Hypoxia Induced HMMR Promotes the NSCLC Progression

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Abstract

Background: Hyaluronan mediated motility receptor (also known as RHAMM) is another one of few defined hyaluronan receptors, play pivotal roles in cell growth. However, the functionof HMMR in lung adenocarcinoma remain unclear.

Methods: HMMR expression was analyzed emoloyed the public databases, the prognosis of HMMR was analysis by prognoscan, KMplot and GEPIA databases. The GO and KEGG pathway was analysis by the DAVID and GSEA software. The correlation between the HMMR expression was analysis by the TIMER databases, the gene and protein networks was analysis by Genemania and STRING databases, the DNA methylation was analysis by the MethSurv and UALCAN databases. The expression of HMMR was analysis by IHC and qPCR, the function of HMMR on cell proliferation and migration was examine by the cell growth curve, clone information, transwell and wound healing assay.

Results: in this study, we find that HMMR was elevated in LUAD and it's highly expression associated with the poor prognosis and lymph node metastasis. Furthermore, the expression of HMMR was induced by hypoxia in LUAD. HMMR expression level not only positively correlation with the different immune cells, but also positively correlation with the expression of immune checkpoints related gene. Finally, depletion of HMMR significantly represses the cell growth and migration of NSCLC. We also found that the HOXB7/TMPO-AS1/Let-7b-5p axis mediated high expression of HMMR in NSCLC, depletion of TMPO-AS1 and over-expression the Let-7b-5p would result in decreased the expression of HMMR in NSCLC cells, the TMPO-AS1 was positively with the HMMR and negatively related to the Let-7b-5p in NSCLC. Overall, this study emphasized the significance of HOXB7/TMPO-AS1/Let-7b-5p axis mediated high expression of HMMR in cancer progression and Immune infiltration of LUAD.

Conclusions: we demonstrated HMMR was elevated in LUAD and positively relation to poor prognosis. We find the hypoxia microenvironment and DNA hypomethylation able to up-regulation of the HMMR expression. Additionally, HMMR expression was positive with the diverse immune cell and immune regulator related gene in LUAD. Finally, we found that depletion of HMMR was inhibits the cell proliferation and migration ability of NSCLC cells. These findings suggest that HMMR could be served as a biomarker for prognosis and immune infiltration in LUAD.

Background

Lung cancer is one of the main causes of cancer death, and It's has brought huge economic and social burden in the worldwide[1]. Lung Cancer mainly contained the small lung squamous cell carcinoma and non-small lung squamous cell carcinoma. While, the NSCLC Composed of lung adenocarcinoma and lung squamous cell carcinoma[1, 2]. Due to the lack of effective diagnostic markers result in most of the patients with lung cancer have been found in advanced stage, missed the best treatment time [2, 3]. Therefore, it's imperative find specificity and sensitivity biomarkers for diagnosis and treatment of lung cancer.

HMMR, named as RHAMM, called hyaluronan mediated motility receptor, its play pivotal roles in cell growth [4]. Studies have shown that HMMR was mainly expressed in the nervous system [5]and the mouse brain [6]. The gene mutation of HMMR usually lead to the neurodevelopmental defects [7]. Research studies have shown that high HMMR expression has been associated with various cancer, such as breast cancer [8], colorectal cancer[9], stomach cancer [10], endometrial cancer [11] and prostate cancer [12]. Previous study indicated that HMMR was essential for maintains the stemness of glioblastoma stem cells[13]. However, the significance and potential mechanisms of HMMR in LUAD progression and tumor-infiltrating remains bewildered.

In this finding, we show that HMMR was highly expresison in lung adenocarcinoma and it's high expression was correlated with poor prognosis, tumor stages and lymph node metastasis. furthermore, up-regulated HMMR expression was markedly positive with the the infiltration levels of B cells, CD4 + T cells, CD8 + T cells, macrophages, neutrophils, and dendritic cells in lung adenocarcinoma. Importantly, the expression of HMMR was positively with the immune related gene expression, HMMR able to influence the prognosis of lung adenocarcinoma patients partially via immune cell infiltration. We use the qRT-PCR and IHC assay show that HMMR was highly expression in NSCLC cells and lung cancer. We also found that HMMR was induced by hypoxia via HIF-1α-dependent transcriptional regulation. Finally, we show that depletion of HMMR significantly inhibits growth and migration of NSCLC cells. These observations indicate that HMMR may plays an crucial role in the progression and immune infiltration of lung adenocarcinoma.

Methods

The expression, prognosis, clinical information and Immune infiltration analysis

We mainly using the following databases to analysis the expression, prognosis, clinical information and Immune infiltration of HMMR in cancers. The detail information of databases used in this study are as follows: Oncomine database [14], TIMER [15],UALCAN database [16], Kaplan-Meier plotter [17] ,TISIDB database [18], the Human Disease Methylation Database [19], the PrognoScan database [20],The LinkedOmics database[21]. GEPIA database [22] and cbioportal[23].

Analysis the correlation between the UBE2C expression and Drug sensitivity

We employed the GDSC and CTRP databases to analysis the correlation between UBE2C expression and drug sensitivity [24, 25].

Analysis the Molecular characteristics of SNHG1

We employed the lncLocator (www.csbio.sjtu.edu.cn/bioinf/lncLocator.) and CPC2 (http://cpc2.cbi.pku.edu.cn) to examine the subcellular localization and the protein coding ability of SNHG1[26, 27].

Cells and cell culture conditions

The BEAS-2B cell line was purchased from cell bank of Kunming Institute of Zoology, and cultured in BEGM media (Lonza, CC-3170). HEK-293T was obtained from ATCC. Lung cancer cell lines, including A549, H1299 and H1975were purchased from Cobioer, China with STR document, A549, H1299 and H1975 cells were all cultured in RPMI1640 medium (Corning) supplemented with 10% fetal bovine serum (Cat# 10099141C, Gibco, USA) and 1% penicillin/streptomycin. HEK-293T cells were cultured in DMEM medium (Corning). The shRNA for HMMR was constructed employ pLKO.1 vector. The shRNA for HMMR primer sequences are list follows: HMMR shRNA#1: AAACAGCTGGAAGATGAAGAAGGAA, HMMR shRNA#2: CAGCTGGAAGATGAAGAAGGAAGAA.

Quantitative real-time PCR

The qRT-PCR assay was performed as documented [28]. The primer sequences are list follows HMMR-F: ATGATGGCTAAGCAAGAAGGC, HMMR-R: T TTTCCCTTGAGACTCTTCGAGA; β-actin-F: CTTCGCGGGCGACGAT, β-actin-R: CCATAGGAATCCTTCTGACC. The expression quantification was obtained with the 2−ΔΔCt method.

The function assays of HMMR in LUAD

The cell migration and invasion abilities were assessed by transwell assays based on published method [29]. For cell proliferation assay, indicated cells were plated into 12-well plates at a density of 2×104, the cell numbers were subsequently counted each day using an automatic cell analyzer countstar (Shanghai Ruiyu Biotech Co., China, IC 1000). For colony formation assay, indicated cells were seeded in 6-well plate with 500 cells per well supplemented with 2 mL cell culture medium, and the cell culture medium was changed every 3 days for 2~3 weeks. Indicated cells were fixed with 4% PFA and stained with 0.5% crystal violet. Cell migration assays. The migration ability of indicated cells was evaluated by wound healing and transwell assays. For wound healing assay, cells were plated in six-well plates at 1×10^6 cells/well in 2 ml of culture medium. Twenty-four hours later, a wound was scratched in the adherent cell monolayers with an Eppendorf tip. Wounds were imaged at 5–10 positions along each well. For transwell assay, 2×10^4 cells in serum-free medium were plated on uncoated insets and incubated using 24-well chemotaxis chambers (Corning cell culture inserts, 8 μm pore size). Fetal bovine serum (10%) was added to the bottom wells of the chambers as chemo-attractant. Cells were allowed to migrate through the membrane for indicated time. The non-migrating cells were removed and migrating

cells to the lower face were stained with cresyl violet (Sigma). Stained cells in the entire fields were counted under an inverted microscope.

Western Blotting and Immunohistochemistry staining

The Western Blotting and Immunohistochemistry staining assay was performed as documented [29].Briefly, Cell lysates were collected, perform the Western blot, primary antibody overnight incubation and second antibody incubation. Finally, develop using instrument.The detail information of antibodies employ in our study are as follows:HMMR antibody (CST Group (HMMR, Rabbit mAb #87129, 1:1000) andβ-actin (Catalog number: 66009-1-Ig , 1:20000, Proteinch, Shanghai, China).

Statistical Analysis

The significance of the data between two assays groups was decided by Student's t-test, $P < 0.05$ (*), $P < 0.01$ (**) and $P < 0.001$ (***), was considered significantly.

Results

The mRNA of HMMR was elevated in human cancer

In order to examine the mRNA of HMMR expression pattern in multifarious cancer, we employed the TIMER tools to analysis the expression of HMMR, the find shown that HMMR was elevated in as follow cancer than the control group, it mainly includes BLCA, BRCA, CHOL, COAD, ESCA, HNSC, KIRC, KIRP, LIHC, LUAD, LUSC, PRAD, READ, STAD, THCA and UCEC (Figure 1A). To further verify the results, we using the combine the TCGA and GTE databases to figure out the HMMR expression. As is show in Figure 1B, the HMMR was significantly up-regulation in ACC, BLCA, BRCA, CESC, COAD, DLBC, ESCA, GBM, HNSC, LIHC, LUAD, LUSC, OV, PAAD, READ, SKCM, STAD, THYM, UCEC and UVM cancer than match healthy tissue, while low expression in LAML and TGCT(Figure 1B). Besides, we found that the HMMR was highly expression in NSCLC cells lines observe in CCLE network tools (Figure 1C). Above all, our findings indicated that the HMMR may play an crucial roles in the progression of cancers.

The prognostic value of HMMR in pan-cancers

Then, through analysis the TCGA databases, we find the expression of HMMR has the great significance for assessment the prognostic value of different cancers. High expression of HMMR was associated with poor OS in ACC, COAD, KICH, KIRC, KIRP, LGG, LIHC), LUAD, MESO, PAAD, PCPG, PRAD, THYM and UVM (Figure 2A), and related to poor DSS in KIRP, LIHC, MESO, SARC and THCA (Figure 2B), and related to poor DFS in ACC, KICH, KIRC, KIRP, LGG, LIHC, LUAD, MESO, PRAD, THCA and UVM (Figure 2C), and linkage to poor PFS in ACC, KICH, KIRC, KIRP), LGG, LIHC, LUAD, MESO, PCPG and UVM (Figure 2D).

Analysis the pathological stage in different cancer

We employ the GEPIA tools to examine the relationship between the expression of HMMR and the human cancers pathological stage. Interestingly, we find that the expression of HMMR was markedly positive with the pathological stage of ACC, BRCA, ESCA, NHCS, KICH, KIRC, KIRP, LAML, LAML, LIHC, LUAD and LUSC (Figures 3). These results suggested that HMMR may as an oncogene in human cancers.

The expression pattern of HMMR in Immune and Molecular Subtypes of cancers

Previous reports shown that the cancer could accord to the molecular characteristics divided into different immune and molecular subtypes. Thus, we adopt the TISIDB tools to analysis the expression of HMMR in immune and molecular Subtypes of different human cancer. Concerning the immune subtypes, the analysis results shown that HMMR has a differential expression pattern in cancers (Figure 4A), a quintessential example should be cited LUAD, the HMMR was elevated in C1 and C2, while, it's low expression was observe in C3. With regard to the molecular subtypes, HMMR also display a distinctive expression pattern (Figure 4B), such as, the HMMR was highly expression in C1 of LUAD, decreased in C3. To summarize, our result indicated that the expression pattern of HMMR has the tissue dependence specificity.

HMMR was highly expression in LUAD

Considering the significance of HMMR in cancers, next, we want to explore the relationship between the expression of HMMR and clinical features in LUAD. First, we find that the RNA and protein of HMMR was significantly elevated in LUAD by perform the UALCAN tools analysis (Figure 5A-5B). In addition, we also find the expression of HMMR was increased with the elevation of stage nodal metastasis (Figure 5C). The expression of HMMR not only highly expression in Ttp53-mutant patients but also upregulated in long term smoking patients. For the gender, the expression of HMMR significantly elevated in male patients than female (Figure 5D-5F). Surprisingly, we find higher expression of HMMR has the poor overall survival, poor OS, PFS and PPS (Figure 5G-5I). Finally, we also evaluate the connection of among the expression of HMMR and MKI67 (famous cell proliferation index), the result suggested that HMMR expression was markedly positive with the expression of mki67 (Figure 5K). These findings strongly imply that the HMMR may involve in the progression of LUAD.

The gene mutation of HMME analysis

For explore the gene mutation information about the HMMR, we employ the cBioportal tools preform comprehensive analysis regard to the HMMR. The result shown that the mutation rate of HMMR reached 2.9% in NSCLC (Figure 6A), we also examine the mutation type and base mutation in NSCLC, we found

that Missense substitution and base G>A reached the highest mutation rate in NSCLC (Figure 6B-6C). The results also display the mutation of HMMR in different NSCLC molecular Subtypes (Figure 6D), In DNA level, gain and diploid was the main reason for the HMMR high expression in NSCLC (Figure 6E). Overall, these results emphasize the gene mutation of HMME may be contribute to the HMMR elevated in NSCLC.

Analysis DNA methylation of HMMR in LUAD

Next, we further explore the connection of among the DNA methylation of and the expression of HMMR, as is shown in the (Figure 7A), we display varies methylation sites locate in the promoter of HMMR by heat map. Through preform the comprehensive and detailed analysis about the DNA methylation state of HMMR, we find the methylation level was significantly low expression in the LUAD, as well as the methylation level was gradually decreased with the elevation of pathological stage in LUAD (Figure 7B-7C). The methylation level was gradually decreased observe in the different lymph node metastasis state, race and tp53mutation (Figure 7D-7G). The overall survival analysis display that elevated the methylation level of HMMR has better prognosis (Figure 7H). These finds suggested that DNA hypomethylation may be reason for the abnormal up-regulation in LUAD.

Analysis The function of HMMR in LUAD

In order to exploration the potential significance of HMMR in LUAD progression. We first employ the linkedomics database to examine the positive gene with HMMR. As is show in the Figures 8A-8C, we choose the most positive gene(r>0.7) display in the form of heat map. Next, we perform the GO and KEGG analysis. The biology processes mainly involve in DNA replication, chromosome segregation, cell division and protein localization (Figures 8D), for KEGG enriched results, the pathway mainly including cell cycle, P53 signaling pathway, non-small cell lung cancer and FOXO signal pathways (Figures 8E). In addition, we analysis the most relevant gene of HMMR by employed the Genemania, the result indicated that xx gene were most relevant, these gene functions mainly involve in cell cycle (Figure 8F). We also using STRING databases to construct the protein interaction network, the protein of interaction with HMMR mainly including PLK4, CD44, AURKA, NEK2, CDK1, FAM83D(Figure 8G). To explore the HMMR related signal pathway in progression of LUAD, we employ the GSEA software perform KEGG pathway enrich. The analysis results show that upregulation HMMR expression mainly involve in the IL2-STAT5 signaling pathway, IL6 JAK-STAT3- signaling pathway, Interferon-γ response, TNFα signaling pathway(Figure 8H). These results suggested that HMMR plays an pivotal roles in the immune response regulation in LAUD.

