

HMMR is a prognostic-related biomarker of p ancreatic ductal adenocarcinoma and promotes the development of p pancreatic ductal adenocarcinoma

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Abstract

Background HMMR is an essential oncogene, which is highly expressed in most tumors and is related to the survival and prognosis of many tumors. Pancreatic adenocarcinoma (PAAD) is a common malignant tumor of the digestive tract. The five-years overall survival (OS) is less than 10%. This study aim to investigate the relationship between HMMR expression and survival prognosis in patients with different tumors, the function of HMMR in pancreatic ductal adenocarinoma (PDAC) cells and explore the possible mechanisms in tumorigensis and development.

Methods The Oncomine, Tumor Immunoassay Resource (TIMER) and GEPIA databases were used for assessing the expression of HMMR, after which PrognoScan database and Kaplan-Meier plotter database was used to explore the relationship between HMMR and tumour outcomes including overall survival (OS), diseases free survival (DFS) and relapse free survival (RFS). Then, we investigated the function of HMMR in PDAC cells. Finally, we conduct HMMR-related gene enrichment analysis.

Results We determined HMMR expression to be significantly correlated with outcome in multiple types of cancer in the Cancer Genome Atlas (TCGA), with the effect being particularly pronounced in pancreatic ductal adenocarinoma. Elevated *HMMR* expression was found to be significantly correlated with PDAC staging and grade. Furthermore, we found that HMMR promotes the proliferation, invasion and migration of PDAC cells. The KEGG data suggest that "Oocyte meiosis", "Cell cycle", "Progesterone-mediated oocyte maturation", "FoxO signaling pathway" and "p53 signaling pathway" might be involved in the effect of HMMR on tumor pathogenesis.

Conclusions *HMMR* is high expression and associated with patient outcome in multiple cancer types, in addition, HMMR is a key factor which governs invasion and migration to PDAC, potentially playing a vital role in governing tumor invasion and migration and thus representing a valuable prognostic biomarker in PDAC patients.

1 Background

Pancreatic cancer (PC) is still one of the deadliest malignant diseases in the world[1]. In the United States, PC is the third leading cause of cancer death, and the 5-year survival rate is less than 10%, with only marginal improvement during the last decades[2]. Pancreatic ductal adenocarcinoma (PDAC) accounts for more than 90% of all PC and is therefore the major contributor to the aforementioned mortality[3]. The incidence and mortality of pancreatic cancer has been increasing year by year worldwide, and 80% of patients have been found to have local metastasis when diagnosed[4]. Surgery is the main treatment for pancreatic cancer; however, 80% of patients are not suitable for surgery, and the recurrence rate among patients who have undergone resection is very high. Further, there are numerous postoperative complications, all of which result in unsatisfying surgical outcomes[5]. In recent years, research on molecular diagnosis and targeted biological therapy of disease has seen certain progress. Diagnosis and therapy have gradually become important means to improve the prognoses of patients with malignant

tumors. Thanks to the rapid development of genomics, tumor-related genes are continuously being discovered. A number of research studies have shown that genes play vital roles in the incidence and development of pancreatic cancer[5].

Hyaluronan mediated motility receptor (HMMR, also known as RHAMM, XRHAMM, IHABP, or CD168) gene, was first identified as a constituent of a novel hyaluronan receptor complex purified from the supernatants of murine cells[12], which was originally detected in human and rats, map to Human Chromosome 5q33.2–qter and Mouse Chromosome 11[12]. Many studies have found that HMMR is highly expressed in various malignant tumors, including bladder cancer[14], pancreatic cancer[15, 16], glioma[17], gastric cancer[18], colorectal cancer[19] and so on. And some studies showed that a high expression of HMMR is associated with worse prognosis as it promotes cancer growth and metastasis. Previous studies have shown that short peptides of HMMR can be effectively presented by DC cells and activate T cell immunity[20-23]. Although some researchers found that HMMR was overexpressed in various cancer types, and considered as a tumor-associated antigen and therapeutic target of immunotherapy. However, there is still no pan-cancer evidence on the relationship between HMMR and various tumor types based on big clinical data, and the mechanisms of HMMR governs tumour progression in pancreatic ductal adenocarcinoma remain unclear.

Herein, we conducted a comprehensive assessment of the relationship between HMMR and patient prognosis using databases including Oncomine, PrognoScan and Kaplan-Meier plotter. We further investigated the function of HMMR in pancreatic ductal adenocarcinoma cells and the potential molecular mechanism of HMMR in the pathogenesis or clinical prognosis of different cancers. Our results offer novel insights into the functional role of HMMR in pancreatic ductal adenocarcinoma cells, and may act as a prognostic-related biomarker of pancreatic ductal adenocarcinoma.

2 Materials And Methods

2.1 Oncomine database analysis

The Oncomine database compiled 86,733 samples and 715 gene expression data sets into a single comprehensive database designed to facilitate data mining efforts[24]. We therefore used this database to assess the association between *HMMR* expression and prognostic outcome in various tumour types (<https://www.oncomine.org/resource/login.html>).

2.2 PrognoScan database analysis

The PrognoScan database is designed to facilitate meta-analyses of gene prognostic value by comparing the relationship between gene expression and relevant outcome including overall survival (OS) in a wide range of published cancer microarray data sets[25]. We therefore used this database to assess the relationship between *HMMR* expression and patient outcome (<http://www.abren.net/PrognoScan/>).

2.3 TIMER2 database analysis

We input HMMR in the “Gene_DE” module of TIMER2 (tumor immune estimation resource, version 2) web (<http://timer.cistrome.org/>) and observed the expression difference of *HMMR* between tumor and adjacent normal tissues for the different tumors or specific tumor subtypes of the TCGA project. Moreover, we used the “Gene_Corr” module of TIMER2 to supply the heatmap data of the selected genes, which contains the partial correlation (cor) and *P*-value in the purity-adjusted Spearman’s rank correlation test.

