com/). HeLa cells were routinely cultured in Dulbecco's modified Eagle's medium (DMEM; Wuhan Servicebio Technology Co., Ltd.), which was supplemented with 10% fetal bovine serum (FBS; Zhejiang Tianhang Biotechnology Co., Ltd.). Cells were grown at 37˚C and 5% CO_2 under humidified conditions and passaged upon reaching 80-90% confluency.

Table I. Sample data of TCGA.

Characteristic	TCGA-CC_1	TCGA-CC_2	TCGA-CC_3	Total
Sex, female	43	40	95	178
Age, years [mean (SD)]	48.14 (13.05)	47.10 (14.08)	47.60 (13.56)	-
Stage				
I	10	21	63	94
II	20	8	18	46
III	5	7	9	21
IV	6	2	4	12
Unknown	2	2	1	5
Distant metastasis				
Yes	2	0	0	2
No	13	16	42	71
Unknown	28	24	53	105
Lymph node metastasis				
Yes	7	10	16	33
No	13	11	59	83
Unknown	23	19	20	62
Response status				
Yes	37	0	-	37
No	6	0	-	6
Unknown	0	40	-	40

CC, cervical cancer; TCGA, The Cancer Genome Atlas.

Cell viability assay. Cell viability was investigated using the Cell Counting Kit-8 (CCK-8; cat. no. C0038; Beyotime Institute of Biotechnology) assay. Cells were seeded at a density of $1x10^4$ cells/ml in a 96-well plate at a volume of 100 ml/well. Various concentrations (0-100,000 nM) of DDP (cat. no. P4394; Sigma-Aldrich; Merck KGaA) were introduced into the culture medium, with a three-fold gradient to systematically probe the cytotoxic effects. After a 96-h incubation at 37˚C, cell viability was quantified using the CCK-8 assay and measuring the absorbance, which was used to calculate the cell survival rate. The subsequent data were fitted to a dose-response curve to determine the IC_{50} of cell proliferation. The equation used to calculate inhibition (%) was: Inhibition (%)=$[(A_c-A_s)/(A_c-A_b)]$ x100. 'A_s' and 'A_b' represent the absorbance of the experimental wells and the wells with the highest concentration, respectively. 'A_c' represents the absorbance of the control wells.

DDP-resistant cells construction. HeLa cells were initially treated with 1 μM DDP which was increased to 2 μM after ~2 months and treatment was continued at this concentration for another 4 months until stabilization, resulting in DDP-resistant cells (HeLa/DDP). Subsequently, HeLa/DDP cells were seeded at a density of $5x10^5$ cells/well into 6-well plates and maintained in culture medium containing 2 μM cisplatin at 37˚C. Next, HeLa/DDP cells were cultured in the presence of increasing concentrations of DPP (cat. no. P4394; Sigma-Aldrich; Merck KGaA) to establish the IC_{50}. The drug sensitivity of the cells were quantified by determining the IC_{50} using a cell viability assay. The resistance index (RI) was calculated as the ratio of the IC_{50} of the resistant cells to the IC_{50} of the parental cells, which served as a measurement of the relative resistance. An RI >3 indicated that the resistant cell line was less sensitive to the drug compared with the parental cell line.

Western blotting (WB). WB was used to detect HYOU1 protein levels in three independent experiments. HeLa and HeLa/DDP cells were harvested and lysed in Whole Protein Extraction kit (cat. no. WLA019, Wanleibio Co., Ltd.) for 5 min. The supernatant was centrifuged at 4˚C and 10,005 x g for 10 min and the protein concentration was determined using a bicinchoninic acid kit. Following this, 40 μg of protein from the supernatant was loaded per lane on a 10% gel and SDS-PAGE was carried out before the proteins were transferred to a PVDF membrane. Subsequently, the membrane was blocked with blocking buffer (cat. no. WLA066; Fast Blocking Western; Wanleibio Co., Ltd.) for 1 h at room temperature and then incubated with either the HYOU1 (cat. no. R383157; 1:500; Chengdu Zen-Bioscience Co., Ltd.) or the β-actin (cat. no. WL01372; 1:1,000; Wanleibio Co., Ltd.) primary antibody overnight at 4˚C. The membranes were then rinsed with TBST (0.15% Tween20; Wanleibio Co., Ltd.) and incubated with a secondary antibody (cat. no. WLA023; 1:5,000; Goat Anti-Rabbit IgG/HRP; Wanleibio Co., Ltd.) for 45 min at 37˚C. Subsequently, the membrane was washed with TBST six times and visualized using Ultrasensitive ECL Chemiluminescence Kit (cat. no. WLA006; Wanleibio Co., Ltd.) (32). The total

Table II. R, P and FDR values for N6-methyladenosine-associated genes.

Type	Genes	R	P-value	FDR
Methyltransferases	*ZC3H13*	0.4566	0.0021	0.0156
	RBM15B	0.4141	0.0058	0.0247
	VIRMA	0.3708	0.0144	0.0479
	ZCCHC4	0.3594	0.0179	0.0538
	CBLL1	0.3150	0.0396	0.0914
	METTL16	-0.2918	0.0576	0.1234
	METTL3	0.2553	0.0985	0.1739
	METTL14	0.2313	0.1356	0.2034
	METTL5	-0.1695	0.2773	0.3618
	WTAP	0.0096	0.9511	0.9908
	RBM15	-0.0079	0.9598	0.9908
Demethylases	*FTO*	0.3329	0.0292	0.0730
	ALKBH5	0.2854	0.0635	0.1271
Reader proteins	*G3BP2*	0.5410	0.0002	0.0040
	PRRC2A	0.5292	0.0003	0.0040
	EIF3A	0.4764	0.0012	0.0124
	YTHDF3	0.4291	0.0041	0.0245
	G3BP1	0.4146	0.0057	0.0247
	YTHDF1	0.4022	0.0075	0.0281
	IGF2BP1	0.3453	0.0234	0.0637
	IGF2BP3	0.2808	0.0682	0.1279
	HNRNPA2B1	0.2484	0.1082	0.1804
	YTHDC2	0.2318	0.1347	0.2034
	IGF2BP2	0.2234	0.1499	0.2142
	YTHDF2	0.1746	0.2629	0.3585
	HNRNPC	0.0988	0.5283	0.6604
	RBMX	0.0925	0.5554	0.6665
	YTHDC1	0.0503	0.7489	0.8321
	ELAVL1	0.0530	0.7359	0.8321
	FMR1	0.0018	0.9908	0.9908

R, Pearson correlation coefficient; FDR, false discovery rate.

protein concentration obtained was 2 μg/μl. The intensity of each band was quantified using Gel-Pro-Analyzer software (version 4.0; Media Cybernetics, Inc.).