Hypoxia induced the HMMR highly expression in LUAD

The GSEA enrich results show that HMMR mainly participate in hypoxia process (Figure 9A), we guess the HMMR expression whether by hypoxia induced. To test this hypothesis, we employ the JASPAR and AnimalTFDB tools to predict the HIF1α HRE in the promoter of HMMR. Next, we analysis

the relationship among HMMR expression and HIF1α expression in TCGA LUAD, the result indicated that HIF1α significantly positive with the expression of HMMR (Figure 9B). In addition, we using the different condition to treat the NSCLC cells, such as hypoxia and cocl2, cocl2 as an chemical inducer of HIF1α, through these condition treat, compared with normoxia, we find the hypoxia and cocl2 was able to increase the expression of HMMR (Figure 9C). We further analysis the relationship among HMMR expression and HIF1α downstream targets gene expression (eg LDHA, PGK1, SLC2A1, GBE1) in TCGA LUAD, the results suggested that HMMR expression was strongly positive with these gene (Figure 9D-9G). Beside, we find that the RNA of HIF1α was significantly elevated in LUAD by perform the UALCAN tools analysis (Figure 9H). We also find the expression of HIF1α was increased with the elevation of pathological stage and lymph node metastasis (Figure 9I-9J). Surprisingly, we find higher expression of HIF1α has the poor overall survival (Figure 9K). These findings strongly imply that the HMMR expression may be induced by the hypoxia.

Analysis the correlation between the expression of HMMR and Immune infiltration in LUAD

We employ the TIMER databases analysis examine the relationship between the expression of HMMR and Immune infiltration in LUAD, the results show that gene copy number change of HMMR was significantly affect the immune infiltration level of B cells, CD4+ T cells, CD8+ T cells, Macrophage cells, dendritic cells and neutrophils in LUAD (Figure 10A). Next, we used TIMER to analyze the relationship between the HMMR level and immune infiltration levels in LUAD. The results showed that HMMR expression is markedly positively associated with B cells (r=0.47, p=7.69e-30), CD4+ T cells (r=0.71, p=1.71e-78), CD8+ T cells (r=0.51, p=1.22e-35), Macrophage cells(r=0.44, p=8.88e-26), dendritic cells (r=0.84, p=5.94e-139) and neutrophils (r=0.77, p=3.54e-103) in LUAD (Figure 10B). To evaluate the relationship among HMMR expression and immune checkpoints related gene, for instance: CD274, CD279, CTLA4, LAG3, PDCD1LG2, TIGIT and HAVCR2. The results show that HMMR expression was memorably positively relation to CD274(r=0.34, P=9.3e-15), CTLA4(r=0.556, P=5), PDCD1(r=0.56, P=0), TIGIT(r=0.71, P=0), PDCD1LG2 (r=0.67, P=0), HAVCR2 (r=0.71, P=0) and LAG3 (r=0.39, P=0) in LUAD (Figure 10C). These results memorably indicated that HMMR play crucial roles in tumor immune infiltration regulator in LUAD.

Analysis the correlation between the expression of HMMR and Immune modulator in LUAD

Considering the significance of HMMR in the immune regulation, we next explored the relationship between the HMMR expression and diverse immune modulator, including tumor infiltrating lymphocytes, immune-stimulator, immune-inhibitor, chemokine, receptor, MHCs in LUAD, the analysis revealed that HMMR expression were positively with the 28 tumor infiltrating lymphocytes (Figure 11A), 24 immune-inhibitor (Figure 11B), 45 immune-stimulator (Figure 11C) , 21 MHCs (Figure 11D), 41 chemokine (Figure

11E) and 18 receptor (Figure 11F) in lung adenocarcinoma. These findings indicated that HMMR plays indispensable roles in the regulation of immune response of lung adenocarcinoma.

The prognostic of HMMR based on the different immune cells

To explore the prognostic of HMMR based on the different immune cells in LUAD. We using the KMplot database analysis found that elevated the HMMR expression as well as enriched the B cells, CD4+ T-cells, CD8+ T cells, macrophages, natural killer T cells, regulatory T cells cohort had a poor prognosis (Figures 12A-L). These data indicated that the immune cell infiltration could significantly affect the prognostic of HMMR in LUAD.

HMMR functions as a target gene for Let-7b-5p

miRNA plays important roles in modulate the gene expression, we further examined the upstream the miRNA that regulated HMMR expression in the progression of lung cancer. We employed the starbase and targetscan to predict the potential miRNAs of HMMR, the Common results show there are 3 miRNAs that binding with the 3'UTR of HMMR, mainly including the let-7b-5p, has-miR-18a-5p, hsa-miR-33a-5p and hsa-miR-369-3p, we first analysis the correlation between the miRNAs and HMMR, among these miRNAs, only let-7b-5p was markedly negative with the HMMR expression in LUAD(Figures 13A and 13C). Next, we using the starbase analysis the target sites between the HMMR and let-7b-5p (Figure 13B), further study show that let-7b-5p was down-regulated in LUAD(Figure 13D), and low expression of let-7b-5p was related to poor prognosis, tumor stage and nodal metastatis status in LUAD(Figure 13E-13G). Additionally, we using the qRT-PCR assay examined the expression of let-7b-5p in NSCLC cells lines, the data indicate that let-7b-5p was decreased in NSCLC cells than the control cells(Figure 13H). For determined the let-7b-5p whether affect the HMMR expression, we overexpression of let-7b-5p in A549 cell, we found that the mRNA and protein of HMMR was significantly reduced after the overexpression of let-7b-5p (Figure 13I-13J). Collectively, these data imply that let-7b-5p may be participated in the regulation the HMMR expression in NSCLC.

TMPO-AS1 functions as a ceRNA for Let-7b-5p

It has been shown that lncRNA plays crucial roles in the regulation the miRNA and mRNA expression. Above findings shown that let-7b-5p may modulate the HMMR expression via biding with the 3'UTR of HMMR. We next explored the upstream lncRNAs of let-7b-5p. we employed the starbase and lncBase predicted 2.0 to examined the potential lncRNA that as an miRNA sponge and control mRNA expression. By perform the related analysis we obtained 3 lncRNAs, including the SNHG12, LINC02242 and TMPO-AS1, we further analysis the correlation between these lncRNAs and has-let-7b-5p, the data indicated that SNHG12 and LINC02242 were positively with the has-let-7b-5p, while, the expression of TMPO-AS1 not

only negative with has-let-7b-5p, but also positive with the HMMR, as a target gene of has-let-7b-5p(Figure 14A-14C). In addition, we found that TMPO-AS1 was high expression in LUAD, and it's high expression were related to the poor OS, PFS and tumor stage in LUAD(Figure 14D-14G). We perform the localization and coding potential analysis by diverse public databases, the subcellular localization of TMPO-AS1 employed the lncLocator tools, the results indicated that TMPO-AS1 was mainly located in the cytoplasm (Figures 14H-14I). We also analyzed the coding potential of TMPO-AS1 by performing the coding potential calculator, the results showed that TMPO-AS1 not possessss the protein coding ability (Figure 14J). Finally, we show that depletion of TMPO-AS1 was markedly reduced the HMMR expression and up-regulation the expression of has-let-7b-5p in A549 cell(Figure 14K). In conclusion, our results suggested that TMPO-AS1 may be as the upstream the lncRNA of has-let-7b-5p and regulation the expression of HMMR in LUAD.

Transcript factor plays indispensable roles in control gene expression [20]. We next explored the transcript factor that potential regulation the transcription of TMPO-AS1, we employed the JASPAR, PROMO, ConTrav3 and UCSC databases. We identify transcription factors HOXB7 that may regulate the expression of TMPO-AS1, we using the JASPAR database predicted the potential binding site in the promoter of TMPO-AS1 (Figure 14L). Next, we employed the GSE46393 dataset to verify above results, the data shown that knock down of HOXB7 was reduced the expression of TMPO-AS1, we also found that HOXB7 was markedly positively with the TMPO-AS1 in LUAD (Figure 14M-14N). For search the potential drug that related to the TMPO-AS1 expression. We find TMPO-AS1 was negative with the drug sensitivity clofarabine, triazolothiadiazine, topotecan, manumycin A, CD-437, BI-2536, LY-2183240, vincristine, SB-743921, cytarabine hydrochloride, etoposide, GSK461364, gemcitabine, Cerulenin, paclitaxel, parbendazole, PX-12, 3-Cl-AHPC, ML311, PRIMA-1, nakiterpiosin, BRD-K70511574, Chlorambucil ,SR-II-138A, narciclasine , BRD-K34222889, doxorubicin, piperlongumine, ciclopirox, KX2-391 and CR-1-31B(Figure 14O and Table 1). Above all, these results show that HOXB7 may be as a transcript factor of TMPO-AS1 in lung cancer.

Table 1

The correlation between the TMPO-AS1 expression and drug sensitivity in diverse cancer cells lines analysis by the CTRP database.

symbol	drug	cor	fdr
TMPO-AS1	clofarabine	-0.2922	4.84E-16
TMPO-AS1	triazolothiadiazine	-0.28996	6.52E-16
TMPO-AS1	topotecan	-0.28797	1.38E-15
TMPO-AS1	manumycin A	-0.29202	1.49E-15
TMPO-AS1	CD-437	-0.28984	2.67E-15
TMPO-AS1	BI-2536	-0.28224	3.64E-15
TMPO-AS1	LY-2183240	-0.28531	4.34E-15
TMPO-AS1	vincristine	-0.2771	6.95E-15
TMPO-AS1	SB-743921	-0.27369	2.89E-14
TMPO-AS1	cytarabine hydrochloride	-0.27399	4.93E-14
TMPO-AS1	etoposide	-0.27312	7.49E-14
TMPO-AS1	GSK461364	-0.27417	9.19E-14
TMPO-AS1	gemcitabine	-0.28068	2.25E-13
TMPO-AS1	cerulenin	-0.26883	4.44E-13
TMPO-AS1	paclitaxel	-0.26453	9.56E-13
TMPO-AS1	parbendazole	-0.25739	1.06E-12
TMPO-AS1	PX-12	-0.26269	1.18E-12
TMPO-AS1	3-Cl-AHPC	-0.26236	1.29E-12
TMPO-AS1	ML311	-0.25774	1.3E-12
TMPO-AS1	PRIMA-1	-0.26688	1.87E-12
TMPO-AS1	nakiterpiosin	-0.25991	1.94E-12
TMPO-AS1	BRD-K70511574	-0.25929	2E-12
TMPO-AS1	chlorambucil	-0.26228	2.21E-12
TMPO-AS1	SR-II-138A	-0.24873	5.3E-12
TMPO-AS1	narciclasine	-0.24971	2.21E-11
TMPO-AS1	BRD-K34222889	-0.24657	2.24E-11
TMPO-AS1	doxorubicin	-0.24194	2.38E-11
TMPO-AS1	piperlongumine	-0.2449	2.48E-11

TMPO-AS1	ciclopirox	-0.24312	2.71E-11
TMPO-AS1	KX2-391	-0.24591	3.31E-11
TMPO-AS1	CR-1-31B	-0.24056	4.2E-11
TMPO-AS1	SB-225002	-0.23908	4.79E-11
TMPO-AS1	ceranib-2	-0.23941	5.51E-11
TMPO-AS1	PF-184	-0.24655	6.58E-11
TMPO-AS1	BRD-K66453893	-0.24134	6.83E-11
TMPO-AS1	GW-843682X	-0.25201	7.36E-11
TMPO-AS1	decitabine	-0.2375	8.37E-11
TMPO-AS1	phloretin	-0.23707	3.44E-10
TMPO-AS1	KW-2449	-0.23155	3.48E-10
TMPO-AS1	COL-3	-0.27236	5.07E-10
TMPO-AS1	PL-DI	-0.23032	6.86E-10
TMPO-AS1	YK 4-279	-0.22471	1.05E-09
TMPO-AS1	dexamethasone	-0.25044	1.19E-09
TMPO-AS1	isoevodiamine	-0.22457	1.22E-09
TMPO-AS1	FQI-2	-0.22198	1.41E-09
TMPO-AS1	docetaxel	-0.3351	1.5E-09
TMPO-AS1	MK-1775	-0.2262	1.91E-09
TMPO-AS1	NSC48300	-0.22453	1.94E-09
TMPO-AS1	valdecoxib	-0.23127	2.25E-09
TMPO-AS1	SNX-2112	-0.21808	2.68E-09
TMPO-AS1	necrosulfonamide	-0.26787	2.9E-09
TMPO-AS1	STF-31	-0.22683	3.12E-09
TMPO-AS1	leptomycin B	-0.2154	3.18E-09
TMPO-AS1	NSC19630	-0.26645	3.89E-09
TMPO-AS1	MGCD-265	-0.22168	4.06E-09
TMPO-AS1	KPT185	-0.2535	4.65E-09
TMPO-AS1	BRD-K26531177	-0.22888	5.1E-09

TMPO-AS1	mitomycin	-0.21774	5.19E-09
TMPO-AS1	GW-405833	-0.21753	6.31E-09
TMPO-AS1	BRD-K92856060	-0.22697	6.99E-09
TMPO-AS1	PHA-793887	-0.21252	7.9E-09
TMPO-AS1	NSC95397	-0.22743	8.99E-09
TMPO-AS1	tivantinib	-0.30764	9.97E-09
TMPO-AS1	pifithrin-mu	-0.2156	1.4E-08
TMPO-AS1	rigosertib	-0.21372	1.68E-08
TMPO-AS1	CHM-1	-0.20773	1.99E-08
TMPO-AS1	AZD7762	-0.20828	2.12E-08
TMPO-AS1	LRRK2-IN-1	-0.24683	2.27E-08
TMPO-AS1	pazopanib	-0.2128	2.27E-08
TMPO-AS1	NVP-231	-0.20944	2.29E-08
TMPO-AS1	BIX-01294	-0.21053	2.37E-08
TMPO-AS1	I-BET151	-0.20408	2.94E-08
TMPO-AS1	BRD-K35604418	-0.21205	2.96E-08
TMPO-AS1	elocalcitol	-0.21062	2.98E-08
TMPO-AS1	epigallocatechin-3-monogallate	-0.23023	3.41E-08
TMPO-AS1	sotrastaurin	-0.24616	3.69E-08
TMPO-AS1	pevonedistat	-0.21061	3.91E-08
TMPO-AS1	ISOX	-0.20523	3.93E-08
TMPO-AS1	SN-38	-0.2268	4.72E-08
TMPO-AS1	apicidin	-0.20091	5.02E-08
TMPO-AS1	GMX-1778	-0.20842	5.96E-08
TMPO-AS1	ouabain	-0.20127	6.27E-08
TMPO-AS1	axitinib	-0.2026	6.37E-08
TMPO-AS1	1S,3R-RSL-3	-0.2062	6.38E-08
TMPO-AS1	tretinoin	-0.21025	6.98E-08
TMPO-AS1	sirolimus	-0.20284	9.97E-08

TMPO-AS1	tipifarnib-P1	-0.20415	1.02E-07
TMPO-AS1	CAY10618	-0.1994	1.15E-07
TMPO-AS1	alisertib	-0.19849	1.28E-07
TMPO-AS1	PAC-1	-0.20326	1.33E-07
TMPO-AS1	bardoxolone methyl	-0.21182	1.64E-07
TMPO-AS1	methylstat	-0.23401	2.83E-07
TMPO-AS1	SU11274	-0.19788	3.55E-07
TMPO-AS1	NSC632839	-0.18979	4E-07
TMPO-AS1	gossypol	-0.19568	5.36E-07
TMPO-AS1	WP1130	-0.1935	6E-07
TMPO-AS1	MST-312	-0.18822	6.75E-07
TMPO-AS1	indisulam	-0.19022	8.14E-07
TMPO-AS1	ML162	-0.19178	8.42E-07
TMPO-AS1	vorinostat	-0.18489	8.82E-07
TMPO-AS1	NSC23766	-0.19118	8.92E-07
TMPO-AS1	AT13387	-0.27365	1.04E-06
TMPO-AS1	teniposide	-0.25348	1.04E-06
TMPO-AS1	StemRegenin 1	-0.18704	1.35E-06
TMPO-AS1	tigecycline	-0.27327	1.39E-06
TMPO-AS1	KU-60019	-0.18369	1.39E-06
TMPO-AS1	belinostat	-0.2586	1.4E-06
TMPO-AS1	barasertib	-0.18303	1.43E-06
TMPO-AS1	BRD-K41597374	-0.18462	1.62E-06
TMPO-AS1	selumetinib	0.19896	1.83E-06
TMPO-AS1	ML031	-0.19778	1.86E-06
TMPO-AS1	obatoclax	-0.17823	2.15E-06
TMPO-AS1	PF-573228	-0.18063	2.17E-06
TMPO-AS1	SMER-3	-0.20039	2.29E-06
TMPO-AS1	olaparib	-0.17902	2.87E-06