2.4 GEPIA2 database analysis

GEPIA2 is an online database which facilitates the standardized analysis of RNA-seq data from 9,736 tumour samples and 8,587 normal control samples in the TCGA and GTEx data sets (<http://gepia.cancer-pku.cn/index.html>)[25]. To further assess the difference in *HMMR* expression levels in those tumour types which did not have normal tissues in TIMER2 database, we use the GEPIA2 database to analysis them. We also employed this database to assess the link between *HMMR* expression and patient prognosis in multiple tumour types.

2.5 Kaplan-Meier plotter analysis

The Kaplan-Meier plotter offers a means of readily exploring the impact of a wide array of genes on patient survival in 21 different types of cancer, with large sample sizes for the breast (n = 6,234), ovarian (n = 2,190), lung (n = 3,452) and gastric (n = 1,440) cancer cohorts[27]. We therefore used this database to explore the association between *HMMR* expression and outcome in patients with gastric, breast, ovarian, lung cancer and pancreatic ductal adenocarcinoma, analysing the impact of both clinicopathological factors and HMMR on patient outcome in pancreatic ductal adenocarcinoma patients (<http://kmplot.com/analysis/>).

2.6 Genetic alteration analysis

Firstly, we logging into the cBioPortal web (<https://www.cbioportal.org/>)[28, 29], and secondly chose the “TCGA Pan Cancer Atlas Studies” in the “Quick select” section and entered “HMMR” for queries of the genetic alteration characteristics of HMMR. The results of the alteration frequency, mutation type and CNA (Copy number alteration) across all TCGA tumors were observed in the “Cancer Types Summary” module. The mutated site information of HMMR can be displayed in the schematic diagram of the protein structure or the 3D (Three-dimensional) structure via the “Mutations” module.

2.7 Gene function analysis

We select human pancreatic ductal adenocarcinoma (PDAC) cell lines for functional analysis of HMMR. The cell lines HPDE6-C7, PANC-1, Capan-2, SW1990 and BxPC-3 were cultured in RPMI 1640 medium containing 10% fetal bovine serum, which was placed in incubator at 37 °C in a humidified atmosphere with 5% CO₂. After 2 to 3 days of cell passage, the cells in the logarithmic growth phase were selected for further experiment. MTS assay. Dispense 5×10^3 per cells into wells of the 96-well plates in a final

volume of 100 μ l. Incubate the plate at 37 °C for 48 - 72 hours in a humidified, 5% CO₂ atmosphere. Add 20 μ l per well of CellTiter 96® AQueous One Solution Reagent. Incubate the plate at 37 °C for 1 - 4 hours in a humidified, 5% CO₂ atmosphere. Record the absorbance at 490nm using a 96-well plate reader. Each experiment was conducted with 3 replicates[30]. For colony formation assays, 500 cells were seeded per well in 6-well plates and cultured for 2 weeks. The colonies were fixed with 4% paraformaldehyde and stained with 1% crystal violet. Only colonies containing more than 50 cells were counted. For wound healing assay, 5×10^5 cells were seeded into 6-well plates and grown to confluence. Mitomycin C (10 μ g/mL) was used to suppress cell proliferation before scratching[31]. Wounds were created by scraping the confluent cell monolayers with a 10 μ l pipette tip. After extensively rinsed to remove cellular debris, cells were cultured in serum-free medium. Observe wound closure rate after incubating for 24 h, and images were taken using an inverted microscope TE-2000S (Nikon, Tokyo, Japan). Transwell invasion assay was performed in a 24-well transwell plate with 8- μ m polyethylene terephthalate membrane filters (Corning Costar Corp, Corning, NY). 1×10^5 cells in 200 μ l of serum-free medium were added to the upper chambers, which contained matrigelcoated membranes (BD Biosciences). Each lower chamber was filed with 500 μ l medium with 10% FBS. After 24 h of incubation, cells that invaded to the bottom chamber were fixed with 4% paraformaldehyde and stained with 0.1% crystal violet. Invasive cells were counted in five randomly chosen fields (magnification, $\times 200$) per well.

Further details of materials and methods are described in the Supporting Materials and Methods.

2.8 HMMR-related gene enrichment analysis

We first searched the STRING website (<https://string-db.org/>) using the query of a single protein name (“HMMR”) and organism (“Homo sapiens”). Subsequently, we set the following main parameters: minimum required interaction score [“Low confidence (0.150)”], meaning of network edges (“evidence”), max number of interactors to show (“no more than 50 interactors” in 1st shell) and active interaction sources (“experiments”). Finally, the available experimentally determined HMMR-binding proteins were obtained. We used the “Similar Gene Detection” module of GEPIA2 to obtain the top 100 HMMR-correlated targeting genes based on the datasets of all TCGA tumor and normal tissues. We also applied the “correlation analysis” module of GEPIA2 to perform a pairwise gene Pearson correlation analysis of HMMR and selected genes. The log₂ TPM was applied for the dot plot. The *P*-value and the correlation coefficient (R) were indicated. Moreover, we used the “Gene_Corr” module of TIMER2 to supply the heatmap data of the selected genes, which contains the partial correlation (cor) and *P*-value in the purity-adjusted Spearman’s rank correlation test.