Transfection. All small interfering RNA (siRNA), with a final concentration of 50 nM, were transiently transfected into HeLa/DDP cells using Lipofectamine®™ 3000 (Invitrogen; Thermo Fisher Scientific, Inc.) for 20 min to form transfection complexes at 37˚C. Following a 6-h incubation, the transfection medium was replaced with fresh growth medium. DDP was added the next day and the culture was continued for 48 h at 37˚C. Transfection efficiency was semi-quantified using WB. The siRNA sequences (Wanleibio Co., Ltd.) used were as follows: *HYOU1* sense: 5'-AAGCUGCUGCGUGAGGCUAAU C-3'; anti-sense: 5'-GAUUAAGCCUCACGAGCAGCUU-3'; *HYOU1* siRNA-2 sense: 5'-AGCUGGGGAAGAACAUCA AU-3'; anti-sense: 5'-AUUGUUCUUCCCAUCAUCG-3'; and siRNA negative control (NC) sense: 5'-AUAAACAUCGAC UCAAU-3'; anti-sense: 5'-AUUGAGCUCGAUUGUUAU-3'.

Statistical analysis. An unpaired student's t-test was used to identify differentially expressed genes (DEGs) between tumor and normal samples. OS was defined as the time from the date of initial surgical resection to the date of mortality or last contact (censored), which was truncated to 60 months. As the number of responders and non-responders may not be equal, the 'surv_cutpoint' algorithm was used to determine the optimal cut-off to distinguish between the high and low expression levels of genes. Survival curves were drawn using the Kaplan-Meier method and statistically compared using the log-rank test. A univariate Cox regression model was used to analyze the association between clinical factors and OS. Hazard ratios (HRs) and 95% confidence intervals (CIs) were calculated using Cox regression models.

Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis was performed using gene set enrichment analysis (GSEA) from the web-based gene set analysis toolkit (WebGestalt; http://www.webgestalt.org) (33), with a cut-off value of <0.05 for the false discovery

rate (FDR). The m^6A sites of genes were predicted using a sequence-based RNA adenosine methylation site predictor (SRAMP) program (http://www.cuilab.cn/sramp/) (34) by inputting the sequences of the genes. The RMBase version 2.0 platform (http://rna.sysu.edu.cn/rmbase/) (35), a comprehensive resource for RNA modification data verified using methylated RNA immunoprecipitation sequencing, m^6A-sequence and/or m^6A-crosslinking immunoprecipitation arrays, was used to validate whether the predicted m^6A sites underwent m^6A modification. Subsequently, the interaction probabilities between predicted m^6A site sequence motifs and the protein sequence of a m^6A-associated gene were retrieved using the RNA-Protein Interaction Prediction (RPISeq) database (http://pridb.gdcb.iastate.edu/RPISeq/) (36). This database calculated the interaction probabilities using random forest (RF) and support vector machine (SVM) methods.

The correlation between gene expression levels and IC_{50} values for DDP in the GDSC database was estimated using Pearson correlation analysis and the ggplot2 package in R (https://cran.r-project.org/web/packages/ggplot2/index.html) was used to visualize the results. Comparisons between two groups were analyzed using the unpaired student's t-test. Comparisons among multiple groups were analyzed using one-way analysis of variance (ANOVA) and Tukey's test. P-values were adjusted using the Benjamini-Hochberg procedure for multiple testing (37) to control for the FDR. FDR <0.05 for multiple testing or P<0.05 was considered to indicate a statistically significant difference.

Results

HYOU1 is a gene that promotes CC survival and DDP resistance. Based on CRISPR-Cas9 screening data from CC cell lines, 699 genes were identified with potential impact on cell proliferation in CC cell lines, in which the CERES scores were <-1 in >75% of CC cell lines. Compared with normal samples, 3,309 DEGs were identified in 43 samples with CC derived from the TCGA-CC_1 set (unpaired student's t-test; FDR <0.05 and $\log_2$(FC)>0; Fig. 1A). Furthermore, 401 DDP resistance genes were identified in the non-response group compared with those in the response group (unpaired student's t-test; P<0.05 and $\log_2$(FC)>0; Fig. 1B). Three genes, including *HYOU1*, *nonsense-mediated mRNA decay factor* (*SMG5*) and *ankyrin repeat and LEM domain containing 2* (*ANKLE2*), were selected as they were significantly upregulated in samples with CC and in the non-response group when compared with normal samples and the response group, respectively (Fig. 1C).

The area under the curve of *HYOU1*, *SMG5* and *ANKLE2* for predicting the response and non-response status was 0.802, 0.815 and 0.775, respectively (Fig. 1D-F). Finally, for each gene, the mean expression level was used to stratify patients into high- and low-expression groups and a survival analysis was performed. The results showed that there was no significant difference in the OS between the two groups for the three genes [*HYOU1* (high vs. low expression, 19 vs. 24; log-rank P=0.5412; HR=1.50; 95% CIs, 0.40-5.62), *SMG5* (high vs. low expression, 23 vs. 20; log-rank P=0.5557; HR=1.51; 95% CIs, 0.38-6.07) and *ANKLE2* (high vs. low expression, 20 vs. 23; log-rank P=0.6183; HR=1.40; 95% CIs, 0.37-5.22); Fig. S1].

It was hypothesized that the mean value may not be suitable for distinguishing patients with different responses to DDP. Therefore, the 'surv_cutpoint' algorithm was used to re-determine the optimal threshold for *HYOU1* expression levels, which was 4.9094. Survival analysis using the TCGA-CC_1 set showed that patients with high *HYOU1* expression levels (>4.9094) had a significantly reduced OS compared with patients with low *HYOU1* expression levels (<4.9094) following DDP treatment (high vs. low expression, 10 vs. 33; log-rank P=0.0456; HR=3.59; 95% CIs, 0.94-13.67; Fig. 1G). Similarly, the 'surv_cutpoint' algorithm was used to re-determine the optimal thresholds for *SMG5* and *ANKLE2*, which were 4.6751 and 3.0310, respectively. However, high or low *SMG5* expression levels (threshold, 4.6751) and *ANKLE2* expression levels (threshold, 3.0310) did not indicate a significantly different OS in the TCGA-CC_1 set [*SMG5* (high vs. low expression, 30 vs. 13; log-rank P=0.0761; HR=291,560,949.96; 95% CIs, 0-infinity (inf); Fig. 1H) and *ANKLE2* (high vs. low expression, 35 vs. 8; log-rank P=0.2170; HR=229,801,985.83; 95% CIs, 0-inf; Fig. 1I)]. Therefore, *HYOU1* was selected for follow-up analyses as a gene associated with the survival of CC cells and DDP resistance.