TMPO-AS1	ML210	-0.1827	3.01E-06
TMPO-AS1	triptolide	-0.17863	3.06E-06
TMPO-AS1	BRD-K66532283	-0.18167	3.39E-06
TMPO-AS1	curcumin	-0.17379	4.38E-06
TMPO-AS1	SKI-II	-0.18811	4.62E-06
TMPO-AS1	Merck60	-0.17086	4.84E-06
TMPO-AS1	ML050	-0.18395	4.9E-06
TMPO-AS1	PI-103	-0.17959	5.1E-06
TMPO-AS1	BRD-K61166597	-0.17761	5.39E-06
TMPO-AS1	panobinostat	-0.16855	5.9E-06
TMPO-AS1	CCT036477	-0.17232	5.96E-06
TMPO-AS1	LE-135	-0.18693	7.29E-06
TMPO-AS1	AT7867	-0.17343	8.75E-06
TMPO-AS1	BRD-A86708339	-0.25246	8.97E-06
TMPO-AS1	serdemetan	-0.17416	9.27E-06
TMPO-AS1	marinopyrrole A	-0.21508	9.61E-06
TMPO-AS1	Ko-143	-0.17045	1.11E-05
TMPO-AS1	avrainvillamide	-0.19999	1.45E-05
TMPO-AS1	dacarbazine	-0.16478	1.55E-05
TMPO-AS1	momelotinib	-0.17075	1.57E-05
TMPO-AS1	PF-3758309	-0.23485	1.58E-05
TMPO-AS1	methotrexate	-0.17237	1.71E-05
TMPO-AS1	SCH-79797	-0.16127	2.25E-05
TMPO-AS1	daporinad	-0.17635	2.77E-05
TMPO-AS1	cucurbitacin I	-0.16452	2.79E-05
TMPO-AS1	Compound 7d-cis	-0.17406	2.9E-05
TMPO-AS1	SID 26681509	-0.17685	2.9E-05
TMPO-AS1	erastin	-0.16652	3.02E-05
TMPO-AS1	PD318088	0.171433	3.05E-05

TMPO-AS1	BRD-K80183349	-0.16006	3.29E-05
TMPO-AS1	PIK-93	-0.16522	3.29E-05
TMPO-AS1	ML239	-0.16487	3.32E-05
TMPO-AS1	GSK525762A	-0.15339	4.3E-05
TMPO-AS1	YM-155	-0.17961	4.4E-05
TMPO-AS1	OSI-930	-0.17358	4.44E-05
TMPO-AS1	BMS-270394	-0.17053	4.53E-05
TMPO-AS1	neuronal differentiation inducer III	-0.15416	4.98E-05
TMPO-AS1	skepinone-L	-0.2387	4.99E-05
TMPO-AS1	fingolimod	-0.15698	4.99E-05
TMPO-AS1	BRD-K13999467	-0.1644	5.09E-05
TMPO-AS1	JQ-1	-0.152	5.11E-05
TMPO-AS1	ABT-737	-0.16834	5.19E-05
TMPO-AS1	HBX-41108	-0.20068	5.62E-05
TMPO-AS1	SRT-1720	-0.16807	5.96E-05
TMPO-AS1	FQI-1	-0.21223	6.75E-05
TMPO-AS1	TPCA-1	-0.15145	7.75E-05
TMPO-AS1	entinostat	-0.15068	7.8E-05
TMPO-AS1	CIL56	-0.22562	7.9E-05
TMPO-AS1	N9-isopropylolomoucine	-0.15593	8.67E-05
TMPO-AS1	alvocidib	-0.21964	8.81E-05
TMPO-AS1	Ch-55	-0.16687	9.87E-05
TMPO-AS1	PF-750	-0.17188	9.92E-05
TMPO-AS1	PDMP	-0.15759	0.000107
TMPO-AS1	niclosamide	-0.15699	0.00011
TMPO-AS1	dinaciclib	-0.21283	0.000111
TMPO-AS1	ABT-199	-0.20021	0.000115
TMPO-AS1	omacetaxine mepesuccinate	-0.17088	0.000134
TMPO-AS1	navitoclax	-0.15269	0.000134

TMPO-AS1	Compound 23 citrate	-0.14785	0.000138
TMPO-AS1	tipifarnib-P2	-0.20082	0.000144
TMPO-AS1	necrostatin-7	-0.15904	0.000161
TMPO-AS1	TG-101348	-0.14651	0.000178
TMPO-AS1	BRD-K51490254	-0.15378	0.000183
TMPO-AS1	necrostatin-1	-0.16339	0.000186
TMPO-AS1	BRD-K45681478	-0.15561	0.000208
TMPO-AS1	bexarotene	-0.15348	0.000227
TMPO-AS1	B02	-0.1453	0.000237
TMPO-AS1	imatinib	-0.14889	0.000248
TMPO-AS1	UNC0638	-0.14742	0.000273
TMPO-AS1	sunitinib	-0.14242	0.000275
TMPO-AS1	TG-100-115	-0.16446	0.000283
TMPO-AS1	tacedinaline	-0.16456	0.000303
TMPO-AS1	foretinib	-0.14466	0.000306
TMPO-AS1	bendamustine	-0.14976	0.00031
TMPO-AS1	BRD-K28456706	-0.14283	0.00038
TMPO-AS1	BRD1812	-0.13884	0.000411
TMPO-AS1	BRD-A94377914	-0.19023	0.00043
TMPO-AS1	vorapaxar	-0.15128	0.000473
TMPO-AS1	BRD-A71883111	-0.14331	0.000478
TMPO-AS1	AM-580	-0.16177	0.000479
TMPO-AS1	RITA	-0.13915	0.000527
TMPO-AS1	tosedostat	-0.14052	0.000608
TMPO-AS1	KU-55933	-0.14105	0.000638
TMPO-AS1	BRD1835	-0.15198	0.000656
TMPO-AS1	BRD-K97651142	-0.13807	0.000664
TMPO-AS1	tacrolimus	-0.13939	0.000669
TMPO-AS1	lenvatinib	-0.13949	0.000715

TMPO-AS1	BRD-K29313308	-0.13822	0.000727
TMPO-AS1	sorafenib	-0.13956	0.0008
TMPO-AS1	Bax channel blocker	-0.14157	0.000826
TMPO-AS1	MK-2206	-0.13926	0.000837
TMPO-AS1	BMS-345541	-0.12984	0.000839
TMPO-AS1	GSK-3 inhibitor IX	-0.1363	0.000883
TMPO-AS1	ruxolitinib	-0.1317	0.001043
TMPO-AS1	AA-COCF3	-0.13351	0.001054
TMPO-AS1	16-beta-bromoandrosterone	-0.14793	0.001066
TMPO-AS1	Mdivi-1	-0.13273	0.001069
TMPO-AS1	TW-37	-0.12997	0.001093
TMPO-AS1	ETP-46464	-0.16172	0.001174
TMPO-AS1	AZD7545	-0.13381	0.001184
TMPO-AS1	darinaparsin	-0.19052	0.001201
TMPO-AS1	AZD8055	-0.12634	0.001374
TMPO-AS1	bosutinib	-0.12839	0.001378
TMPO-AS1	brivanib	-0.12743	0.001393
TMPO-AS1	BRD-K11533227	-0.13433	0.001404
TMPO-AS1	XL765	-0.15958	0.001454
TMPO-AS1	ELCPK	-0.18164	0.00158
TMPO-AS1	BIBR-1532	-0.13431	0.001621
TMPO-AS1	BIRB-796	-0.13541	0.001635
TMPO-AS1	tubastatin A	-0.18161	0.001664
TMPO-AS1	CD-1530	-0.15588	0.001726
TMPO-AS1	trametinib	0.200959	0.001743
TMPO-AS1	oligomycin A	-0.1225	0.001888
TMPO-AS1	R428	-0.14955	0.001941
TMPO-AS1	bortezomib	-0.11971	0.002291
TMPO-AS1	SNS-032	-0.12389	0.00231

TMPO-AS1	KHS101	-0.12551	0.002348
TMPO-AS1	NVP-BEZ235	-0.14628	0.002482
TMPO-AS1	BRD9647	-0.13619	0.0029
TMPO-AS1	linsitinib	-0.12346	0.002913
TMPO-AS1	MLN2238	-0.11534	0.003019
TMPO-AS1	KU-0063794	-0.11853	0.003134
TMPO-AS1	nintedanib	-0.13992	0.00326
TMPO-AS1	linifanib	-0.11348	0.00331
TMPO-AS1	zebularine	-0.11508	0.003416
TMPO-AS1	crizotinib	-0.11336	0.003588
TMPO-AS1	BRD-K88742110	-0.11836	0.003598
TMPO-AS1	RG-108	-0.12145	0.003728
TMPO-AS1	BRD-K24690302	-0.11791	0.00416
TMPO-AS1	GSK-J4	-0.40481	0.004373
TMPO-AS1	BRD-K85133207	-0.1198	0.004826
TMPO-AS1	masitinib	-0.11431	0.004971
TMPO-AS1	AZ-3146	-0.11415	0.005081
TMPO-AS1	BRD6340	-0.11034	0.005196
TMPO-AS1	CHIR-99021	-0.11348	0.005742
TMPO-AS1	NVP-BSK805	-0.10897	0.005767
TMPO-AS1	BRD-K19103580	-0.12487	0.006035
TMPO-AS1	CAL-101	-0.13331	0.006323
TMPO-AS1	nutlin-3	-0.10771	0.007115
TMPO-AS1	VAF-347	0.15456	0.007395
TMPO-AS1	Ki8751	-0.11255	0.007428
TMPO-AS1	ML029	-0.11092	0.009405
TMPO-AS1	PRIMA-1-Met	-0.16531	0.009664
TMPO-AS1	MI-2	-0.16307	0.0103
TMPO-AS1	BRD-K51831558	-0.17065	0.010861

TMPO-AS1	ML320	-0.11098	0.011173
TMPO-AS1	tozasertib	-0.3783	0.011566
TMPO-AS1	KU 0060648	-0.10118	0.011924
TMPO-AS1	VER-155008	-0.10303	0.012391
TMPO-AS1	NSC 74859	-0.11903	0.012618
TMPO-AS1	HLI 373	-0.10155	0.013425
TMPO-AS1	purmorphamine	-0.11255	0.01343
TMPO-AS1	temsirolimus	-0.1457	0.013512
TMPO-AS1	Compound 1541A	-0.12957	0.014633
TMPO-AS1	OSI-027	-0.09941	0.016651
TMPO-AS1	AZD1480	-0.15717	0.016766
TMPO-AS1	BRD-K55116708	-0.10277	0.017708
TMPO-AS1	erlotinib	0.100513	0.018497
TMPO-AS1	ML203	-0.11661	0.02014
TMPO-AS1	azacitidine	-0.10707	0.0202
TMPO-AS1	fluorouracil	-0.09026	0.020471
TMPO-AS1	ML006	-0.11811	0.020828
TMPO-AS1	lomeguatrib	-0.11794	0.02108
TMPO-AS1	neopeltolide	-0.20143	0.021273
TMPO-AS1	DBeQ	-0.09542	0.025849
TMPO-AS1	CIL70	-0.12616	0.026263
TMPO-AS1	AZD6482	-0.10993	0.027142
TMPO-AS1	cabozantinib	-0.09921	0.028318
TMPO-AS1	regorafenib	-0.10055	0.032835
TMPO-AS1	BRD-K02251932	-0.10174	0.033198
TMPO-AS1	saracatinib	0.092732	0.038126
TMPO-AS1	PYR-41	-0.10006	0.046137

Depletion of HMMR inhibits the cell proliferation and migration of NSCLC cells

To further definite the function of HMMR in NSCLC progression, we first using the IHC and qRT-PCR assays examine the expression of HMMR in different NSCLC cells lines, the results show that HMMR significantly elevated in the lung cancers and NSCLC cells (Figures 15A-15B), especially in A549 and H1299 cells. Next, we construction the HMMR knockdown cell lines in A549 and H1299 cells as well as employ the qRT-PCR and Western blot examine the knock down efficiency(Figures 15-15D), through the loss of functions shown that depletion of HMMR inhibits the cell growth and migration of NSCLC cells(Figures 15E-15H). These findings display that HMMR boost the cell growth and migration of NSCLC cells.

Analysis the correlation between the HMMR expression and Drug sensitivity

Above results suggested that HMMR may plays oncogene roles in the cancer progression, so we next explored the correlation between HMMR expression and different drug sensitivity in different cancer cell lines from the GDSC and CTRP database. The result indicated that HMMR expression was positively correlated with drug sensitivity of Trametinib, selumetinib, RDEA119, SB590885, PD-0325901, PLX4720, and markedly negative related to the drug sensitivity of Vorinostat, ZSTK474, AZD7762, NPK76-II-72-1, CP466722, TPCA-1, MK-2206, Genentech Cpd 10 (Table 2). In CTRP database, we observed HMMR expression was negatively correlated with the drug sensitivity of bosutinib, skepinone-L, docetaxel, PF-3758309, tivantinib, VER-155008, 3-Cl-AHPC, Elocalcitol, methotrexate, MK-1775, phloretin, pevonedistat and dasatinib (Table 3). In summary, these result suggested that HMMR was significantly related to diverse drug sensitivity in the different cancer cell lines.