We used Jvenn, an interactive Venn diagram viewer[32], to conduct an intersection analysis to compare the HMMR-binding and interacted genes. Moreover, we combined the two sets of data to perform KEGG (Kyoto encyclopedia of genes and genomes) pathway analysis. In brief, we uploaded the gene lists to DAVID (Database for annotation, visualization, and integrated discovery) with the settings of selected identifier (“OFFICIAL_GENE_SYMBOL”) and species (“Homo sapiens”) and obtained the data of the functional annotation chart. The enriched pathways were finally visualized with the “dplyr” and “ggplot2”

R packages. In addition, we applied the "clusterProfiler" R package to conduct GO (Gene ontology) enrichment analysis. The data for BP (Biological process), CC (Cellular component), and MF (Molecular function) were visualized as cnetplots, using the cnetplot function (circular = F, colorEdge = T, node_label = T). The R language software [R-4.0.4, 64-bit] (<https://www.r-project.org/>) was used in this analysis. Two-tailed $P < 0.05$ was considered statistically significant.

3 Results

3.1 Assessment of HMMR expression in different cancer and normal tissues

We first assessed the expression of *HMMR* in multiple tumour and normal tissue types using the Oncomine database, revealing that expression of this gene was elevated relative to normal tissue controls for bladder, brain, breast, cervical, rectal, colorectal, oesophageal, gastric, head and neck, renal, liver, lung, lymphoma, ovarian, pancreatic, sarcoma and prostate cancers. We also found that relative to normal tissue controls, *HMMR* expression was lower in leukemia and other cancer tissues (Figure 1A). Detailed findings in particular tumour types are compiled in Table S1. We also used the TCGA and TIMER databases to assess how *HMMR* expression differs in particular tumour types. We found that the expression of *HMMR* was significantly elevated relative to normal controls in bladder urothelial carcinoma (BLCA), breast invasive carcinoma (BRCA), cervical squamous cell carcinoma and endocervical adenocarcinoma (CESC), cholangiocarcinoma (CHOL), colon adenocarcinoma (COAD), esophageal carcinoma (ESCA), glioblastoma multiforme (GBM), head and neck squamous cell carcinoma (HNSC), kidney renal clear cell carcinoma (KIRC), kidney renal papillary cell carcinoma (KIRP), liver hepatocellular carcinoma (LIHC), lung adenocarcinoma (LUAD), lung squamous cell carcinoma (LUSC), prostate adenocarcinoma (PRAD), rectum adenocarcinoma (READ), stomach adenocarcinoma (STAD), thyroid carcinoma (THCA) and uterine corpus endometrial carcinoma (UCEC). Differences between the expression of *HMMR* in tumours and normal adjacent tissue samples in the TCGA data set are shown in Figure 1B.

To further evaluate the expression difference of *HMMR* between the normal tissues and tumor tissues, we use the GTEx dataset as controls, which shown that high *HMMR* expression in tumor than the normal tissue in adrenocortical carcinoma (ACC), lymphoid neoplasm diffuse large B-cell lymphoma (DLBC), ovarian serous cystadenocarcinoma (OV), pancreatic adenocarcinoma (PAAD) (Figure 1C, $P < 0.01$), and sarcoma (SARC), thymoma (THYM), uterine carcinosarcoma (UCS), (Figure S1A-C, $P < 0.01$). However, we did not obtain a significant difference in kidney chromophobe (KICH), brain lower grade glioma (LGG) and pheochromocytoma and paraganglioma (PCPG), as shown in Figure S1D-F. What more, we found the expression of *HMMR* was high in normal tissues than tumor tissues in acute myeloid leukemia (LAML) and testicular germ cell tumors (TGCT) are shown in Figure S1G, H.

3.2 The association between HMMR expression and cancer patient prognosis

We next explored the link between the expression of *HMMR* and cancer patient outcome using the PrognoScan database (Table 1 and Tables S2–S4). We found that multiple cancer types exhibited a

significant association between patient prognosis and *HMMR* expression including bladder, brain, blood, breast, colon, ovarian skin and lung cancer (Figure 2A–H and Figure S2). We additionally employed the Kaplan-Meier plotter database in order to assess how *HMMR* expression relates to prognosis in a range of cancer types, revealing its elevation to be significantly linked with a poorer prognosis in ovarian cancer (OS HR = 1.32, 95% CI = 1.15–1.5, $P = 4e-5$; PFS HR = 1.3, 95% CI = 1.14–1.48, $P = 7e-5$), lung cancer (OS HR = 1.7, 95% CI = 1.49–1.93, $P = 4e-16$; PFS HR = 1.7, 95% CI = 1.67–2.47, $P = 6.5e-13$) and pancreatic ductal adenocarcinoma (PAAD) (OS HR = 2.31, 95% CI = 1.51–3.55, $P = 8e-5$; RFS HR = 3.62, 95% CI = 1.4–9.41, $P = 0.0048$) (Figure 2I–L and Table 2). However, we found reduced *HMMR* expression to be correlated with poorer patient prognosis in gastric cancer (OS HR = 0.59, 95% CI = 0.48–0.71, $P = 4.7e-8$; PFS HR = 0.63, 95% CI = 0.5–0.8, $P = 8.2e-5$) (Figure 2M–N). There was not any significant relationship between the expression of *HMMR* and the OS prognosis of breast cancer patients (Figure 2O), but the high *HMMR* expression level have a significant relationship with the RFS prognosis of breast cancer patients (RFS HR = 1.32, 95% CI = 1.19–1.46, $P = 8.1e-8$) (Figure 2P). In addition, we found *HMMR* expression to be linked with poor pancreatic ductal adenocarcinoma patients prognosis, by use the Kaplan-Meier plotter database to assess the relationship between *HMMR* expression and patient clinicopathological findings. We found that *HMMR* expression correlated significantly with OS, DFS and patient gender, stage, grade of pancreatic ductal adenocarcinoma patients (Table 2). We further used the GEPIA2 database to assess how *HMMR* expression relates to patient prognosis, analysing 33 TCGA cancer types and revealing that *HMMR* expression correlated both with OS and DFS in ACC, BLCA, KIRP, LGG, LIHC, LUAD and PAAD (Figure S3 and Figure S4).