Validation of the association of HYOU1 with DDP resistance in independent datasets. In the TCGA-CC_2 set, the 'surv_cutpoint' algorithm was used to determine the optimal threshold for *HYOU1*, which was 4.9094. The 8 patients with high *HYOU1* expression levels (>4.9094) demonstrated a significantly reduced OS compared with the 32 patients with low *HYOU1* expression levels following DDP treatment (log-rank P=0.0012; HR=7.09; 95% CIs, 1.81-27.70; Fig. 2A). Using the TCGA-CC_3 set, high and low *HYOU1* expression levels did not indicate a significantly different OS in patients that did not receive DDP treatment (high vs. low expression, 19 vs. 76; log-rank P=0.6254; HR=1.49; 95% CIs, 0.30-7.38; Fig. 2B). Additionally, according to the GDSC database, the expression levels of *HYOU1* were significantly positively correlated with the IC_{50} values of DDP in CC cell lines (Pearson's correlation analysis; P=0.0384; r=0.58; Fig. 2C).

To validate the effect of *HYOU1* on the DDP resistance of CC, HeLa/DDP cells were constructed. The parental HeLa cells exhibited an IC_{50} of 1.65 μM. By contrast, the resistant cells had an IC_{50} of 15.51 μM, corresponding to an RI of 9. The IC_{50} values were determined using dose-response curves generated from cell viability assays (Fig. 2D). Using western blotting, the protein bands revealed an increased HYOU1 expression level in HeLa/DDP cells across three experiments compared with that in HeLa cells (Fig. 2E) and the semi-quantification values in Table SI further elucidates this. The results showed that the protein expression of HYOU1 was significantly increased in HeLa/DDP cells compared with that in parental HeLa cells (unpaired student's t-test; P=0.0002; Fig. 2F). To confirm the efficacy of *HYOU1* knockdown, knockdown efficiency was assessed. Using WB analysis, a significant reduction in protein expression levels of HYOU1 was observed in the knockdown groups (one-way ANOVA; P<0.001; Fig. S2), indicating the success of *HYOU1* knockdown. Based on this effective knockdown, it was further revealed that *HYOU1* knockdown significantly reduced the viability of DDP treated cells compared with the control (one-way ANOVA; P<0.001;

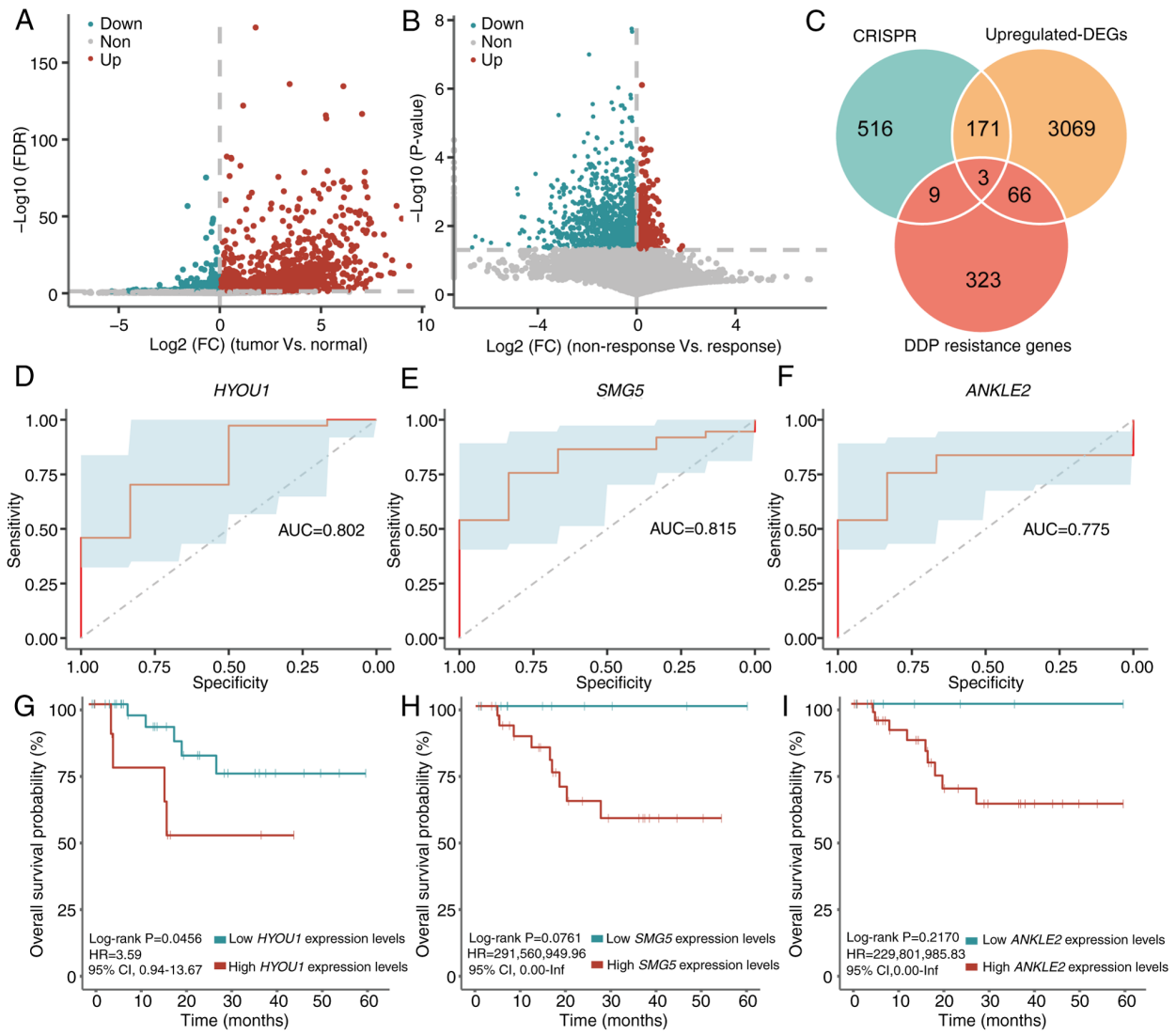

Figure 1. Identification of key genes in promoting CC survival and DDP resistance. Volcano plot of significant DEGs in (A) the tumor group compared with the normal group, and (B) the non-response group compared with the response group. (C) Venn diagram of the genes obtained from CRISPR, CC-associated differential genes and DDP response-associated differential genes. Receiver operating characteristic curves of (D) *HYOU1*, (E) *SMG5* and (F) *ANKLE2* in TCGA-CC$_1$ dataset. Kaplan-Meier curve of overall survival stratified by (G) *HYOU1*, (H) *SMG5* and (I) *ANKLE2* expression in TCGA-CC$_1$ set, respectively. DDP, cisplatin; AUC, area under the curve; CC, cervical cancer; HR, hazard ratio; CI, confidence interval; FDR, false discovery rate; FC, fold change; DEGs, differentially expressed genes; inf, infinity; TCGA, The Cancer Genome Atlas; CRISPR, clustered regularly interspaced short palindromic repeats; HYOU1, hypoxia-upregulated 1 gene; SMG5, nonsense-mediated mRNA decay factor; ANKLE2, ankyrin repeat and LEM domain containing 2.

Fig. 2G). These results suggest that high *HYOU1* expression levels are associated with resistance to DDP.