Table 2

The correlation between the HMMR expression and drug sensitivity in diverse cancer cells lines analysis by the GDSC database.

symbol	drug	cor	fdr
HMMR	Trametinib	0.20436	5.16E-09
HMMR	selumetinib	0.191752	2.61E-08
HMMR	RDEA119	0.175921	3.53E-07
HMMR	SB590885	0.162942	5.3E-05
HMMR	PD-0325901	0.139021	0.000243
HMMR	PLX4720	0.13456	0.000293
HMMR	ZSTK474	-0.11702	0.00103
HMMR	17-AAG	0.120172	0.001054
HMMR	Vorinostat	-0.11747	0.001235
HMMR	(5Z)-7-Oxozeaenol	0.120586	0.001351
HMMR	NPK76-II-72-1	-0.11081	0.001523
HMMR	CP466722	-0.10773	0.002378
HMMR	AZD7762	-0.11582	0.00264
HMMR	TPCA-1	-0.10248	0.003646
HMMR	Dabrafenib	0.113295	0.003955
HMMR	FTI-277	0.113548	0.004045
HMMR	I-BET-762	-0.09951	0.004436
HMMR	KIN001-102	-0.10024	0.004555
HMMR	BMS345541	-0.10066	0.004725
HMMR	Genentech Cpd 10	-0.10078	0.005367
HMMR	AT-7519	-0.09879	0.005455
HMMR	TG101348	-0.09879	0.005478
HMMR	Navitoclax	-0.10057	0.006543
HMMR	PIK-93	-0.09512	0.007105
HMMR	PHA-793887	-0.09461	0.007208
HMMR	CAL-101	-0.09739	0.008126
HMMR	GSK690693	-0.09586	0.008427
HMMR	JW-7-24-1	-0.08998	0.01177

HMMR	Afatinib	0.089274	0.014337
HMMR	QL-XI-92	-0.08798	0.01466
HMMR	Methotrexate	-0.08896	0.016373
HMMR	Lapatinib	0.14445	0.016658
HMMR	NSC-207895	-0.0976	0.016895
HMMR	Y-39983	-0.08773	0.017497
HMMR	BX-912	-0.08472	0.017548
HMMR	GSK429286A	-0.09094	0.020014
HMMR	QL-X-138	-0.08495	0.020195
HMMR	GSK1070916	-0.08445	0.021466
HMMR	MK-2206	-0.10161	0.028189
HMMR	CI-1040	0.089898	0.029125
HMMR	Bleomycin (50 uM)	0.078716	0.030118
HMMR	TGX221	0.131545	0.032572
HMMR	AICAR	-0.08299	0.034697
HMMR	WZ3105	-0.07534	0.036231
HMMR	Nutlin-3a (-)	0.090637	0.036799
HMMR	AR-42	-0.0752	0.037553
HMMR	NSC-87877	0.103992	0.038835
HMMR	AKT inhibitor VIII	0.087731	0.039294
HMMR	QL-XII-47	-0.08001	0.039593
HMMR	BIX02189	-0.07535	0.040042
HMMR	FK866	-0.07345	0.045112
HMMR	TAK-715	-0.07271	0.046695
HMMR	BMS-754807	0.094325	0.048321
HMMR	PI-103	-0.07172	0.049444

Table 3

The correlation between the HMMR expression and drug sensitivity in diverse cancer cells lines analysis by the CTRP database.

symbol	drug	cor	fdr
HMMR	bosutinib	-0.15436	0.000103
HMMR	3-Cl-AHPC	-0.11774	0.002207
HMMR	austocystin D	0.133786	0.002245
HMMR	elocalcitol	-0.1146	0.003174
HMMR	VER-155008	-0.1178	0.003986
HMMR	MK-1775	-0.10531	0.007232
HMMR	phloretin	-0.1041	0.007999
HMMR	methotrexate	-0.10625	0.009483
HMMR	pevonedistat	-0.10316	0.009548
HMMR	manumycin A	-0.09887	0.010005
HMMR	AZD7762	-0.09741	0.011861
HMMR	triazolothiadiazine	-0.09213	0.014641
HMMR	PF-3758309	-0.13222	0.018417
HMMR	nakiterpiosin	-0.09022	0.020467
HMMR	tivantinib	-0.13184	0.020555
HMMR	CHIR-99021	-0.09589	0.021041
HMMR	CD-437	-0.08956	0.02138
HMMR	clofarabine	-0.08762	0.021383
HMMR	dasatinib	-0.099	0.023207
HMMR	decitabine	-0.08654	0.023555
HMMR	docetaxel	-0.13326	0.025147
HMMR	zebularine	-0.08873	0.025806
HMMR	LE-135	-0.09512	0.02618
HMMR	etoposide	-0.08473	0.028145
HMMR	skepinone-L	-0.13393	0.028456
HMMR	SR-II-138A	-0.0814	0.030462
HMMR	nutlin-3	0.08694	0.0323
HMMR	BRD-K51490254	-0.08871	0.035147

HMMR	YM-155	-0.09598	0.037679
HMMR	GSK-3 inhibitor IX	-0.08742	0.038374
HMMR	ML320	-0.09214	0.038688
HMMR	serdemetan	0.08418	0.039039
HMMR	StemRegenin 1	-0.08299	0.040909
HMMR	BRD-K35604418	-0.08101	0.042929
HMMR	momelotinib	-0.08279	0.043985

Discussion

Normal hypoxic environments play a key role in pathologic and physiologic conditions [30]. Previous study have indicated that hypoxia can induce the expression of varies oncogene and promote the cancer development [30]. Hypoxia was necessary for the self-renewal of cancer stem cells [31]. In addition, numerous results have shown that HIF-1α can also directly upregualtion the expression of VEGFR1 and activation cancer-related signaling pathway, result in the progression of cancers. While, the expression of HMMR whether induced by hypoxia need to be further explored.

HMMR was report plays crucial roles in the progression of human cancer [13]. For example, studies have shown that HMMR was elevated in gbm, high expression of HMMR could boost the self-renewal of GSC [13]. In breast cancer, elevated the levels of HMMR are related to poor outcome [32]. At the present time, important evidence is still lacking about the significance of HMMR in the progression of LUAD. We also adopt STRING databases to construct the protein interaction network, the protein of interaction with HMMR mainly including PLK4, CD44, AURKA, NEK2, CDK1 and FAM83D. It has been show that PLK4 was elevated in NSCLC and high expression was related to the tumor size and lymph node metastasis [33]. Recent one study found that CD44 able to elevated the expression of PDL1 by regulation the CD274 transcription, result in restrain the tumor-intrinsic function of PD-L1 [34]. It has been report that the alisertib, as an inhibitor of AURKA, combined with PD-L1 blockade able to employ treat the mammary tumors [35]. NEK2 was report that elevated in lung cancer, regulated by EGFR mutation, over-expression of NEK2significantly promotes the cell growth and induce the cell cycle progression in NSCLC cells [36]. The above results indicate that HMMR may plays a central regulate role in the cancer progression.

In this study, we employ varies bioinformatics database conduct a comprehensive and detailed analysis about the expression, prognosis, clinical significance, Immune infiltration and biological function in LUAD. We find the HMMR was highly expression in various cancer tissues, such as ACC, BRCA, KIRC, KIRP, LIHC, LUAD, LUSC, OV, SKCM, TGCT, THCA and THYM,high expression of HMMR was significantly correlated with the poor prognosis, tumor stages and lymph node metastasis of LUAD.

Hypoxia microenvironment and epigenetic modication play an crucial role in control gene expression [37]. Therefore, Hypoxia microenvironment and DNA hypomethylation may be a cause for HMMR elevated in

LUAD. Here, we first revealed that elevated the HMMR expression was relation to the immune infiltration of B cells, CD4 + T cells, CD8 + T cells, neutrophils, macrophages, and dendritic cells. Moreover, HMMR expression was also positively relation to various immune check points related gene was observed in lung cancer. Interestingly, HMMR affect the existence state of LUAD patients partially via immune cell infiltration. Our GSEA enrichment analysis results showed that HMMR is closely associated with cell immune-related pathways such as interferon γ response, TNF α signaling pathway, IL6-JAK-STAT3 signaling pathway and IL2-STAT5 signaling pathway. These findings suggested that HMMR could be as a immune-related biomarker in LUAD. Finally, we find that knockdown of HMMR significantly reduced the ability of proliferation and migration of NSCLC cells. These results demonstrate that HMMR promote the cell growth and migration of NSCLC cells.

Conclusions

In our study, we demonstrated HMMR was elevated in LUAD and positively relation to poor prognosis. We find the hypoxia microenvironment and DNA hypomethylation able to up-regulation of the HMMR expression. Moreover, our finding also suggested that HMMR promote the LUAD progression by boosts the ability of cell proliferation and migration in LUAD.

Abbreviations

cancer type	Full name
ACC	Adrenocortical carcinoma
BLCA	Bladder Urothelial Carcinoma
BRCA	Breast invasive carcinoma
CESC	Cervical squamous cell carcinoma and endocervical adenocarcinoma
CHOL	Cholangiocarcinoma
COAD	Colon adenocarcinoma
DLBC	Lymphoid Neoplasm Diffuse Large B-cell Lymphoma
ESCA	Esophageal carcinoma
GBM	Glioblastoma multiforme
HNSC	Head and Neck squamous cell carcinoma
KICH	Kidney Chromophobe
KIRC	Kidney renal clear cell carcinoma
KIRP	Kidney renal papillary cell carcinoma
LAML	Acute Myeloid Leukemia
LGG	Brain Lower Grade Glioma
LIHC	Liver hepatocellular carcinoma
LUAD	Lung adenocarcinoma
LUSC	Lung squamous cell carcinoma
MESO	Mesothelioma
OV	Ovarian serous cystadenocarcinoma
PAAD	Pancreatic adenocarcinoma
PCPG	Pheochromocytoma and Paraganglioma
PRAD	Prostate adenocarcinoma
READ	Rectum adenocarcinoma
SARC	Sarcoma
SKCM	Skin Cutaneous Melanoma
STAD	Stomach adenocarcinoma
TGCT	Testicular Germ Cell Tumors

THCA	Thyroid carcinoma
THYM	Thymoma
UCEC	Uterine Corpus Endometrial Carcinoma
UCS	Uterine Carcinosarcoma
UVM	Uveal Melanoma

Declarations

Availability of data and materials

All data generated or analyzed during this study are included in this article.

Ethics approval and consent to participate

The studies involving human participants were reviewed and approved by Ethics Committee of The Third Affiliated Hospital of Kunming Medical University.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

Funding

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Authors' contributions

X.L-J, L.T and Y.X-Y designed this work, performed related assay and analyzed data. J.W., Q.Q-L and X.L-Z contributed to study materials, L.C-D supervised and wrote the manuscript. All authors have read and

approved the final version of the manuscript.

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Figures

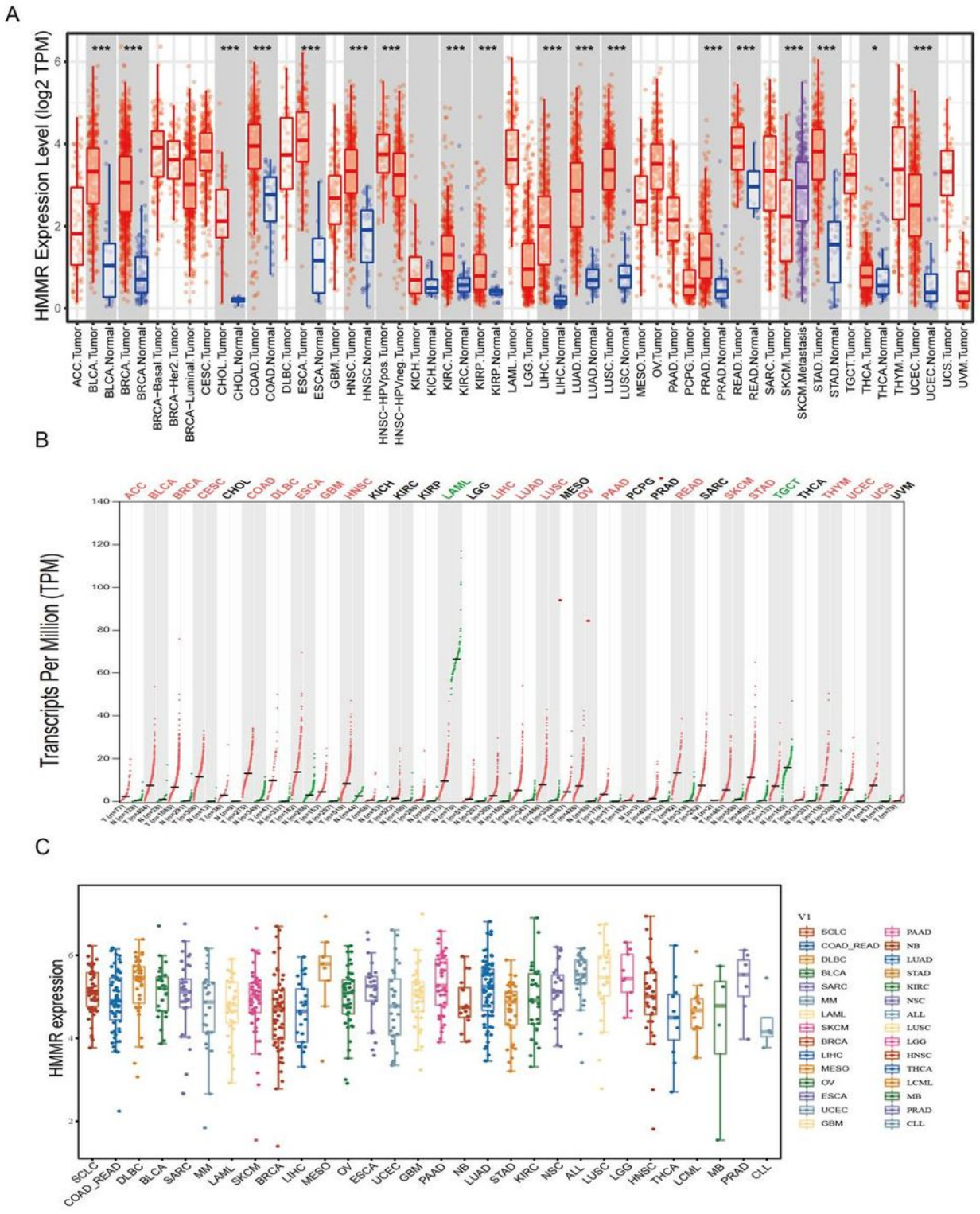

Figure 1

Analysis of the HMMR expression level in human cancers (a) Analysis the HMMR expression in pan-cancers by employed the TIMER tools (b) Analysis the HMMR expression in pan-cancers by employed the GEPIA tools (c) Analysis the HMMR expression in tumor cells lines by employed the CCLE database.

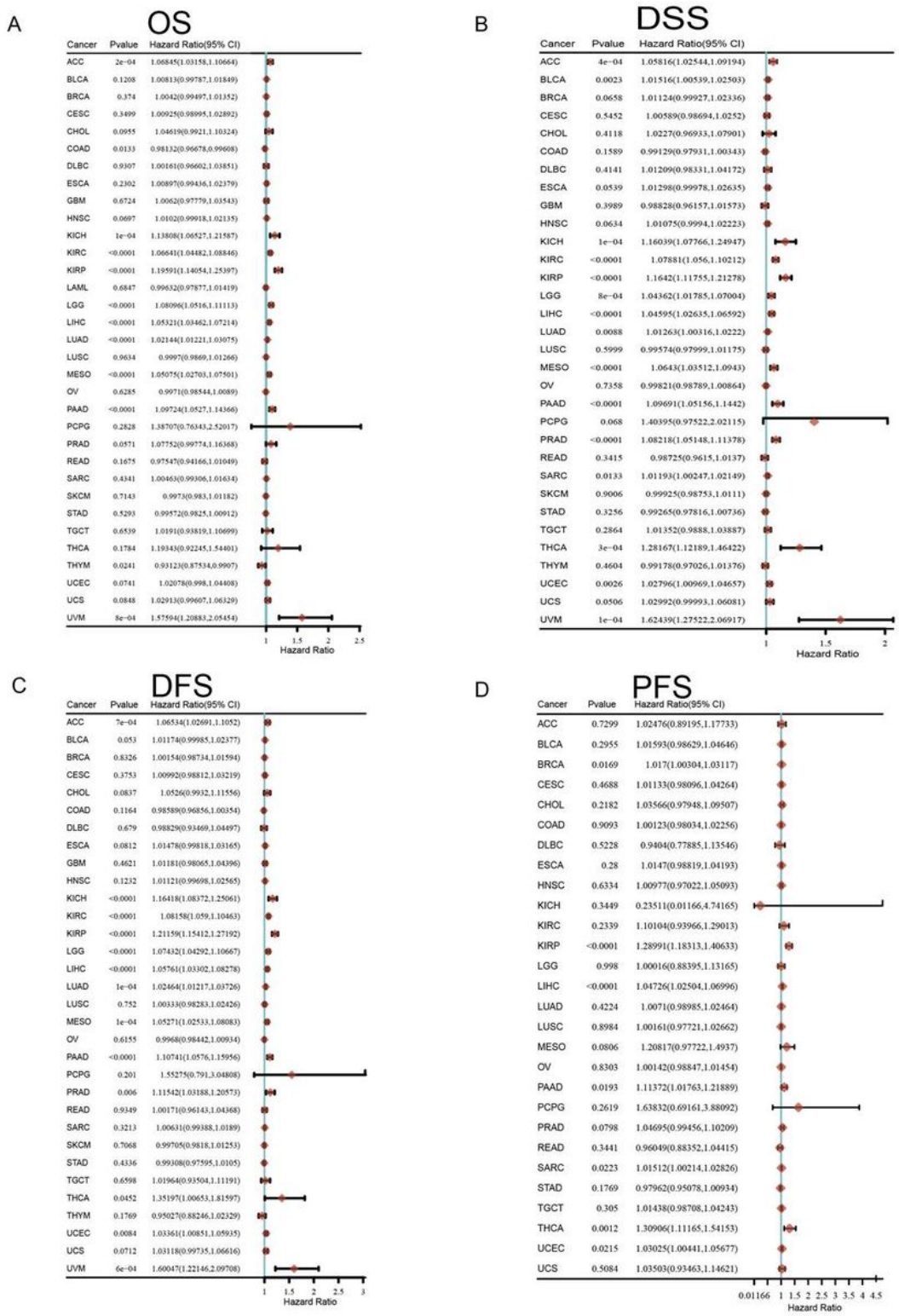

Figure 2

Analysis the prognosis of HMMR expression in Multiple cancers (a–d) Analysis the OS (A), DSS (B), DFS (C), PFS (D) of HMMR in Pan-cancers.