We also conducted a analyses by Sangerbox tool to show the correlation between high expression *HMMR* and poor OS for different tumors, as shown in (Figure S5), high *HMMR* expression was associated with poor OS for LUAD, UCEC, BLCA, PAAD, KIRP, LIHC, MESO, KIRC, HNSC, LGG, KICH, ACC and UVM (all $P < 0.05$). The above results clearly demonstrate that *HMMR* expression significantly correlated with poorer outcome in multiple tumour types.

3.3 Genetic alteration analysis of HMMR

We observed the genetic alteration status of *HMMR* in different tumor samples of the TCGA cohorts. As shown in (Figure 3A), the highest alteration frequency of *HMMR* (> 6%) appears for patients with UCEC with “mutation” as the primary type. The “amplification” type of CNA was the primary type in the KIRC and CHOL cases, which show an alteration frequency of > 6% and ~3%, respectively (Figure 3A). The types, sites and case number of the *HMMR* genetic alteration are further presented in (Figure 3B). We found that missense mutation of *HMMR* in the Q52L site was detected in 1 cases of PAAD (Figure 3B), is able to induce a frame shift mutation of the *HMMR* gene. We can observe the Q52L site in the 3D structure of *HMMR* protein (Figure 3C). In addition, we analyzed the correlation between *HMMR* expression and TMB (Tumor mutational burden)/MSI (Microsatellite instability) across all tumors of TCGA. As shown in (Figure S6), we observed a positive correlation for GBM ($P = 1.7e-06$), LUAD ($P = 0.00092$), PRAD ($P = 3.4e-29$), UCEC ($P = 0.0074$), COAD ($P = 0.013$), STAD ($P = 2e-04$), SKCM ($P = 4.5e-07$), KIRC ($P = 0.003$), HNSC ($P = 0.042$), LAML ($P = 0.029$), KICH ($P = 4.3e-08$), and ACC ($P = 0.0039$). *HMMR* expression is

negatively correlated with MSI of DLBC ($P = 4.4e-05$) but is positively correlated with that of GBM ($P = 5.9e-05$), PRAD ($P = 0.032$), UCEC ($P = 8.8e-05$), SARC (Sarcoma) ($P = 1e-07$), COAD ($P = 0.00091$), and STAD ($P = 2.2e-05$) (Figure S7). This result deserves more in-depth research.

3.4 Gene function analysis

We select human pancreatic ductal adenocarcinoma (PDAC) cell lines for functional analysis of HMMR. The cell lines HPDE6-C7, PANC-1, Capan-2, SW1990 and BxPC-3 were cultured in RPMI 1640 medium containing 10% fetal bovine serum, which was placed in incubator at 37 °C in a humidified atmosphere with 5% CO₂. After 2 to 3 days of cell passage, the cells in the logarithmic growth phase were selected for further experiment.

To study expression of *HMMR* in PDAC cells, real-time polymerase chain reaction (PCR) and western blotting were performed. Compared with HPDE6-C7 cells, which are immortalized human normal pancreatic ductal epithelium cells, HMMR messenger RNA (mRNA) and protein were highly expressed in PDAC cells (Figure 4A and Figure 4B). Notably, *HMMR* expression in high-metastasis potential cell lines, such as Capan-2 (Figure 4A and Figure 4B).

To understand the function of HMMR in PDAC cells, we manipulated *HMMR* expression in Capan-2 by short hairpin RNA (shRNA) knockdown. Three shRNA (shRNA1, shRNA2, and shRNA3) were designed to silence *HMMR* expression in Capan-2 cells named as Capan-2-shHMMR subsequently. Expression level of HMMR was identified by real-time PCR and western blotting; shRNA2 was the most effective one and was chosen for further study (Figure S8A, B). Compared to Capan-2, Capan-2-shHMMR cells had a lower absorbance in methyl thiazol tetrazolium assay, which indicated a lower proliferation rate (Figure S8C). Consistently, Capan-2 cells also formed more colonies compared to Capan-2-shHMMR in colony formation assay (Figure 4C).

The wound-healing and transwell assays were used to investigate migration and invasion capacity. Results showed that Capan-2-control cells had a faster wound closure rate and more invasion cells than Capan-2-shHMMR cells (Figure 4D, 4E). It suggests that HMMR promotes PDAC cells proliferation, migration, and invasion capacity in vitro.

3.5 Enrichment analysis of HMMR-related partners

To further investigate the molecular mechanism of the *HMMR* gene in tumorigenesis and development, we attempted to screen out the targeting HMMR-binding proteins and the *HMMR* expression-correlated genes for a series of pathway enrichment analyses. Based on the STRING tool, we obtained a total of 29 HMMR-binding proteins, which were supported by experimental evidence. Figure 5A shows the interaction network of these proteins. We used the GEPIA2 tool to combine all tumor expression data of TCGA and obtained the top 100 genes that correlated with *HMMR* expression. As shown in Figure 5B, the *HMMR* expression level was positively correlated with that of KIF11 (Kinesin Family Member 11) ($R = 0.77$), BUB1 (Budding Uninhibited By Benzimidazoles 1) ($R = 0.77$), CCNA2 (Cyclin A2) ($R = 0.77$), AURKA (Aurora

Kinase A) ($R = 0.70$), BRCA1 (Breast Cancer 1) ($R = 0.63$), and TPX2 (Targeting Protein For Xklp2) ($R = 0.73$) genes (all $P < 0.001$). The corresponding heatmap data also showed a positive correlation between *HMMR* and the above six genes in the majority of detailed cancer types (Figure 5C). An intersection analysis of the above two groups showed three common member, namely, AURKA, BRCA1 and TPX2 (Figure 5D).