To further investigate the function of *HYOU1*, 2,952 genes that significantly correlated with the expression of *HYOU1* were identified (Pearson correlation analysis; FDR <0.05; |r|>0.3). These genes were notably enriched in 12 functional pathways (GSEA; FDR <0.05; Fig. 2H). Among these functional pathways, 'protein processing in endoplasmic reticulum' and 'N-glycan biosynthesis' were significantly enriched in genes that positively correlated with *HYOU1* and were involved in DDP resistance (9,13) (Fig. 2I). These results suggest that upregulated expression of *HYOU1* is associated with the accumulation of unfolded proteins, and may enhance the stress response in the ER and induce DDP resistance.

m^{6}A modification is enriched in HYOU1 and increases the stability of the transcript. Previous preliminary studies report that m^{6}A modifications are present in almost all types of RNA molecules in the cell, and regulate the transcriptome to influence RNA splicing, translation, export, localization and stability (18-20). To investigate whether the expression of *HYOU1* was regulated by m^{6}A modification, the online tool SRAMP was used to predict m^{6}A modification sites on *HYOU1*. This revealed six *HYOU1* sequence motifs with high confidence (Fig. 3A; Table III).

The correlation between m^{6}A-associated genes and the expression of *HYOU1* using the TCGA-CC$_1$ set was analyzed and eight m^{6}A-associated genes were found that significantly correlated with the expression of *HYOU1* (Pearson's correlation analysis; FDR <0.05; |r|>0.4; Fig. 3B; Table II). Among these genes, the expression of *EIF3A* was significantly upregulated in the non-response group compared with that of the response group (unpaired student's t-test; P=0.0399; FC=1.07; Fig. 3C). Furthermore, the 'surv_cutpoint' algorithm was used

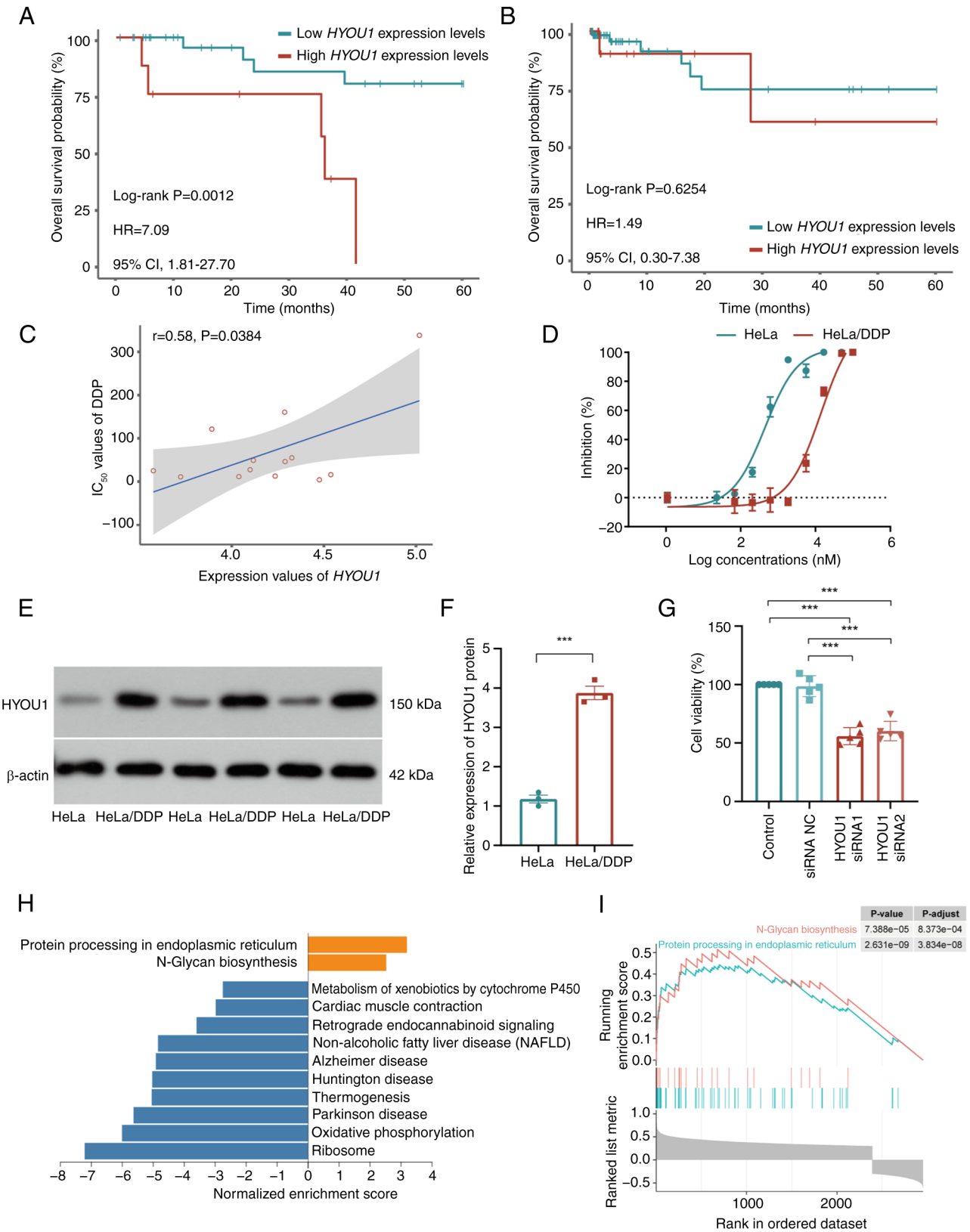

Figure 2. Validation of *HYOU1* in promoting CC survival and DDP resistance. Kaplan-Meier OS analysis of patients with high and low expression levels of *HYOU1* in the (A) TCGA-CC_2 and (B) TCGA-CC_3 datasets. (C) Point plot of the correlation analysis between the mRNA expression level values of *HYOU1* and IC_{50} values of DDP in the Genomics of Drug Sensitivity in Cancer database. (D) Survival curves of parental HeLa and HeLa/DDP cells that were subjected to different concentrations of DDP, as measured using the CCK-8 assay (n=5). (E) Representative western blot showing the HYOU1 protein expression levels in HeLa and HeLa/DDP cells. (F) Semi-quantified expression levels of HYOU1 in HeLa and HeLa/DDP cells (n=3). (G) Proliferation of HeLa/DDP cells treated with DDP and siRNA (HYOU1 siRNA or siRNA NC) or DDP alone using the CCK-8 assay (n=5), using one-way analysis of variance. (H) Bar plot of GSEA of *HYOU1*-associated genes; orange represents the activation pathway and blue represents the inhibition pathway. (I) GSEA results for the activation pathways. ***P<0.001. The statistical difference between two group was analyzed using the unpaired student's t-test, whereas the statistical difference among multiple groups was analyzed using one-way analysis of variance and Tukey's test. CC, cervical cancer; DDP, cisplatin; GSEA, gene set enrichment analysis; IC_{50}, half-maximal inhibitory concentration; OS, overall survival; TCGA, The Cancer Genome Atlas; NC, negative control; siRNA, small interfering RNA; HYOU1, hypoxia-upregulated 1 gene; HR, hazard ratio; CI, confidence interval; CCK-8, Cell Counting Kit-8; HeLa/DDP, DDP-resistant HeLa cells.