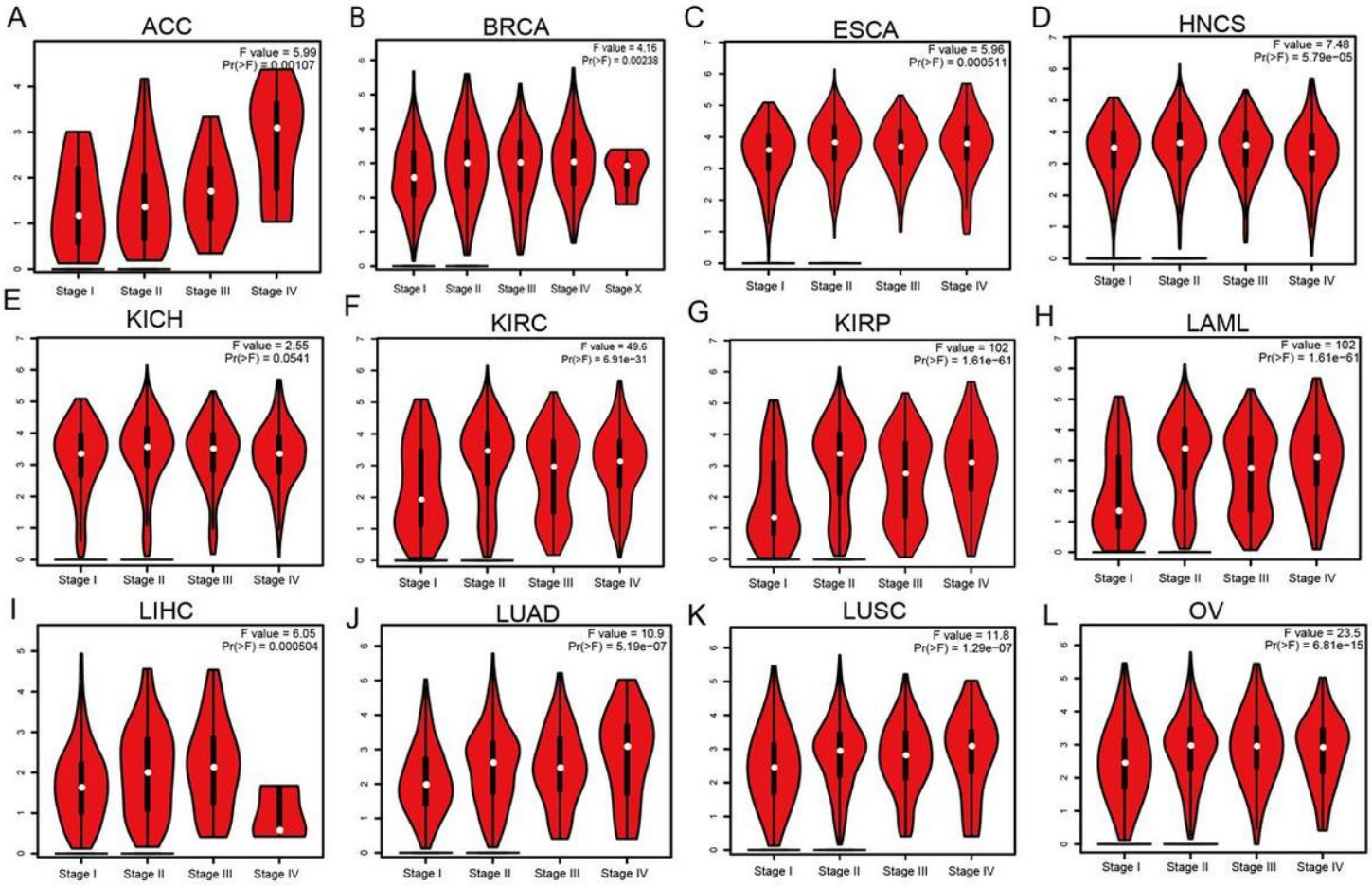

Figure 3

The pathological stage analysis for HMMR in various human cancers (a–k) The pathological stage of HMMR in ACC (A), BRCA (B), ESCA(C), HNSC (D), KICH(E), KIRC(F), KIRP(G), LAML(H), LIHC(I), LUAD (J) , LUSC(K) and OV(L)

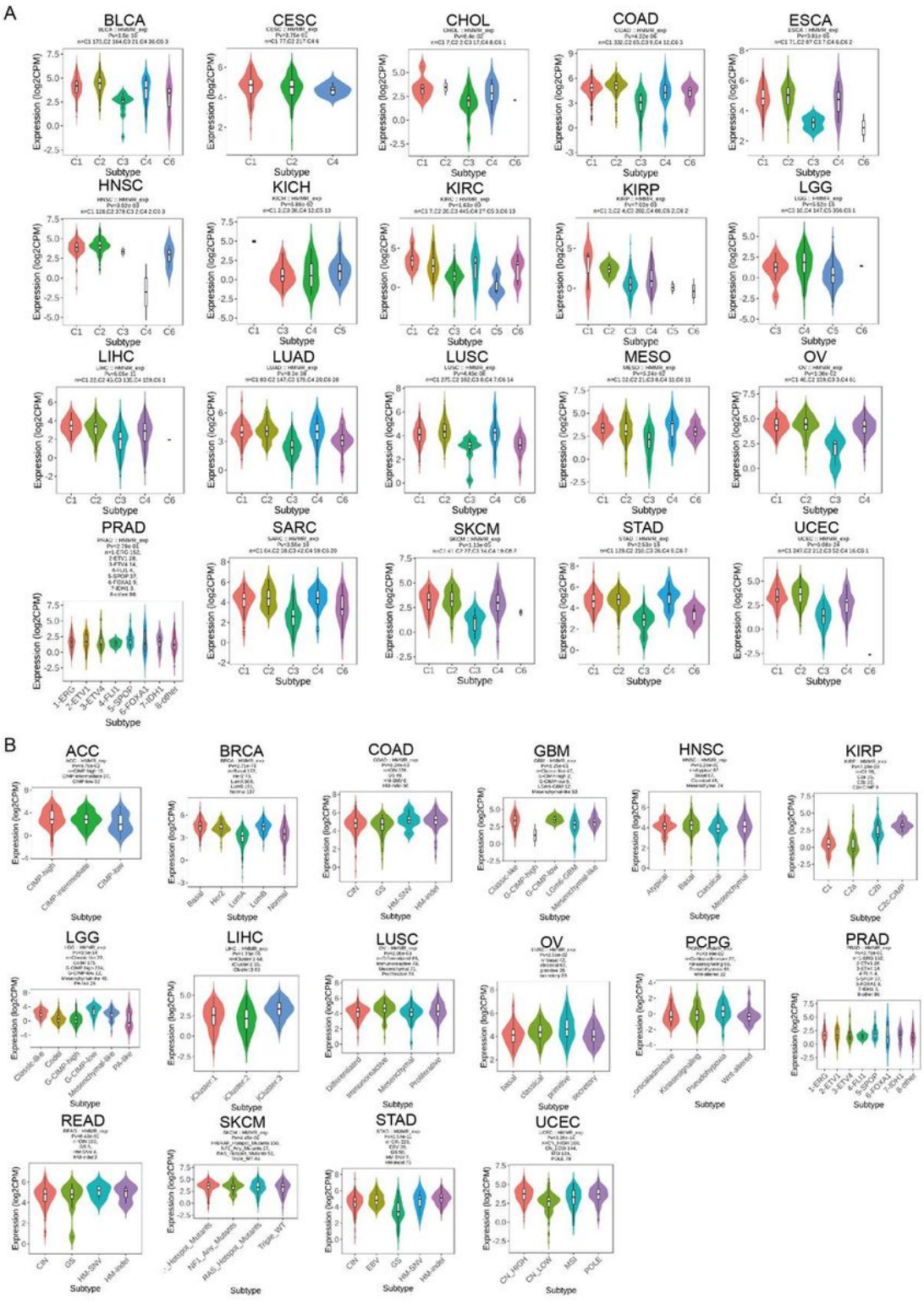

Figure 4

HMMR Expression was associated with Immune and Molecular Subtypes in Human Cancers (a) The expression of HMMR Expression in different Immune Subtypes of Multiple cancers (b) The expression of HMMR Expression in different Molecular Subtypes of Multiple cancers

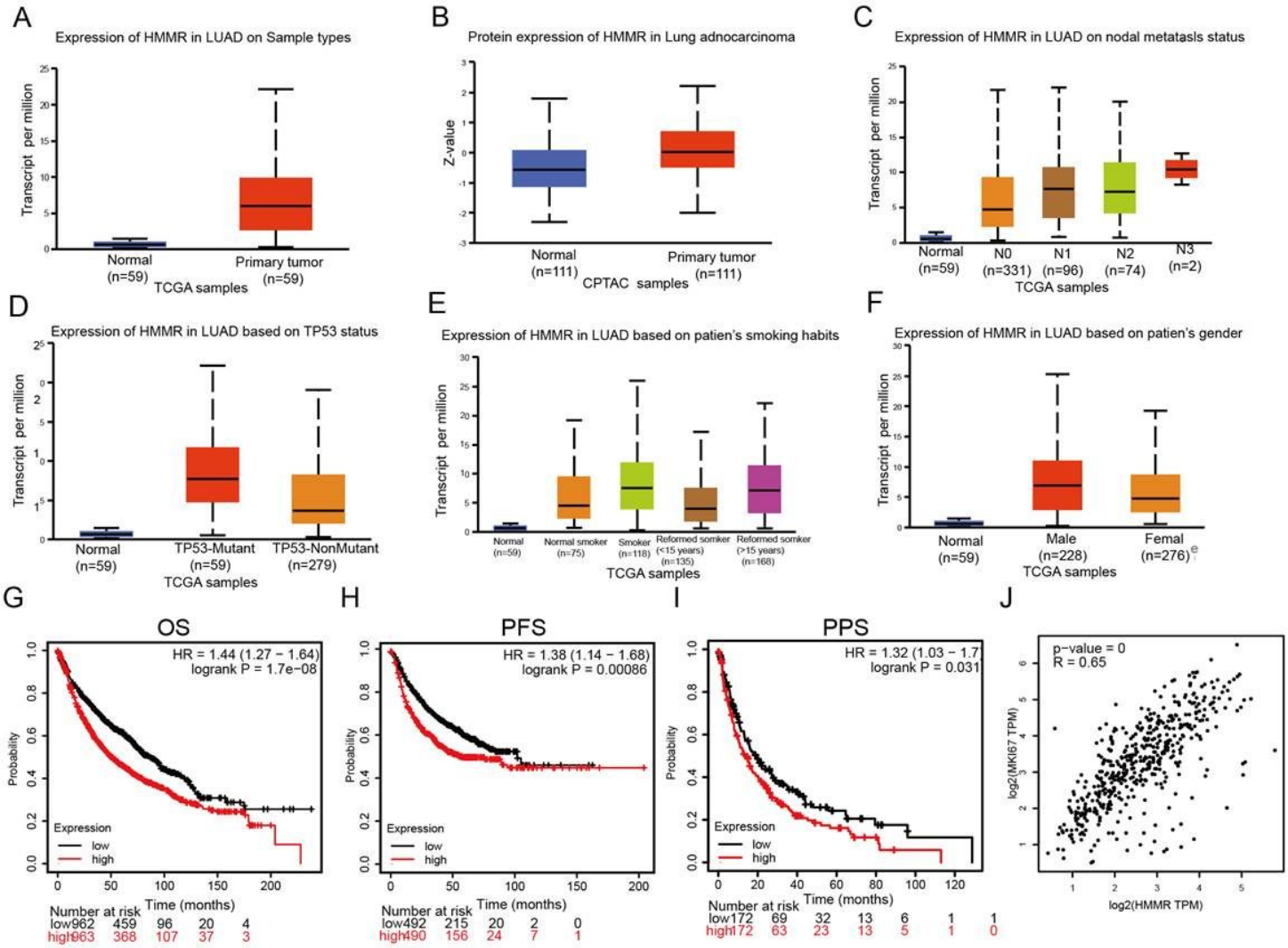

Figure 5

HMMR is elevated in LUAD (a) The mRNA of HMMR in TCGA LUAD by UALCAN databases (b) The protein of HMMR in TCGA LUAD by UALCAN databases (c) The lymph node metastatic stage analysis for HMMR in LUAD determined by GEPIA database (d) HMMR expression in group of TP53 mutation by UALCAN database (e) HMMR expression in group of smoking habits by UALCAN database (f) HMMR expression in group of sexs by UALCAN database (g-i) The OS (G), PFS (H) and PPS (I) of HMMR in LUAD by KMplot database (j) The relationship of Between the HMMR expression and MKI67 expression in TCGA-LUAD