We combined the two datasets to perform KEGG and GO enrichment analyses. The KEGG data of Figure 6A suggest that "Oocyte meiosis", "Cell cycle", "Progesterone-mediated oocyte maturation", "FoxO signaling pathway" and "p53 signaling pathway" might be involved in the effect of *HMMR* on tumor pathogenesis. The GO enrichment analysis data further indicated that most of these genes are linked to the pathways or cellular biology of microtubule motor activity, such as microtubule binding, microtubule cytoskeleton organization, and protein kinase regulator activity, such as protein serine/threonine kinase activator activity, protein serine/threonine kinase activity and cyclin-dependent protein kinase holoenzyme complex, and others. (Figure 6B and Figure S9).

4 Discussion

HMMR is a receptor for hyaluronic acid (HA), which is ubiquitous component of the extracellular matrix (ECM) and is highly expressed in the tumor microenvironment[33-35]. When hyaluronan binds to *HMMR*, the phosphorylation of a number of proteins, including PTK2/FAK1 occurs. *HMMR* playing broad regulatory roles and controlling key physiological processes including cell motility, transformation and metastasis formation, via regulatory extracellular-regulated kinase (ERK) activity, *HMMR* also be involved in signal transduction via Src tyrosine kinase family, expressed in the cytoplasm, nucleus and cell surface, five isoforms (58, 60, 64, 70, 84kDa), regulating cell motility and cell cycle. Many study have shown that *HMMR* can be used as a prognostic marker in some cancer patients, such as hepatocellular carcinoma[36, 37], head and neck squamous cell carcinomas[38], colorectal cancer[38], esophageal adenocarcinoma[40], lung adenocarcinoma[41], renal cell carcinoma[42] etc. However, no studies have investigated *HMMR* as a prognostic marker in pancreatic cancer, so this study to investigate the the relationship between *HMMR* and different tumors, the functions in PDAC cells and the feasibility of *HMMR* as a biomarker for survival and prognosis of pancreatic cancer patients.

In this report, we assessed the expression of *HMMR* in 33 different types of cancers using the independent Oncomine, TIMER2 and GEPIA databases, revealing clear differences between tumour and normal tissue expression of *HMMR* in many cancers. Oncomine data revealed elevated *HMMR* levels in bladder, brain, breast, cervical, rectal, colorectal, oesophageal, gastric, head and neck, renal, liver, lung, lymphoma, ovarian, pancreatic, sarcoma and prostate cancers. We also found that relative to normal tissue controls, *HMMR* expression was lower in leukemia and other cancer tissues (Figure 1A). TCGA dataset analysis indicated that there was elevated *HMMR* expression in BLCA, BRCA, CESC, CHOL, COAD, ESCA, GBM, HNSC, KIRC, KIRP, LIHC, LUAD, LUSC, PRAD, READ, STAD, THCA and UCEC relative to adjacent controls (Figure 1B). GEPIA dataset analysis showed that elevated *HMMR* levels in ACC, DLBC, OV and PAAD patients (Figure 1C). Altered *HMMR* expression in a range of different cancers may be due to the

different means of data collection in different studies, or it may relate to differences in the underlying biological mechanisms. Across GAPIE and Kaplan-Meier plotter databases, we consistently observed a correlation between elevated HMMR expression and a poor PAAD prognosis (Figure S3J, Figure S4J and Table 2). These results together thus suggest that HMMR may have value as a PAAD prognostic biomarker.

In this study, we first presented evidence of the potential correlation between *HMMR* expression and MSI or TMB across all TCGA tumors. Furthermore, we integrated the information on HMMR-binding components and *HMMR* expression-related genes across all tumors for a series of enrichment analyses and identified the potential impact in the etiology or pathogenesis of cancers.

An additional key finding in this study is that the expression of *HMMR* correlated with the proliferation, invasion and migration of pancreatic cancer cells. We found that HMMR expression was increase remarkably in Capan-2 compare with HPDE6-C7 cells (Figure 4A and Figure 4B). We further found compared to Capan-2, Capan-2-shHMMR cells had a lower absorbance in methyl thiazol tetrazolium assay and lesser colonies (Figure S8C and Figure 4C). The wound-healing and transwell assays results showed that Capan-2-control cells had a faster wound closure rate and more invasion cells than Capan-2-shHMMR cells (Figure 4D, 4E). All of these results suggests that HMMR promotes PDAC cells proliferation, migration, and invasion capacity in vitro.

It was reported that HMMR as a homeostasis, mitosis, and meiosis regulator rather than a hyaluronan receptor[43]. Some research showed that HMMR regulates the proliferation of certain stem cell populations and may enable certain hallmarks of cancer[44, 45], and the expression of HMMR is cell cycle-regulated with peak expression between late G2 phase and early mitosis[46]. HMMR is a largely coiled-coil protein that can bind to microtubules directly through its N-terminus[47] and localize to the centrosome through a C-terminal bZip motif[48], which is structurally very similar to the C-terminal bZip motif in Xklp2 that enables an interaction with TPX2[48, 49]. The similarity in these domain structures seeded the hypothesis that HMMR also interacts with TPX2, which has been confirmed experimentally through the study of HMMR/RHAMM in human mitotic cells[48, 50, 51]. The interaction between HMMR and TPX2 is important for Ran-dependent microtubule assembly near chromosomes[52, 53]. HMMR was predicted to be regulated by proliferation associated transcription factors, FOXM1, E2F4, and MYC[52, 53] and, experimentally, by YAP-TEAD of the Hippo pathway[55]. It also be reported that HMMR expression is transcriptionally downregulated by the tumor suppressor TP53[56]. In this study, we analyzed the biological functions and signaling pathways of HMMR by KEGG analysis and GO enrichment, and the results were consistent with those reported by other scholars (Figure 6 and Figure S9).