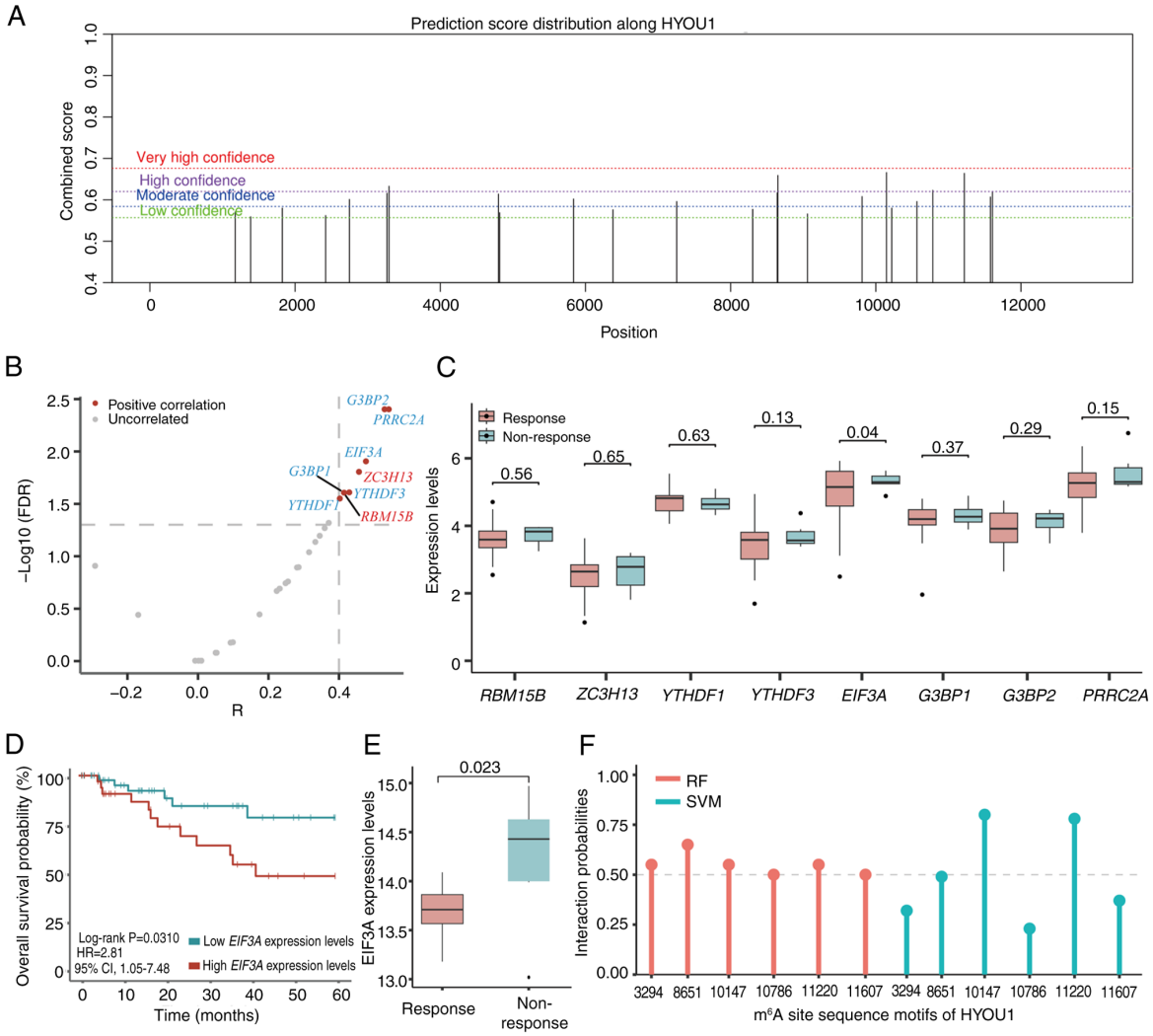

Figure 3. Analysis of predicted *HYOU1* m^{6}A sites and m^{6}A-associated genes. (A) m^{6}A sites of *HYOU1* were predicted using the sequence-based RNA adenosine methylation site predictor program (https://www.cuilab.cn/sramp). (B) Volcano plot of m^{6}A-associated genes that significantly correlated with the expression of *HYOU1*. Red, methyltransferases and blue, reader proteins. (C) Boxplot of m^{6}A-associated gene expression levels in the non-responsive and responsive groups. (D) Kaplan-Meier curves of the overall survival stratified by the *EIF3A* expression levels of patients from the TCGA-CC$_1$ or TCGA-CC$_2$ datasets. (E) Boxplot of the *EIF3A* expression levels in non-responsive and responsive groups in the GSE56363 dataset (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE56363). (F) Lollipop chart of the interaction probabilities of EIF3A with the six m^{6}A site sequence motifs according to RPISeq predictions. TCGA, The Cancer Genome Atlas, RF, random forest; SVM, support vector machine; HYOU1, hypoxia-upregulated 1 gene; m^{6}A, N^6-methyladenosine; EIF3A, eukaryotic translation initiation factor 3 subunit A; CC, cervical cancer; HR, hazard ratio; CI, confidence interval; R, Pearson correlation coefficient; FDR, false discovery rate.

to determine the optimal thresholds for *EIF3A*, which was 5.2442. Survival analysis indicated that patients with high *EIF3A* expression levels (>5.2442) had a significantly reduced OS compared with patients with low *EIF3A* expression levels (<5.2442) following DDP treatment using TCGA-CC data integrated with TCGA-CC$_1$ and TCGA-CC$_2$ sets (high vs. low expression, 35 vs. 48; log-rank P=0.0310; HR=2.81; 95% CIs, 1.05-7.48; Fig. 3D). In an independent dataset of patients with CC (GSE56363), the expression of *EIF3A* was significantly increased in the non-response group compared with the response group (unpaired student's t-test; P=0.0228; FC=1.04; Fig. 3E).

Sequence docking prediction analyses with the RPISeq database confirmed, with high probabilities and confidence, that the EIF3A reader may bind with the six m^{6}A site motifs of *HYOU1* (interaction probabilities >0.5; Table IV; Fig. 3F), including the '3294', '8651', '10147', '10786', '11220' and '11607' sites. Furthermore, searching for the *HYOU1* gene on the RMBase version 2.0 platform revealed that the m^{6}A site ('3294') of *HYOU1*, which exhibited a high probability of binding with EIF3A, was modified by m^{6}A modification (Table III).