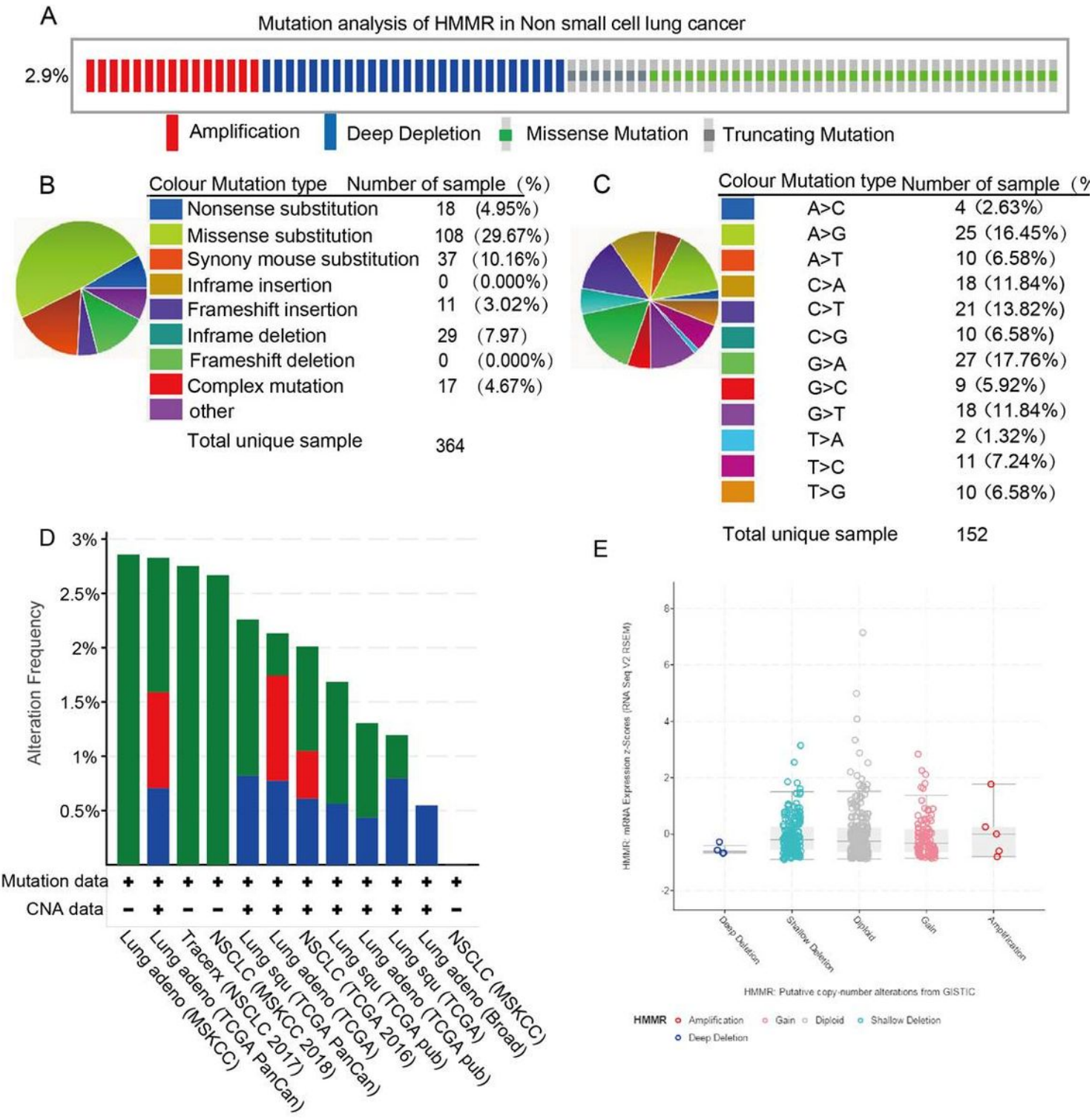

Figure 6

Mutation, Copy Number Variation Analysis of HMMR (a)The gene mutations of HMMR in lung cancer adopt the cBioportal tools (b-c) The Somatic Mutations HMMR in lung cancer adopted the Cancer database. (d) HMMR is frequently amplified in lung cancers. (e) HMMR expression in different CNV groups.

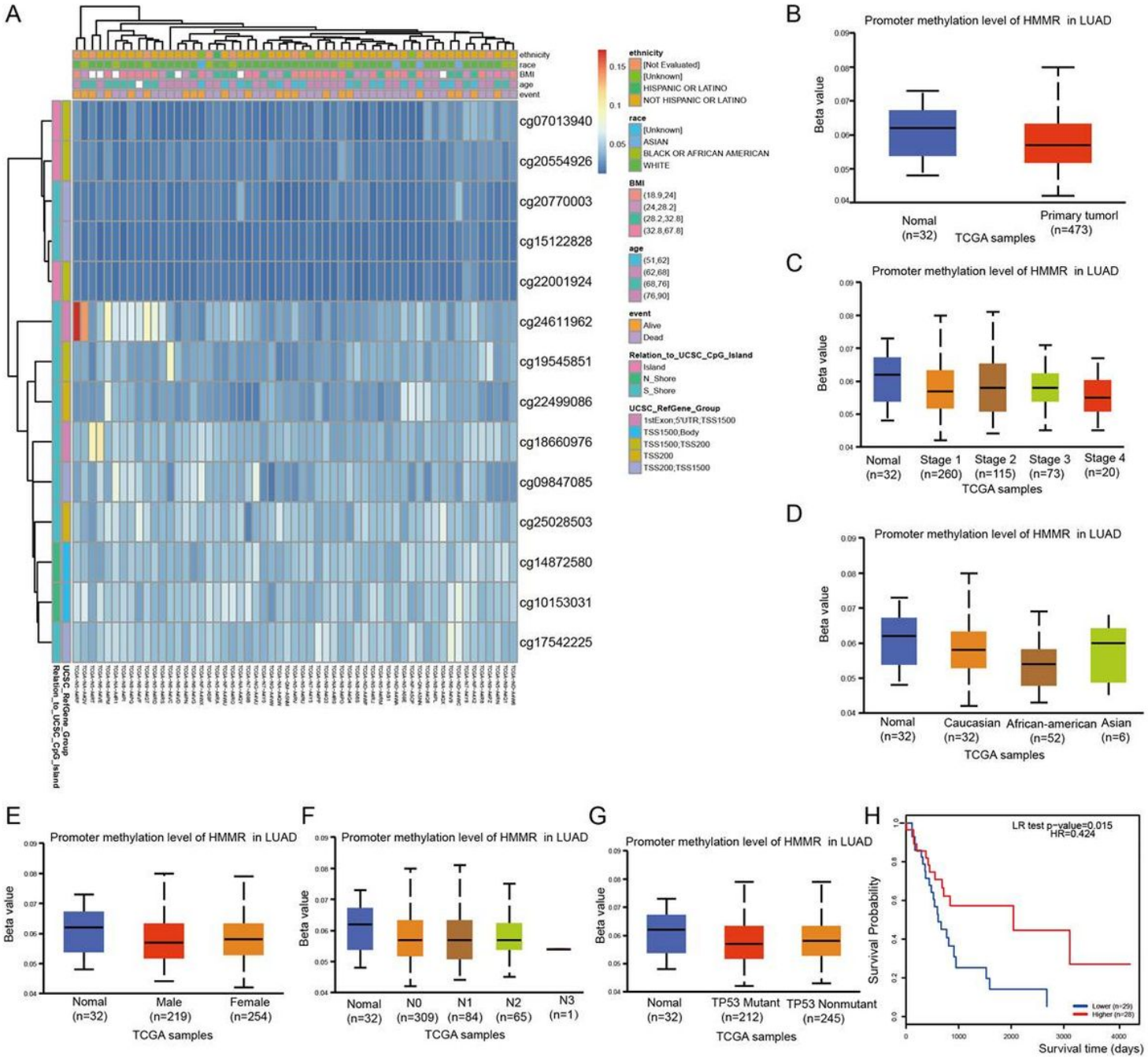

Figure 7

Analysis the DNA Methylation of HMMR （a）The differential methylation regions related to HMMR were presented as heatmaps. （b）The methylation of HMMR in LUAD. （c）The methylation of HMMR in different stage in the TCGA dataset. （d）The methylation level of HMMR in different ethnicity in the TCGA dataset. （e）The methylation level of HMMR in different group of sex in the TCGA dataset. （f）The methylation level of HMMR in different group of metastasis status in the TCGA dataset. （g）The methylation level of HMMR in different group of TP53 type in the TCGA dataset. （h）The OS of the methylation level of HMMR in the TCGA LUAD dataset.

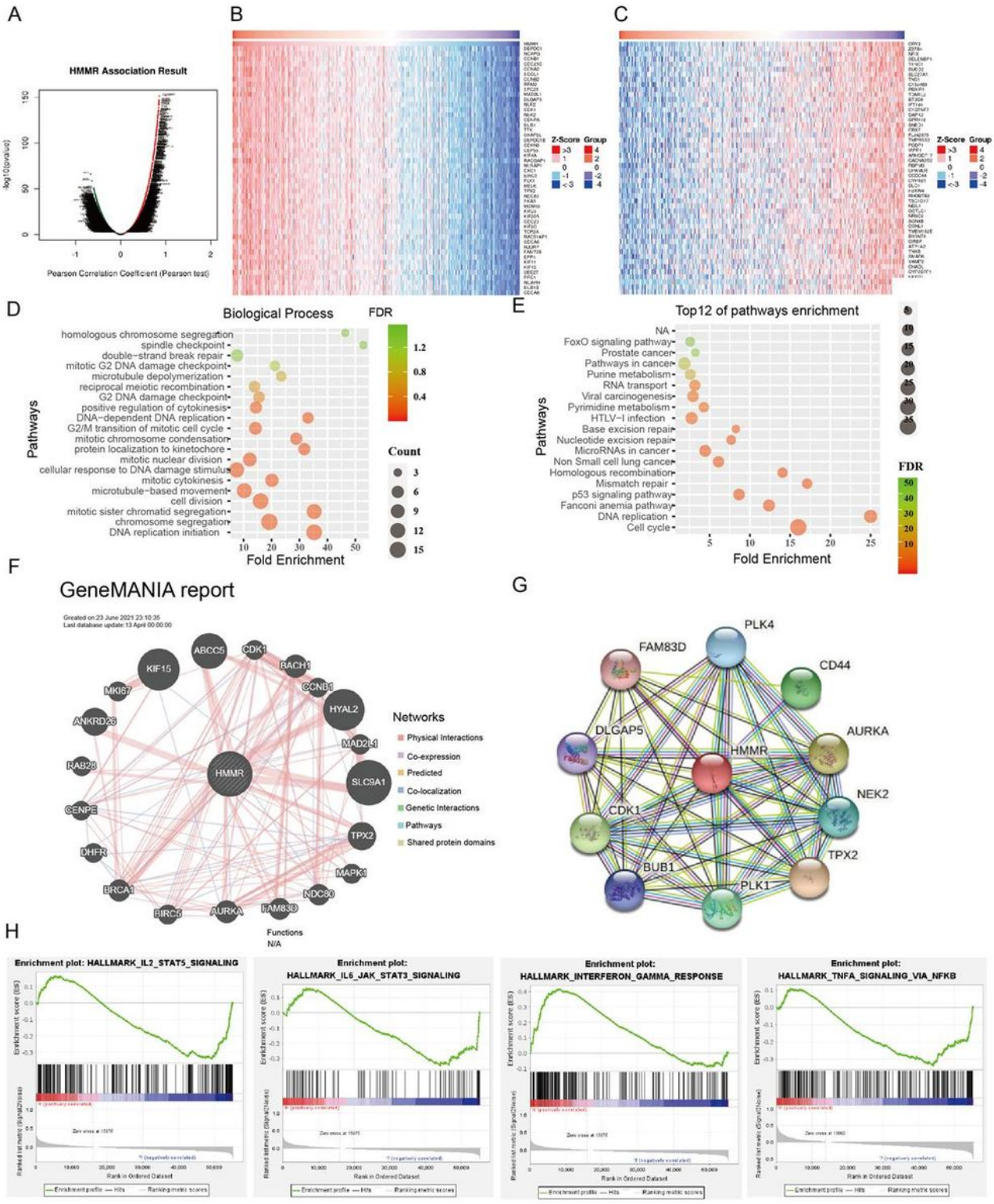

Figure 8

KEGG Pathways Networks of HMMR in LUAD (a)The differentially expressed gene was related to display by Volcano plot (b-c) The positively and negatively related to h HMMR display by Heat maps (d) Analysis the Biology process of HMMR (e) Analysis the KEGG pathway of HMMR (f) Employed the GeneMania to construction the gene interaction network of HMMR (g) Employed the STRING to construction the protein interaction network of HMMR (h) The signaling pathway enriched employed the GSEA software.

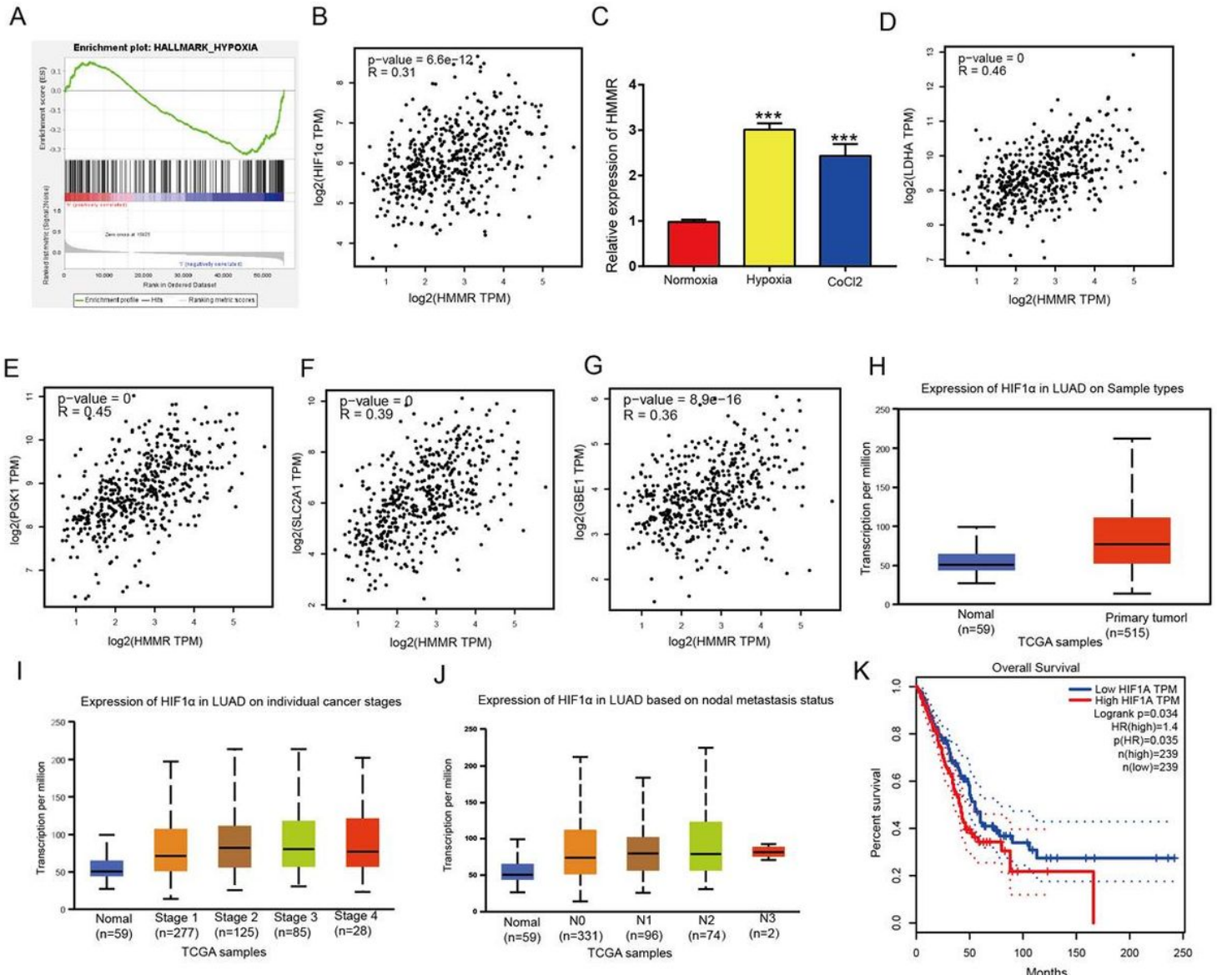

Figure 9

HMMR is transcriptionally regulated by HIF-1α under hypoxia condition (a)The hypoxia pathway by GSEA analyses (b)The correlation between HIF-1α expression and HMMR expression in TCGA-LUAD (c) The expression of HMMR under different treat conditions (d) The correlation between HMMR expression and LDHA expression in TCGA-LUAD (e) The correlation between HMMR expression and PGK1 expression in TCGA-LUAD (f) The correlation between HMMR expression and SLC2A1 expression in TCGA-LUAD (g) The correlation between HMMR expression and GBE1 expression in TCGA-LUAD (h)The expression of HIF-1α in LUAD (i) The pathological stage analysis for HIF-1α in LUAD determined by UALCAN database (j) The lymph node metastatic stage analysis for HMMR in LUAD determined by UALCAN database (k) The overall survival (OS) analysis for HIF-1αin LUAD determined by UALCAN database