Conclusion

In this study, we first pan-cancer analyses of *HMMR* indicated statistical correlations of *HMMR* expression with clinical prognosis, tumor mutational burden or microsatellite instability across multiple

tumors. HMMR may be an important oncogene regulator of cells proliferation, invasion and migration and a valuable prognostic biomarker in pancreatic cancer patients.

Declarations

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Authors' Contributions

NT and GDL conceived the project and wrote the manuscript. XLD, XY and YXZ participated in data analysis. XLD and XY participated in discussion and language editing. NT and GDL reviewed the manuscript.

Ethics declaration

Ethics approval and consent to participate

Not applicable

Patient consent for publication

Identifying information, including names, initials, data of birth or hospital numbers, images or statements are not included in the manuscript.

Publication of clinical datasets

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Tables

Table 1 Relation between HMMR expression and patient prognosis of different cancer (Overall Survival) in Prognoscan database.

Cancer type	Dataset	<i>P</i> -value	Hazard ratio (95% CI)
Bladder cancer	GSE5287(N=30)	0.0496	1.75 (1.00 - 3.07)
	GSE13507(N=165)	0.0169	1.32 (1.05 - 1.65)
Blood cancer	GSE4475(N=158)	0.0073	0.56 (0.37 - 0.86)
	GSE12417(N=79)	0.9010	0.98 (0.77 - 1.25)
	GSE16131(N=180)	0.5453	0.90 (0.63- 1.27)
Brain cancer	GSE4271(N=77)	0.0001	1.56 (1.24 - 1.95)
	GSE7696(N=70)	0.9569	0.99 (0.64 - 1.52)
	GSE4412(N=74)	0.0310	1.31 (1.03 - 1.68)
	GSE16581(N=67)	0.7507	1.25 (0.32 - 4.83)
Breast cancer	GSE3143(N=158)	0.1585	1.39 (0.88 - 2.20)
	GSE8993(N=155)	0.0465	0.73 (0.53 - 0.99)
	GSE1456(N=159)	0.0026	2.80 (1.37 - 2.96)
	GSE7390(N=198)	0.0360	1.36 (1.02 - 1.82)
Colorectal cancer	GSE12945(N=62)	0.2821	1.56 (0.69 - 3.52)
	GSE17536(N=177)	0.4609	0.85 (0.55 - 1.31)
	GSE17537(N=55)	0.5496	0.77 (0.32 - 1.84)
Esophagus cancer	GSE11595(N=34)	0.9670	0.98 (0.45 - 2.13)
Lung cancer	GSE13213(N=117)	0.0008	1.64 (1.23 - 2.18)
	GSE31210(N=204)	0.0013	2.14 (1.35 - 3.39)

	GSE3141(N=111)	0.5969	1.10 (0.78 - 1.54)
	GSE4573(N=129)	0.1673	1.47 (0.85 - 2.55)
	GSE17710(N=56)	0.2067	1.35 (0.85 - 2.14)
Ovarian cancer	GSE9891(N=278)	0.2727	1.10 (0.93 - 1.29)
	GSE8841(N=81)	0.0078	2.60 (1.29 - 5.27)
	GSE26712(N=185)	0.2331	1.19 (0.92 - 1.55)
	GSE17260(N=110)	0.0594	1.37 (0.99 - 1.89)
	GSE14764(N=80)	0.5266	1.19 (0.69 - 2.07)
Prostate cancer	GSE16560(N=281)	0.1522	1.11 (0.96 - 1.27)
Skin cancer	GSE19234(N=38)	0.0070	4.35 (1.49 - 12.64)

Table 2 Kaplan-Meier plotter to determine the effect of different clinicopathological factors on the expression of *HMMR* gene and clinical prognosis in PAAD.

Clinicopathological characteristics	OS (n = 177)			RFS (n = 69)		
	N	Hazard ratio	P-value	N	Hazard ratio	P-value
All	177	2.31(1.51-3.55)	8e-05	69	3.62(1.4-9.41)	0.0048
Gender						
Female	80	2.08(1.13-3.84)	0.016	32	2.75(0.94-8.05)	0.055
Male	97	2.45(1.34-4.47)	0.0025	37	7.03(1.3-37.9)	0.0096
Stage						
1	21	8.22(0.96-66.86)	0.022	12		
2	146	1.97(1.25-3.1)	0.0028	55	2.45(0.82-7.3)	0.097
Grade						
1	31	1.1e+9(0-Inf)	0.0079	13		
2	94	1.76(0.97-3.16)	0.058	38	9.41(1.21-72.95)	0.0087
3	48	2.57(1.22-5.4)	0.01	16		