Discussion

Resistance to DDP-based chemotherapy is the leading cause of mortality for patients with CC. By integrating multidimensional publicly available data of CC, the present study identified *HYOU1* as an important gene, the overexpression of which was associated with DDP resistance in patients with CC. The association between high *HYOU1* expression levels and DDP resistance was revealed using data from 53 patients with CC and cell lines. Mechanistic analyses suggested that *EIF3A* overexpression might be associated with *HYOU1*

Table III. Hypoxia-upregulated 1 gene sequence motifs with high confidence.

Position	Sequence context (5'-3')	Score (binary)	Score (KNN)	Score (spectrum)	Score (combined)	Decision	RMBase version 2.0[a]	
							Motif score[b]	Support Num[c]
3,294	UGGGAAAACUGGAAGACAUGGAAC UUUCAAAAUGUAUUCUAAGGA	0.71	0.56	0.53	0.63	m^{6}A site (high confidence)	294.79	8
8,651	CCUUUGUCCCAUAGACUUCAGGAC UUGACACUCCGAGACCUGGAG	0.72	0.74	0.57	0.66	m^{6}A site (high confidence)	-	-
10,147	UCCGUCUCAAAAAAAAAAAAGGAC UAUUCAAGGGGUUUGUUCAGU	0.76	0.80	0.52	0.67	m^{6}A site (high confidence)	-	-
10,786	UUCAGAACCUGAGAAAGUAGAGAC UGGUGAGUUGGAGCAACCAUG	0.72	0.70	0.48	0.62	m^{6}A site (high confidence)	-	-
11,220	CACUCCAGCCUGGGCAACAGAGAC UCUGUCUCAAAAAACAGAGUA	0.70	0.77	0.61	0.66	m^{6}A site (high confidence)	-	-
11,607	AGCGGCCUUUGAAGAACGACGAAC UAUAACCCCCACCUCUGUUUU	0.69	0.56	0.53	0.62	m^{6}A site (high confidence)	-	-

[a]http://rna.sysu.edu.cn/rmbase. [b]An alignment score to evaluate the accuracy of identified motif regions of m^{6}A (range, 0-500). [c]The number of supporting experiments or studies for each modification site. Num, number; KNN, k-nearest neighbor algorithm; m^{6}A, N6-methyladenosine.

Table IV. Probability of binding based on predictions using the RNA-protein interaction prediction database (http://pridb.gdcb.iastate.edu/RPISeq/).

HYOU1 sequence motif position	Method of interaction probabilities	EIF3A reader
3,294	RF	0.55
	SVM	0.32
8,651	RF	0.65
	SVM	0.49
10,147	RF	0.55
	SVM	0.80
10,786	RF	0.50
	SVM	0.23
11,220	RF	0.55
	SVM	0.78
11,607	RF	0.50
	SVM	0.37

HYOU1, hypoxia-upregulated 1 gene; RF, random forest; SVM, support vector machine; EIF3A, eukaryotic translation initiation factor 3 subunit A.

depending on the m^{6}A modification and was associated with DDP resistance.

HYOU1 belongs to the heat shock protein 70 family and is expressed in numerous cell types, such as epithelial cells, neuronal cells and cardiomyocytes (38,39). It is induced by various types of stress, such as hypoxia, ER stress, ischemia and glucose deprivation (40). Previous studies reveal that *HYOU1* is upregulated in various tumors (such as ovarian cancer and breast cancer) and is involved in tumorigenesis and tumor growth (41,42). The study by Liu and Wang (43) demonstrates that *HYOU1* is upregulated in CC cell lines. In addition, the study by Zhou *et al* (44) indicates the expression of *HYOU1* in the tissues of nasopharyngeal carcinoma, which is associated with poor prognosis. Additionally, *HYOU1* is associated with the expansion and metastatic activity of epithelial ovarian tumor cell lines (41). However, the association of *HYOU1* with DDP resistance has not yet been investigated. The present study was the first to demonstrate that *HYOU1* was associated with DDP resistance in patients with CC. An independent cohort of patients with CC was used to indicate that high *HYOU1* expression levels were associated with poor prognosis only in the patients that received DDP treatment. Additionally, pharmacogenomic data indicated that high *HYOU1* expression levels were associated with high IC_{50} values of DDP. However, the correlation was not strong, which may be due to the small sample size and should be further validated in a large-scale dataset. In addition, the present study demonstrated that high *HYOU1* expression levels were associated with resistance to DDP using WB experiments and knockdown experiments of *HYOU1* in HeLa/DDP cells.

The m^{6}A modification serves an important role in regulating RNA stability and participates in biological activities (such as response to stress and RNA stability) and clinical outcomes in patients with cancer (45,46). The present study found that m^{6}A modifications were enriched within *HYOU1* and that *HYOU1* expression levels were significantly associated with the m^{6}A reader, *EIF3A*. Analysis of TCGA-CC data showed that *EIF3A* was significantly associated with DDP resistance and poor survival in patients treated with DDP. Sequence docking indicated that *EIF3A* had docking activity with the m^{6}A site sequence motifs of *HYOU1*. EIF3A is the largest subunit of EIF3, which is an important factor in translation initiation. EIF3A can bind with the 5'-untranslated region to promote the translation of cap-independent mRNAs (47). Expression of *EIF3A* may influence cancer cell proliferation as this malignant phenotype can be reversed by knocking down *EIF3A* in cancer cells (48). Previously, the study by Su *et al* (49), using ribosome profiling with HEK293T upon CRISPR-Cas9-induced methyltransferase-like protein 16 (METTL16; a methyltransferase) knockdown (GSE156796), reports that METTL16 directly interacts with EIF3A/B, thereby promoting the translation of >4,000 mRNA transcripts. The analysis of the data (49) reveals that METTL16 knockdown suppresses the translation efficiency of *HYOU1* (log2(FC)=-1.21), suggesting that the dysregulation of *HYOU1* might be dependent on the m^{6}A modification. The study by Xu *et al* (50) demonstrates that variation in *EIF3A* contributes to platinum-based chemotherapy resistance in patients with lung cancer. To the best of our knowledge, the role of *EIF3A* in the DDP resistance of patients with CC has not been studied before. In the present study, it was demonstrated that *EIF3A* may promote DDP resistance in CC by inducing *HYOU1* overexpression depending on the m^{6}A modification.

However, there were limitations in the present study. Firstly, the associations of HYOU1 with DDP resistance needs to be validated using a larger number of patients with CC in future studies. Secondly, the underlying regulatory mechanism was only preliminarily investigated and it was found that *EIF3A* may promote DDP resistance in CC by inducing *HYOU1* overexpression depending on the m^{6}A modification. Further m^{6}A RNA immunoprecipitation experiments in EIF3A-transfected and knockout cells are needed to validate the findings.