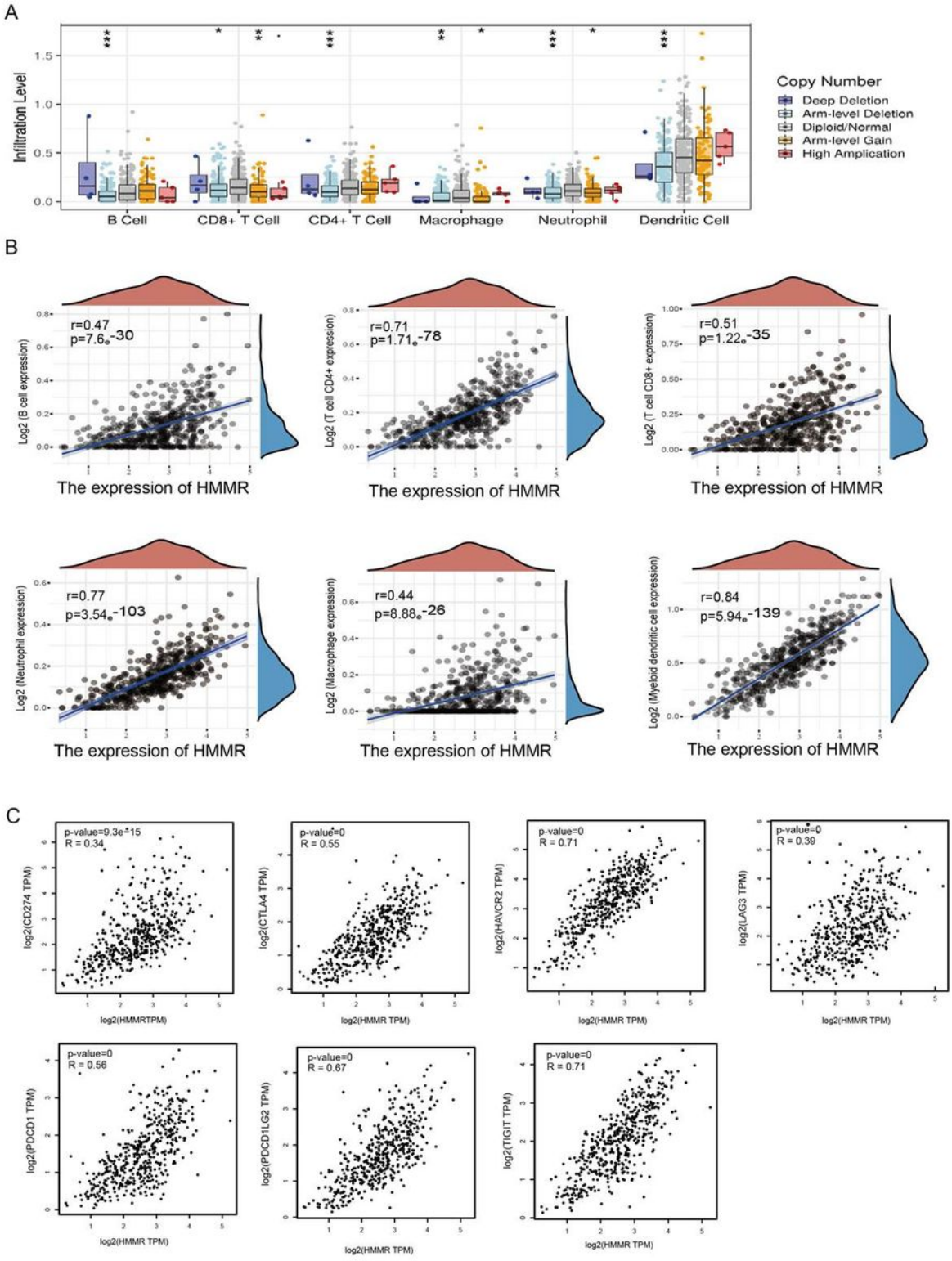

Figure 10

HMMR Expression was associated with Immune Infiltration in LUAD (a) The correlation between HMMR expression and immune cells in LUAD (b) The correlation between HMMR expression and immune infiltration in LUAD (c) The correlation between HMMR expression and immune checkpoints related gene in LUAD

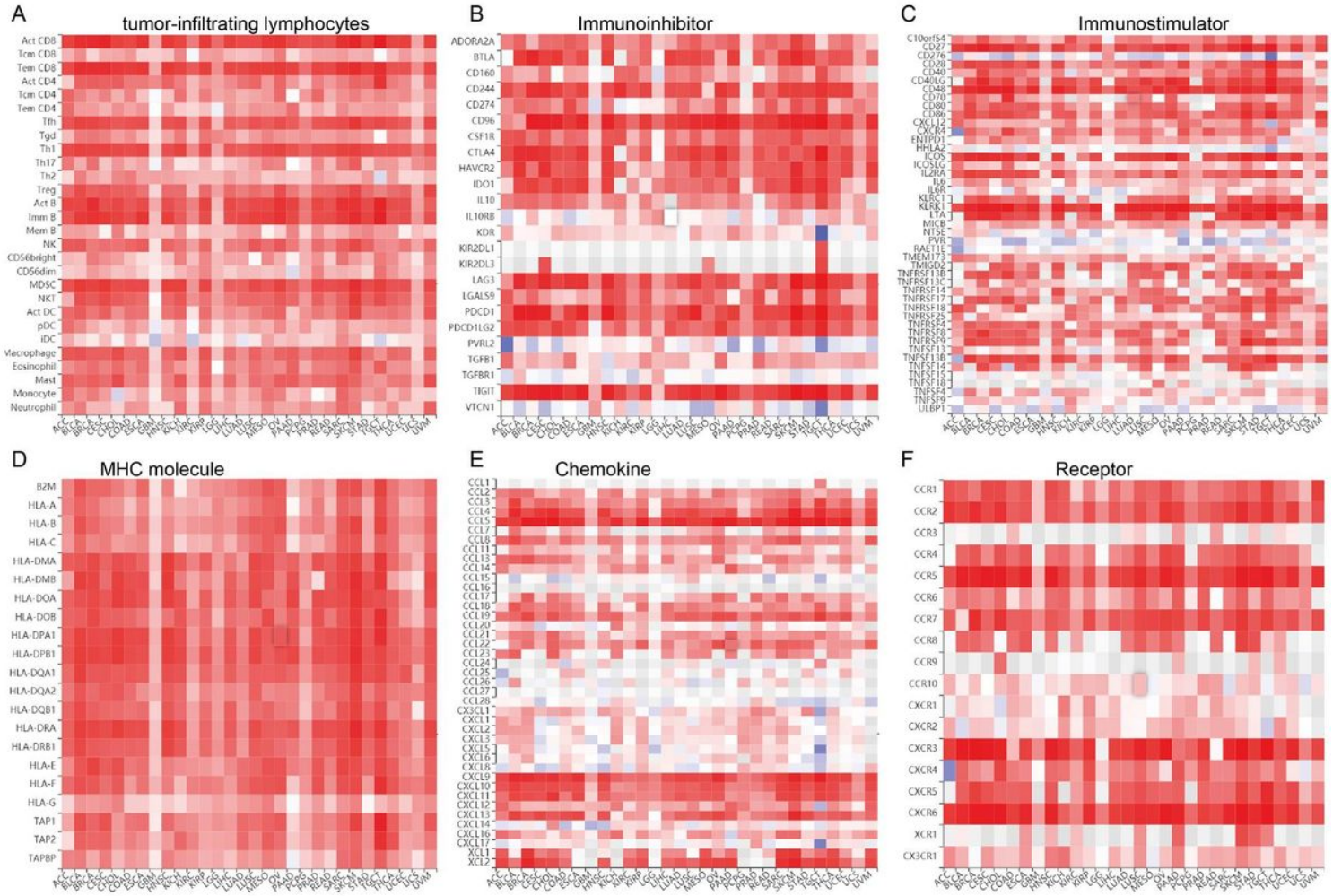

Figure 11

HMMR Expression was associated with diverse immune modulator in LUAD (A)The correlation between the HMMR expression and 28 tumor infiltrating lymphocytes analysis in pan-cancer by the TISIDB database. (B)The correlation between the HMMR expression and 24 immune-inhibitor in pan-cancer analysis by the TISIDB database. (C)The correlation between the HMMR expression and 45 immune-stimulator in pan-cancer analysis by the TISIDB database. (D) The correlation between the HMMR expression and 21 MHCs in pan-cancer analysis by the TISIDB database. (E) The correlation between the HMMR expression and 41 chemokine in pan-cancer analysis by the TISIDB database. (F)The correlation between the U HMMR expression and 18 receptor in pan-cancer analysis by the TISIDB database.

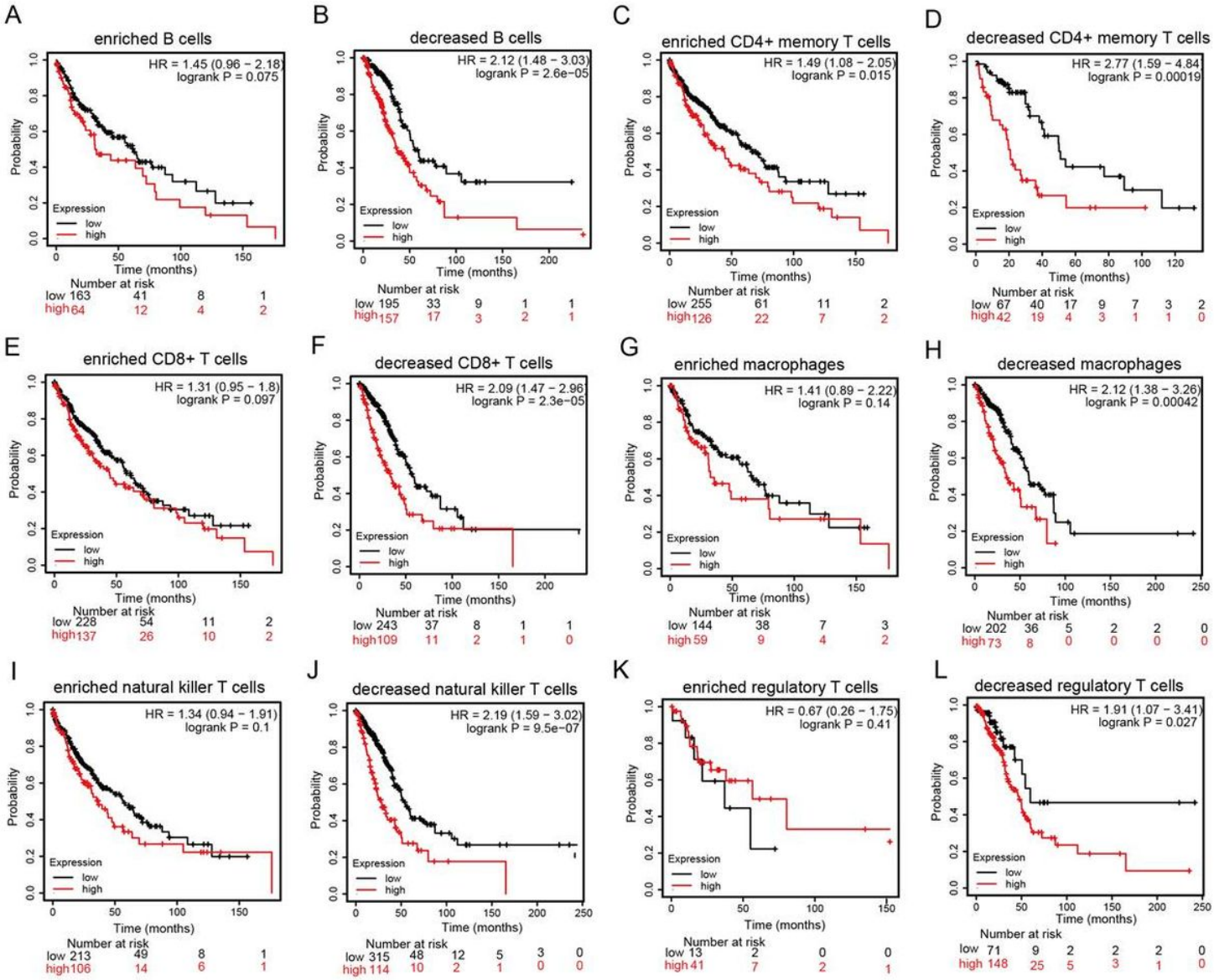

Figure 12

Prognostic Potential of HMMR Expressions in Different Tumors Based on Immune Cells (a-l) The relationship between the HMMR expression and OS based on immune cell subgroups emploted the KMplot *p < 0.05, **p < 0.01, ***p < 0.001.

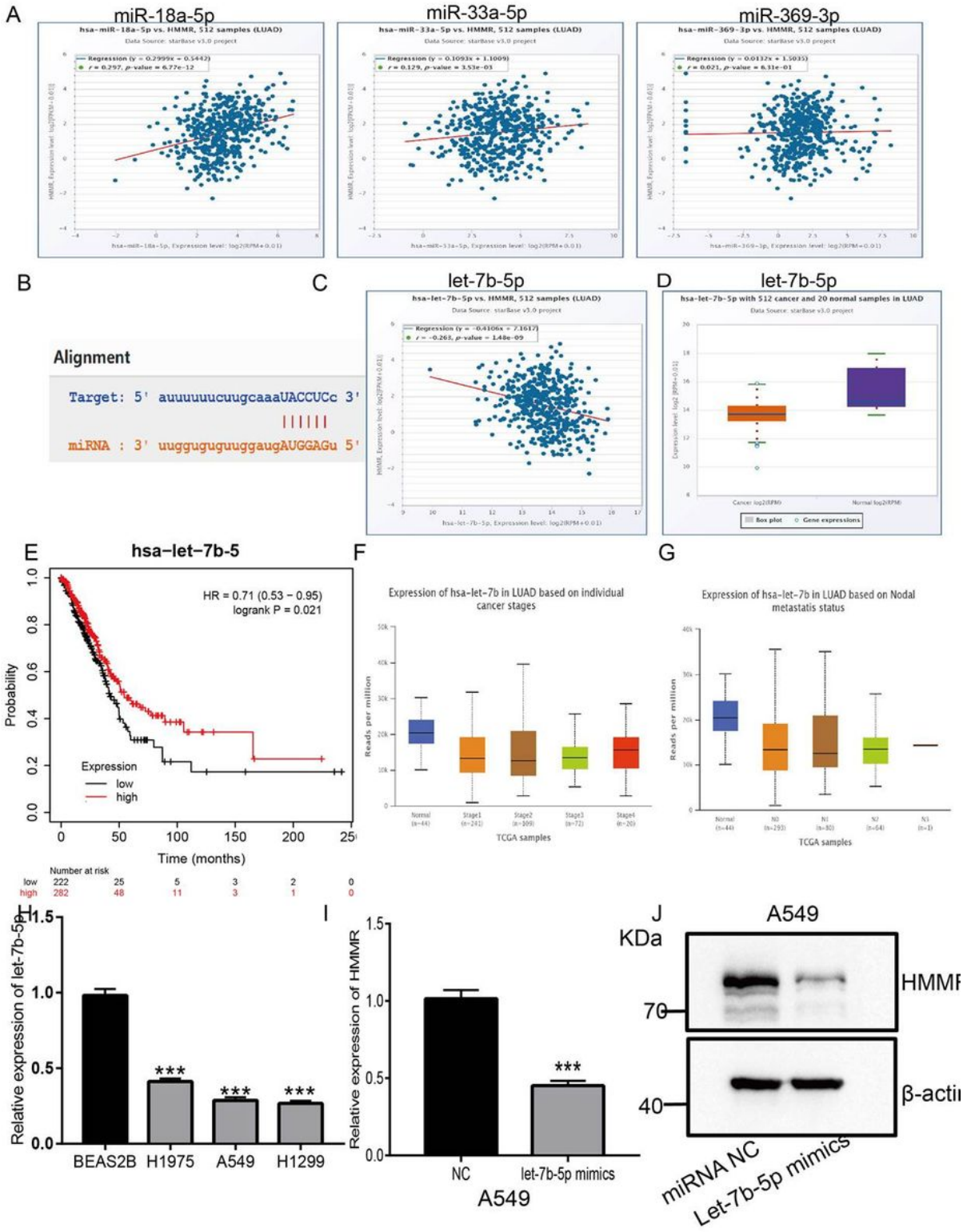

Figure 13

Predicted and analysis the upstream miRNAs of HMMR in LUAD (A)The correlation between the HMMR expression and miRNA-18a-5p, miR-33a-5p and miR-369-3p analysis by employed starbase. (B) The target sites between the HMMR and hsa-let-7b-5p were predicted by employed STarBase. (C) The correlation between the HMMR expression and hsa-let-7b-5p analysis by employed starbase. (D)The expression of hsa-let-7b-5p in LUAD analysis by employed starbase. (E)The prognosis of hsa-let-7b-5p in

LUAD analysis by employed kmplot. (F) The expression of hsa-let-7b-5p in NSCLC Cells analysis by employed qRT-PCR assay. (G) The expression of HMMR after overexpression of hsa-let-7b-5p in NSCLC Cells analysis by employed qRT-PCR assay. (H) The expression of HMMR after overexpression of hsa-let-7b-5p in NSCLC Cells analysis by employed Western blot assay.