Figures

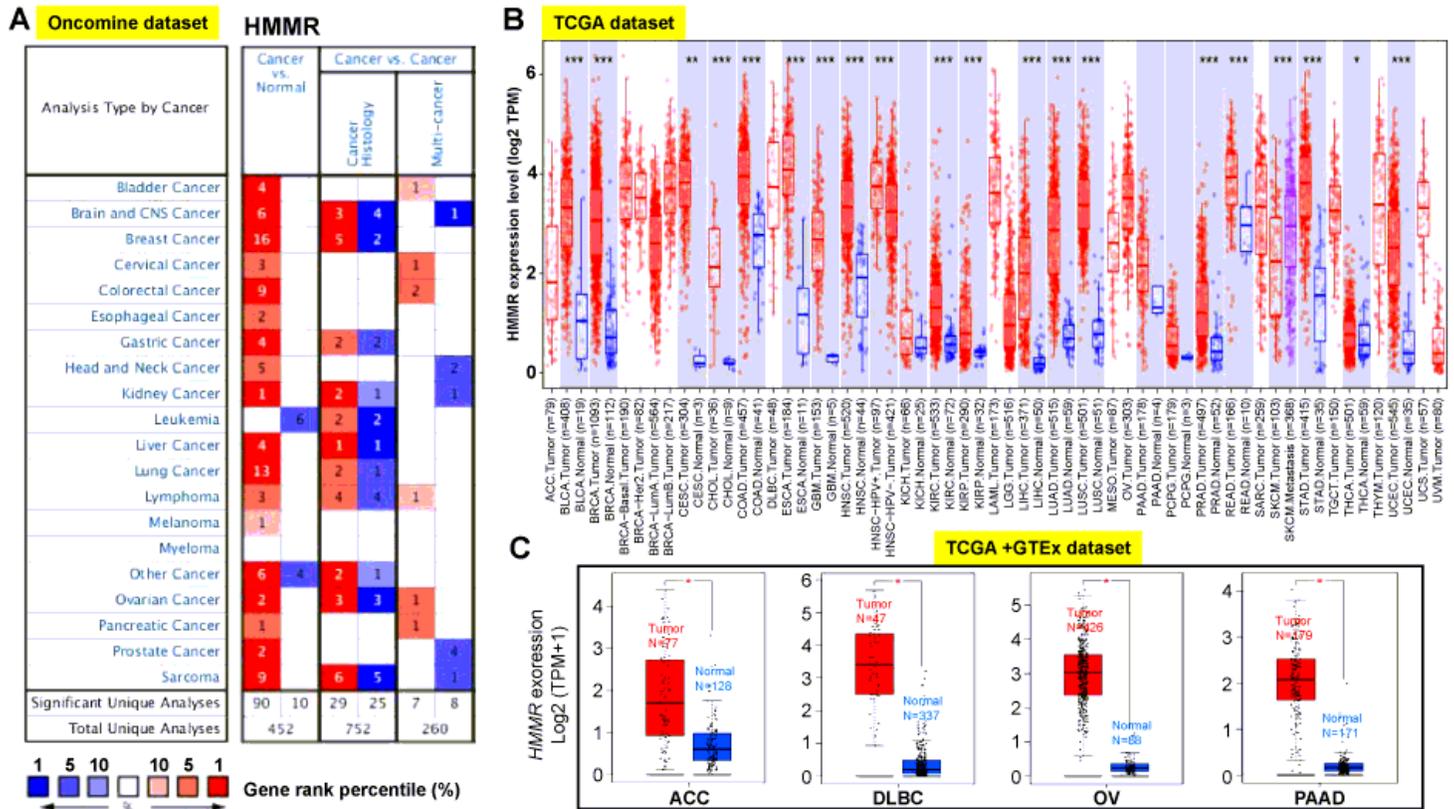


Figure 1

Expression level of HMMR gene in different types of tumor tissues and normal tissues. The expression level of HMMR in different types of tumor tissues and normal tissues in the Oncomine database (A). The expression status of the HMMR gene in different types of tumor tissues and normal tissues in TIMER2 database. (* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$) (B). For the type of ACC, DLBC, OV, and PAAD in the TCGA project, the corresponding normal tissues of the GTEx database were included as controls. The box plot data were supplied. * $P < 0.05$ (C).