In conclusion, *HYOU1* was identified as a key gene associated with DDP resistance in CC. *HYOU1* expression levels may serve as an indicator for assessing the suitability of DDP treatment as a therapeutic strategy. Mechanistically, *EIF3A* may induce *HYOU1* overexpression depending on the m^{6}A modifications in CC cells and may be a candidate to target for the treatment of patients with CC.

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Availability of data and materials

The data generated in the present study may be requested from the corresponding author.

Authors' contributions

RW, JD, MZ, ZW, SW, SL and LQ contributed to the conception and design of the present study. Material preparation, data collection and analysis were performed by RW, JD and MZ. ZW and SW prepared Figs. 1-3. LQ and SL confirm the authenticity of all the raw data. The first draft of the manuscript was written by LQ and SL and all authors commented on previous versions of the manuscript. All authors read and approved the final version of the 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.

References

1. Siegel RL, Miller KD, Wagle NS and Jemal A: Cancer statistics, 2023. CA Cancer J Clin 73: 17-48, 2023.
2. Siegel RL, Miller KD, Fuchs HE and Jemal A: Cancer statistics, 2021. CA Cancer J Clin 71: 7-33, 2021.
3. Zhu H, Luo H, Zhang W, Shen Z, Hu X and Zhu X: Molecular mechanisms of cisplatin resistance in cervical cancer. Drug Des Devel Ther 10: 1885-1895, 2016.
4. Lorusso D, Petrelli F, Coinu A, Raspagliesi F and Barni S: A systematic review comparing cisplatin and carboplatin plus paclitaxel-based chemotherapy for recurrent or metastatic cervical cancer. Gynecol Oncol 133: 117-123, 2014.
5. Kishimoto S, Kawazoe Y, Ikeno M, Saitoh M, Nakano Y, Nishi Y, Fukushima S and Takeuchi Y: Role of Na+, K+-ATPase alpha1 subunit in the intracellular accumulation of cisplatin. Cancer Chemother Pharmacol 57: 84-90, 2006.
6. Bai ZL, Wang YY, Zhe H, He JL and Hai P: ERCC1 mRNA levels can predict the response to cisplatin-based concurrent chemoradiotherapy of locally advanced cervical squamous cell carcinoma. Radiat Oncol 7: 221, 2012.
7. Yang X, Fraser M, Abedini MR, Bai T and Tsang BK: Regulation of apoptosis-inducing factor-mediated, cisplatin-induced apoptosis by Akt. Br J Cancer 98: 803-808, 2008.
8. Ashrafizadeh M, Zarrabi A, Hushmandi K, Kalantari M, Mohammadinejad R, Javaheri T and Sethi G: Association of the epithelial-mesenchymal transition (EMT) with cisplatin resistance. Int J Mol Sci 21: 4002, 2020.
9. Avril T, Vauleon E and Chevet E: Endoplasmic reticulum stress signaling and chemotherapy resistance in solid cancers. Oncogenesis 6: e373, 2017.
10. Visioli F, Wang Y, Alam GN, Ning Y, Rados PV, Nör JE and Polverini PJ: Glucose-regulated protein 78 (Grp78) confers chemoresistance to tumor endothelial cells under acidic stress. PLoS One 9: e101053, 2014.
11. Hu R, Warri A, Jin L, Zwart A, Riggins RB, Fang HB and Clarke R: NF-kappaB signaling is required for XBP1 (unspliced and spliced)-mediated effects on antiestrogen responsiveness and cell fate decisions in breast cancer. Mol Cell Biol 35: 379-390, 2015.
12. Le Mercier M, Lefranc F, Mijatovic T, Debeir O, Haibe-Kains B, Bontempi G, Decaestecker C, Kiss R and Mathieu V: Evidence of galectin-1 involvement in glioma chemoresistance. Toxicol Appl Pharmacol 229: 172-183, 2008.
13. Rao S, Oyang L, Liang J, Yi P, Han Y, Luo X, Xia L, Lin J, Tan S, Hu J, *et al*: Biological function of HYOU1 in tumors and other diseases. Onco Targets Ther 14: 1727-1735, 2021.
14. Jiang X, Liu B, Nie Z, Duan L, Xiong Q, Jin Z, Yang C and Chen Y: The role of m6A modification in the biological functions and diseases. Signal Transduct Target Ther 6: 74, 2021.
15. Fu Y, Dominissini D, Rechavi G and He C: Gene expression regulation mediated through reversible m(6)A RNA methylation. Nat Rev Genet 15: 293-306, 2014.
16. Knuckles P, Lence T, Haussmann IU, Jacob D, Kreim N, Carl SH, Masiello I, Hares T, Villaseñor R, Hess D, *et al*: Zc3h13/Flacc is required for adenosine methylation by bridging the mRNA-binding factor Rbm15/Spenito to the m(6)A machinery component Wtap/Fl(2)d. Genes Dev 32: 415-429, 2018.
17. Pendleton KE, Chen B, Liu K, Hunter OV, Xie Y, Tu BP and Conrad NK: The U6 snRNA m(6)A Methyltransferase METTL16 regulates SAM synthetase intron retention. Cell 169: 824-835 e14, 2017.
18. Mendel M, Chen KM, Homolka D, Gos P, Pandey RR, McCarthy AA and Pillai RS: Methylation of structured rna by the m(6)A writer METTL16 Is essential for mouse embryonic development. Mol Cell 71: 986-1000 e11, 2018.
19. Wei J, Liu F, Lu Z, Fei Q, Ai Y, He PC, Shi H, Cui X, Su R, Klungland A, *et al*: Differential m(6)A, m(6)A(m), and m(1)A demethylation mediated by FTO in the cell nucleus and cytoplasm. Mol Cell 71: 973-985 e5, 2018.
20. Mauer J, Luo X, Blanjoie A, Jiao X, Grozhik AV, Patil DP, Linder B, Pickering BF, Vasseur JJ, Chen Q, *et al*: Reversible methylation of m(6)A(m) in the 5' cap controls mRNA stability. Nature 541: 371-375, 2017.
21. Su Y, Wang B, Huang J, Huang M and Lin T: YTHDC1 positively regulates PTEN expression and plays a critical role in cisplatin resistance of bladder cancer. Cell Prolif 56: e13404, 2023.
22. Wu S, Yun J, Tang W, Familiari G, Relucenti M, Wu J, Li X, Chen H and Chen R: Therapeutic m(6)A eraser ALKBH5 mRNA-Loaded exosome-liposome hybrid nanoparticles inhibit progression of colorectal cancer in preclinical tumor models. ACS Nano 17: 11838-11854, 2023.
23. Niu Y, Wan A, Lin Z, Lu X and Wan G: N (6)-Methyladenosine modification: A novel pharmacological target for anti-cancer drug development. Acta Pharm Sin B 8: 833-843, 2018.
24. Qi L, Li Y, Qin Y, Shi G, Li T, Wang J, Chen L, Gu Y, Zhao W and Guo Z: An individualised signature for predicting response with concordant survival benefit for lung adenocarcinoma patients receiving platinum-based chemotherapy. Br J Cancer 115: 1513-1519, 2016.
25. Behan FM, Iorio F, Picco G, Gonçalves E, Beaver CM, Migliardi G, Santos R, Rao Y, Sassi F, Pinnelli M, *et al*: Prioritization of cancer therapeutic targets using CRISPR-Cas9 screens. Nature 568: 511-516, 2019.
26. Meyers RM, Bryan JG, McFarland JM, Weir BA, Sizemore AE, Xu H, Dharia NV, Montgomery PG, Cowley GS, Pantel S, *et al*: Computational correction of copy number effect improves specificity of CRISPR-Cas9 essentiality screens in cancer cells. Nat Genet 49: 1779-1784, 2017.
27. Yang W, Soares J, Greninger P, Edelman EJ, Lightfoot H, Forbes S, Bindal N, Beare D, Smith JA, Thompson IR, *et al*: Genomics of drug sensitivity in cancer (GDSC): A resource for therapeutic biomarker discovery in cancer cells. Nucleic Acids Res 41(Database issue): D955-D961, 2013.
28. Li Y, Xiao J, Bai J, Tian Y, Qu Y, Chen X, Wang Q, Li X, Zhang Y and Xu J: Molecular characterization and clinical relevance of m(6)A regulators across 33 cancer types. Mol Cancer 18: 137, 2019.
29. Liu J, Harada BT and He C: Regulation of gene expression by N(6)-methyladenosine in cancer. Trends Cell Biol 29: 487-499, 2019.
30. Huang H, Weng H and Chen J: m(6)A modification in coding and non-coding RNAs: Roles and therapeutic implications in cancer. Cancer Cell 37: 270-288, 2020.
31. Nombela P, Miguel-Lopez B and Blanco S: The role of m(6)A, m(5)C and Ψ RNA modifications in cancer: Novel therapeutic opportunities. Mol Cancer 20: 18, 2021.
32. Qiu F, Liu Q, Xia Y, Jin H, Lin Y and Zhao X: Circ_0000658 knockdown inhibits epithelial-mesenchymal transition in bladder cancer via miR-498-induced HMGA2 downregulation. J Exp Clin Cancer Res 41: 22, 2022.
33. Liao Y, Wang J, Jaehnig EJ, Shi Z and Zhang B: WebGestalt 2019: Gene set analysis toolkit with revamped UIs and APIs. Nucleic Acids Res 47: W199-W205, 2019.
34. Zhou Y, Zeng P, Li YH, Zhang Z and Cui Q: SRAMP: Prediction of mammalian N6-methyladenosine (m6A) sites based on sequence-derived features. Nucleic Acids Res 44: e91, 2016.
35. Xuan JJ, Sun WJ, Lin PH, Zhou KR, Liu S, Zheng LL, Qu LH and Yang JH: RMBase v2.0: Deciphering the map of RNA modifications from epitranscriptome sequencing data. Nucleic Acids Res 46(D1): D327-D334, 2018.