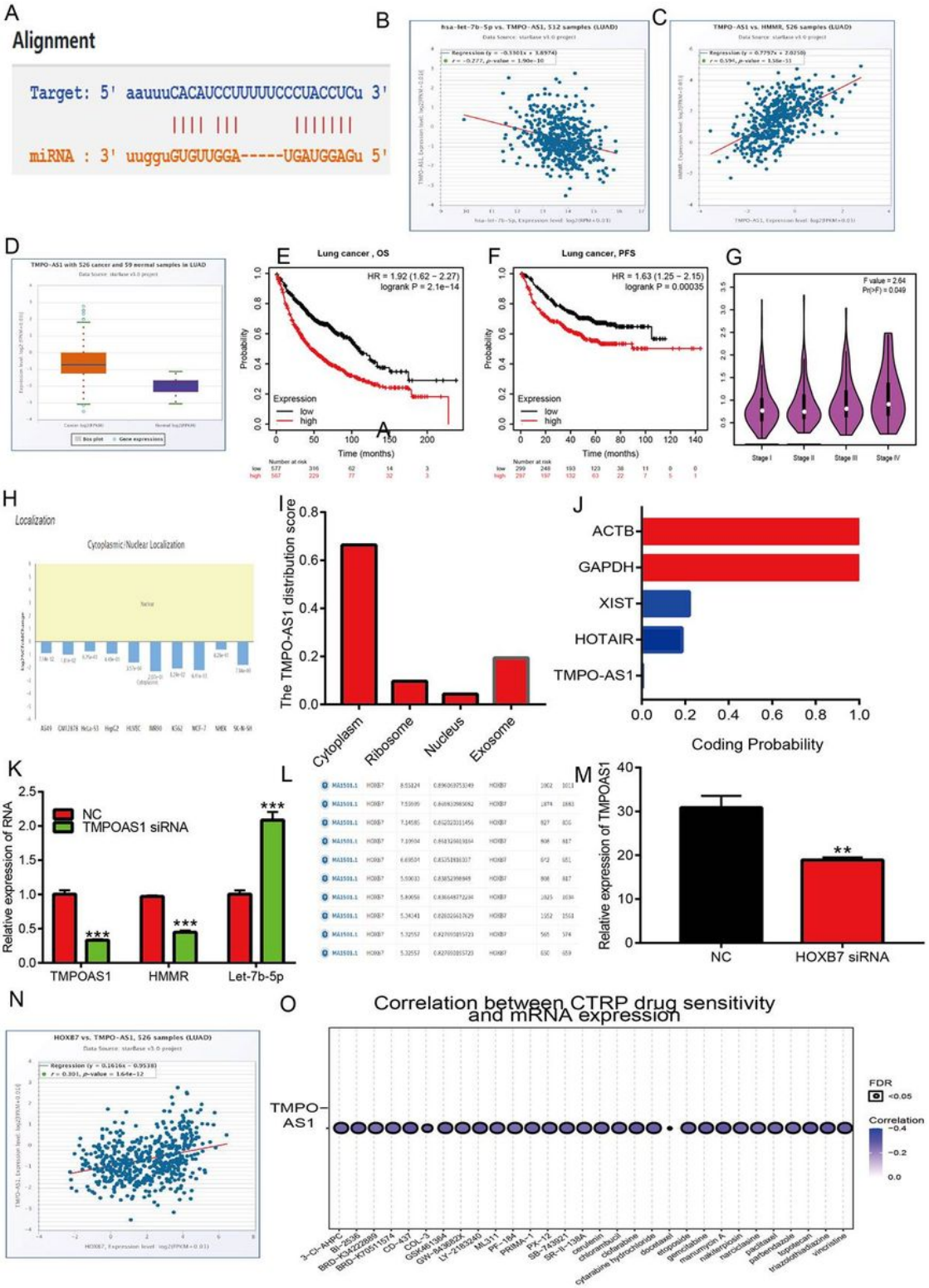

Figure 14

Predicted and analysis the upstream LncRNAs of let-7b-5p in LUAD (A) The target sites between the TMPO-AS1 and hsa-let-7b-5p were predicted by employed STarBase. (B) The correlation between the hsa-let-7b-5p expression and TMPO-AS1 analysis by employed starbase. (C) The correlation between the HMMR expression and TMPO-AS1 analysis by employed starbase. (D) The expression of TMPO-AS1 in LUAD analysis by employed starbase. (E-F) The prognosis of TMPO-AS1 in LUAD analysis by employed kmplot. (G)The correlation between the TMPO-AS1 expression and tumor stage of LUAD analysis by employed GEPIA database. (H-I)The subcellular localization of TMPO-AS1 analysis by the lncLocator tools. (J) The coding potential of TMPO-AS1 analysis by the coding potential calculator. (K)The HMMR and hsa-let-7b-5p expression after depletion of TMPO-AS1 in NSCLC Cells analysis by employed qRT-PCR assay. (L)The potential bind site of HOXB7 in the promoter of TMPO-AS1 analysis by employed the JASTAR database. (M) The TMPO-AS1 expression after depletion of HOXB7 analysis by employed GEO datas. (N) The correlation between the HOXB7 expression and TMPO-AS1 analysis by employed starbase. (O) The correlation between the TMPO-AS1 expression and Drug sensitivity in diverse human cancer analysis by CTRP database.

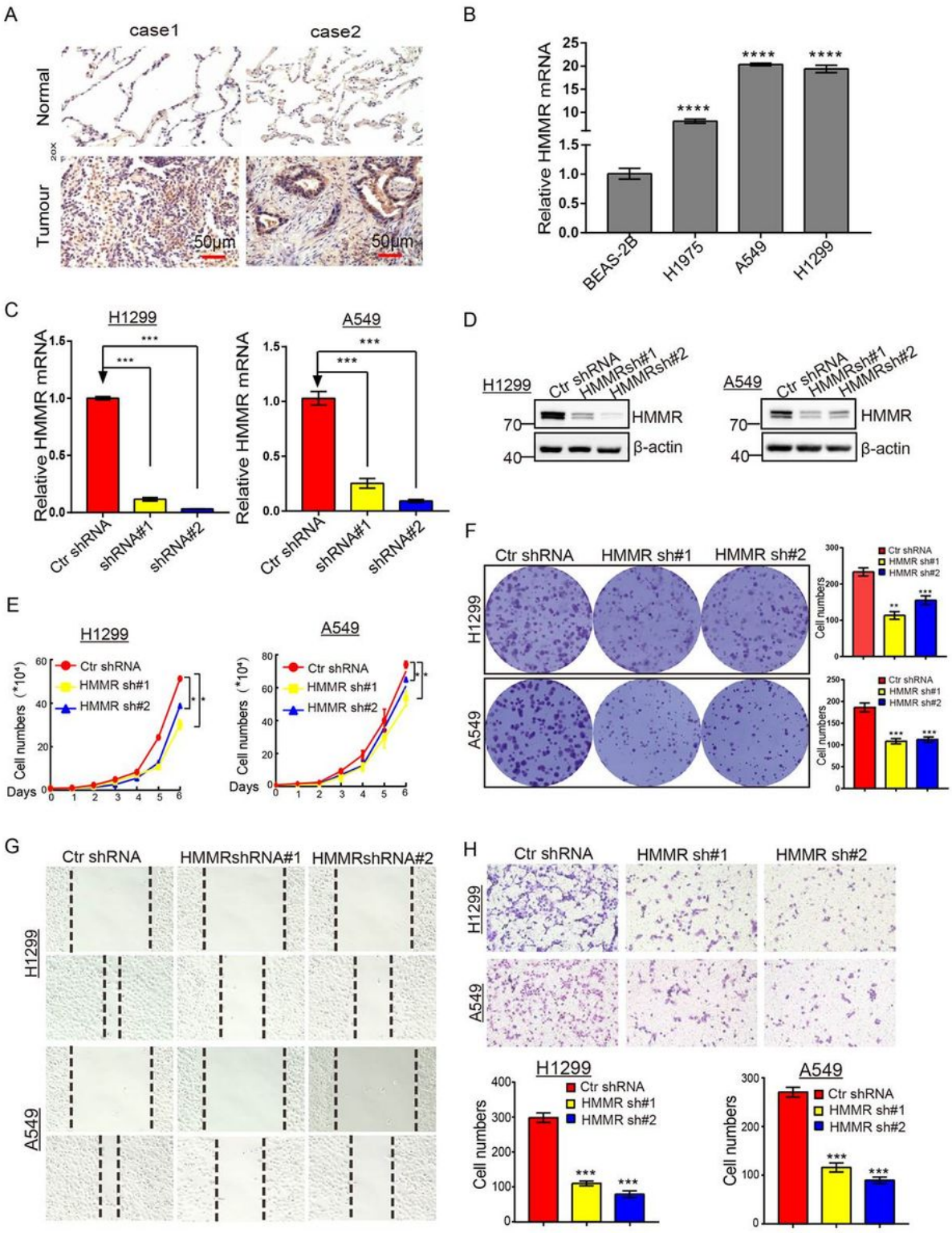

Figure 15

Depletion of HMMR inhibits growth and migration of NSCLC cells in vivro (a) IHC analysis of HMMR in LUAD. (b) The expression of HMMR in NSCLC cell lines by qRT-PCR (c-d) Establishment of HMMR knockdown in A549 cell lines and verified by qRT-PCR and Western blot (e) The growth curve assay was employed detect the proliferation of NSCLC cells. (f) The colony formation assay was employed detect

the proliferation of NSCLC cells. (g) The transwell assay was employed detect the migartion of NSCLC cells. (h)The wound healing assay was employed detect the migartion of NSCLC cells.

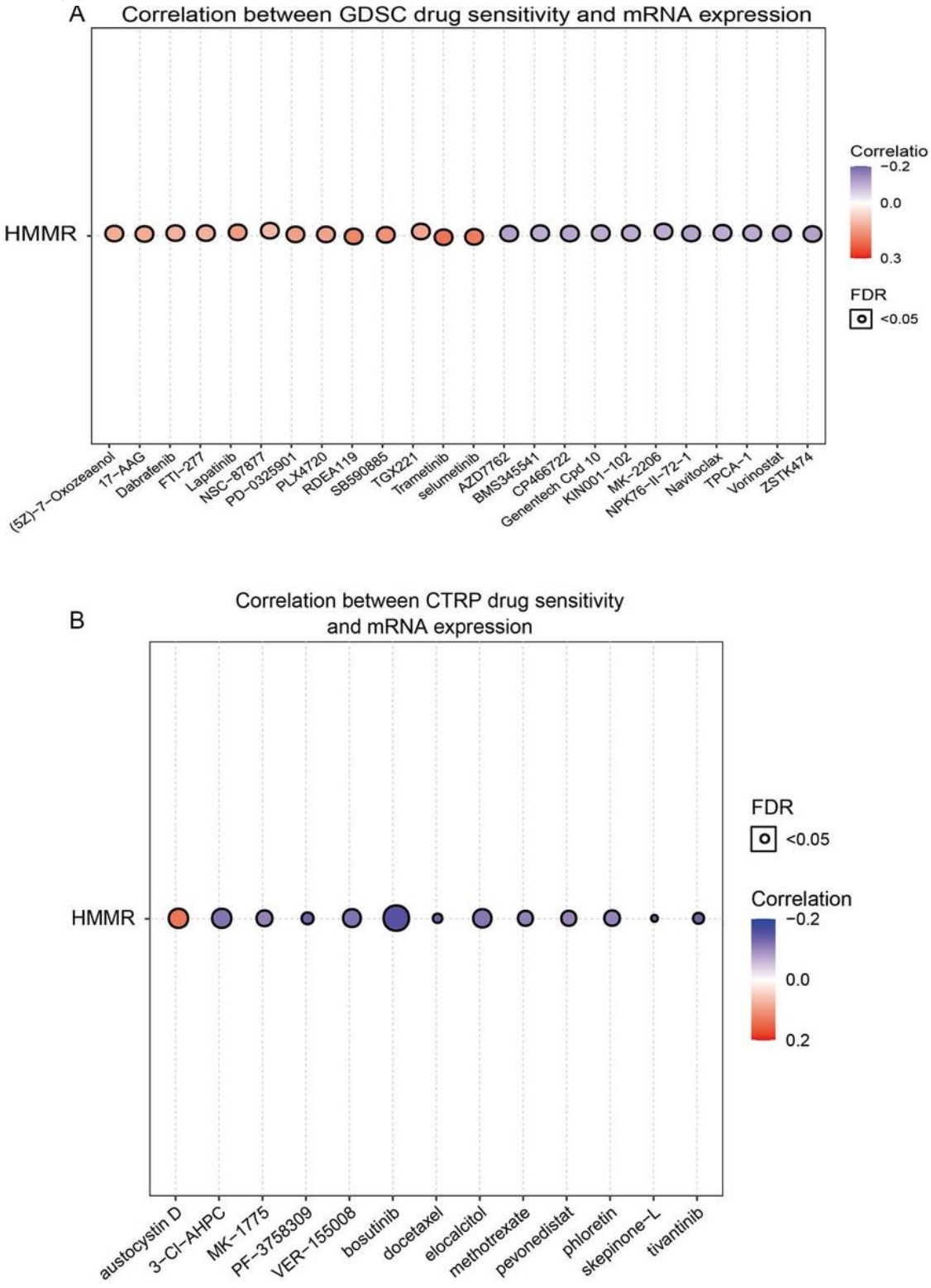

Figure 16

Analysis the correlation between the HMMRC expression and Drug sensitivity in diverse human cancer. (A) The correlation between the HMMR expression and Drug sensitivity in diverse human cancer analysis

by GDSC database. (B) The correlation between the HMMR expression and Drug sensitivity in diverse human cancer analysis by CTRP database.