36. Yi Y, Zhao Y, Huang Y and Wang D: A brief review of RNA-protein interaction database resources. Noncoding RNA 3: 6, 2017.
37. Hochberg Y and Benjamini Y: More powerful procedures for multiple significance testing. Stat Med 9: 811-818, 1990.
38. Tsukamoto Y, Kuwabara K, Hirota S, Ikeda J, Stern D, Yanagi H, Matsumoto M, Ogawa S and Kitamura Y: 150-kD oxygen-regulated protein is expressed in human atherosclerotic plaques and allows mononuclear phagocytes to withstand cellular stress on exposure to hypoxia and modified low density lipoprotein. J Clin Invest 98: 1930-1941, 1996.
39. Giffin L, Yan F, Major MB and Damania B: Modulation of Kaposi's sarcoma-associated herpesvirus interleukin-6 function by hypoxia-upregulated protein 1. J Virol 88: 9429-9441, 2014.
40. Kuwabara K, Matsumoto M, Ikeda J, Hori O, Ogawa S, Maeda Y, Kitagawa K, Imuta N, Kinoshita T and Stern DM: Purification and characterization of a novel stress protein, the 150-kDa oxygen-regulated protein (ORP150), from cultured rat astrocytes and its expression in ischemic mouse brain. J Biol Chem 271: 5025-5032, 1996.
41. Li X, Zhang NX, Ye HY, Song PP, Chang W, Chen L, Wang Z, Zhang L and Wang NN: HYOU1 promotes cell growth and metastasis via activating PI3K/AKT signaling in epithelial ovarian cancer and predicts poor prognosis. Eur Rev Med Pharmacol Sci 23: 4126-4135, 2019.
42. Stojadinovic A, Hooke JA, Shriver CD, Nissan A, Kovatich AJ, Kao TC, Ponniah S, Peoples GE and Moroni M: HYOU1/Orp150 expression in breast cancer. Med Sci Monit 13: BR231-BR239, 2007.
43. Liu J and Wang Y: Long non-coding RNA KCNQ1OT1 facilitates the progression of cervical cancer and tumor growth through modulating miR-296-5p/HYOU1 axis. Bioengineered 12: 8753-8767, 2021.
44. Zhou Y, Liao Q, Li X, Wang H, Wei F, Chen J, Yang J, Zeng Z, Guo X, Chen P, *et al*: HYOU1, regulated by LPLUNC1, is up-regulated in nasopharyngeal carcinoma and associated with poor prognosis. J Cancer 7: 367-376, 2016.
45. Desrosiers R, Friderici K and Rottman F: Identification of methylated nucleosides in messenger RNA from Novikoff hepatoma cells. Proc Natl Acad Sci USA 71: 3971-3975, 1974.
46. Saletore Y, Meyer K, Korlach J, Vilfan ID, Jaffrey S and Mason CE: The birth of the Epitranscriptome: Deciphering the function of RNA modifications. Genome Biol 13: 175, 2012.
47. Meyer KD, Patil DP, Zhou J, Zinoviev A, Skabkin MA, Elemento O, Pestova TV, Qian SB and Jaffrey SR: 5' UTR m(6)A promotes cap-independent translation. Cell 163: 999-1010, 2015.
48. Dong Z, Liu LH, Han B, Pincheira R and Zhang JT: Role of eIF3 p170 in controlling synthesis of ribonucleotide reductase M2 and cell growth. Oncogene 23: 3790-3801, 2004.
49. Su R, Dong L, Li Y, Gao M, He PC, Liu W, Wei J, Zhao Z, Gao L, Han L, *et al*: METTL16 exerts an m(6)A-independent function to facilitate translation and tumorigenesis. Nat Cell Biol 24: 205-216, 2022.
50. Xu X, Han L, Yang H, Duan L, Zhou B, Zhao Y, Qu J, Ma R, Zhou H and Liu Z: The A/G allele of eIF3a rs3740556 predicts platinum-based chemotherapy resistance in lung cancer patients. Lung Cancer 79: 65-72, 2013.