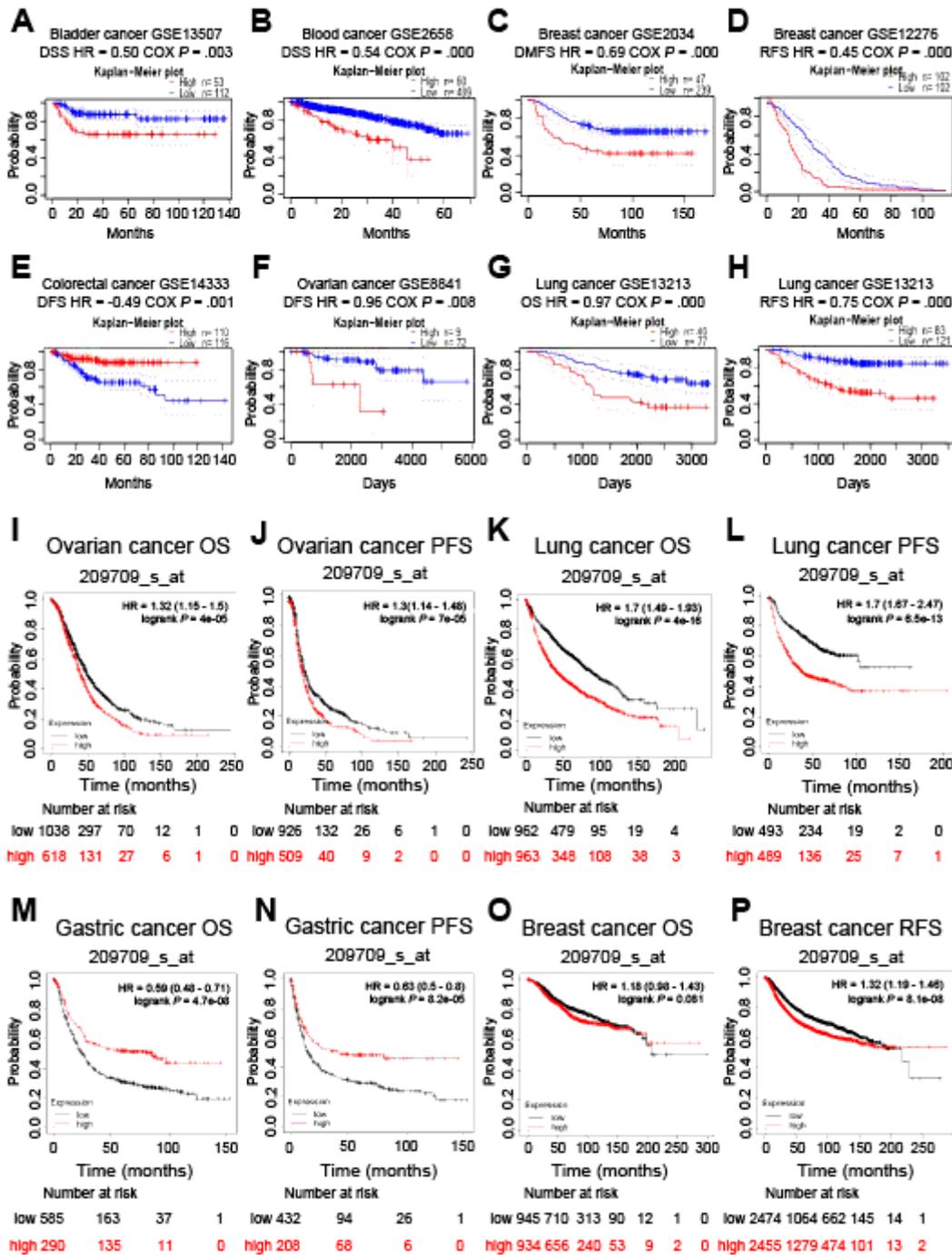


Figure 2

Correlation between HMMR expression and survival prognosis of various types of cancers. Correlation between HMMR and prognosis of various types of cancer in the Prognoscan (A–H) Correlation between HMMR and prognosis of various types of cancer in the Kaplan-Meier plotter database (I–P). OS, overall survival; PFS, Kaplan-Meier plotter database; RFS, recurrence-free survival.

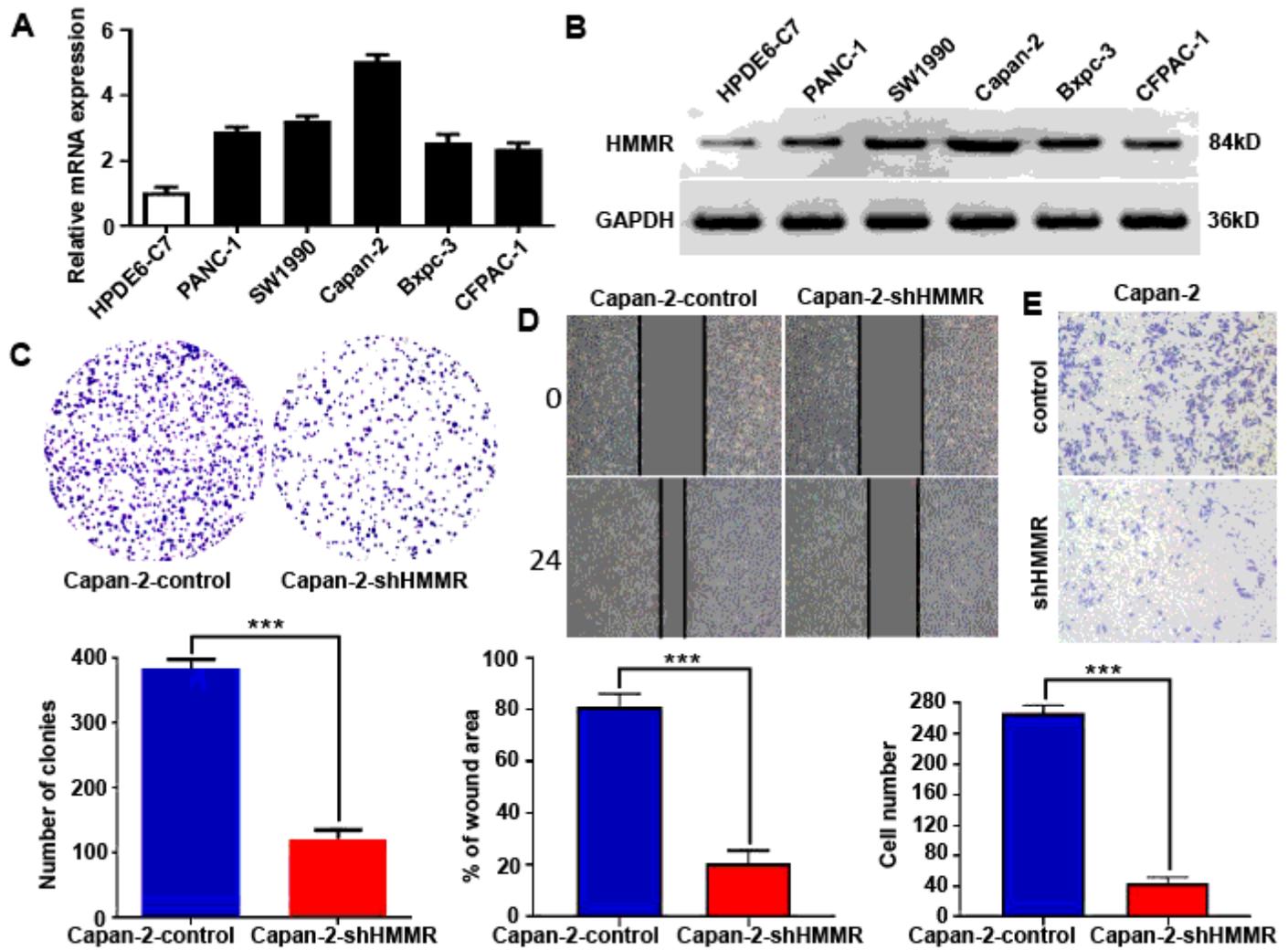
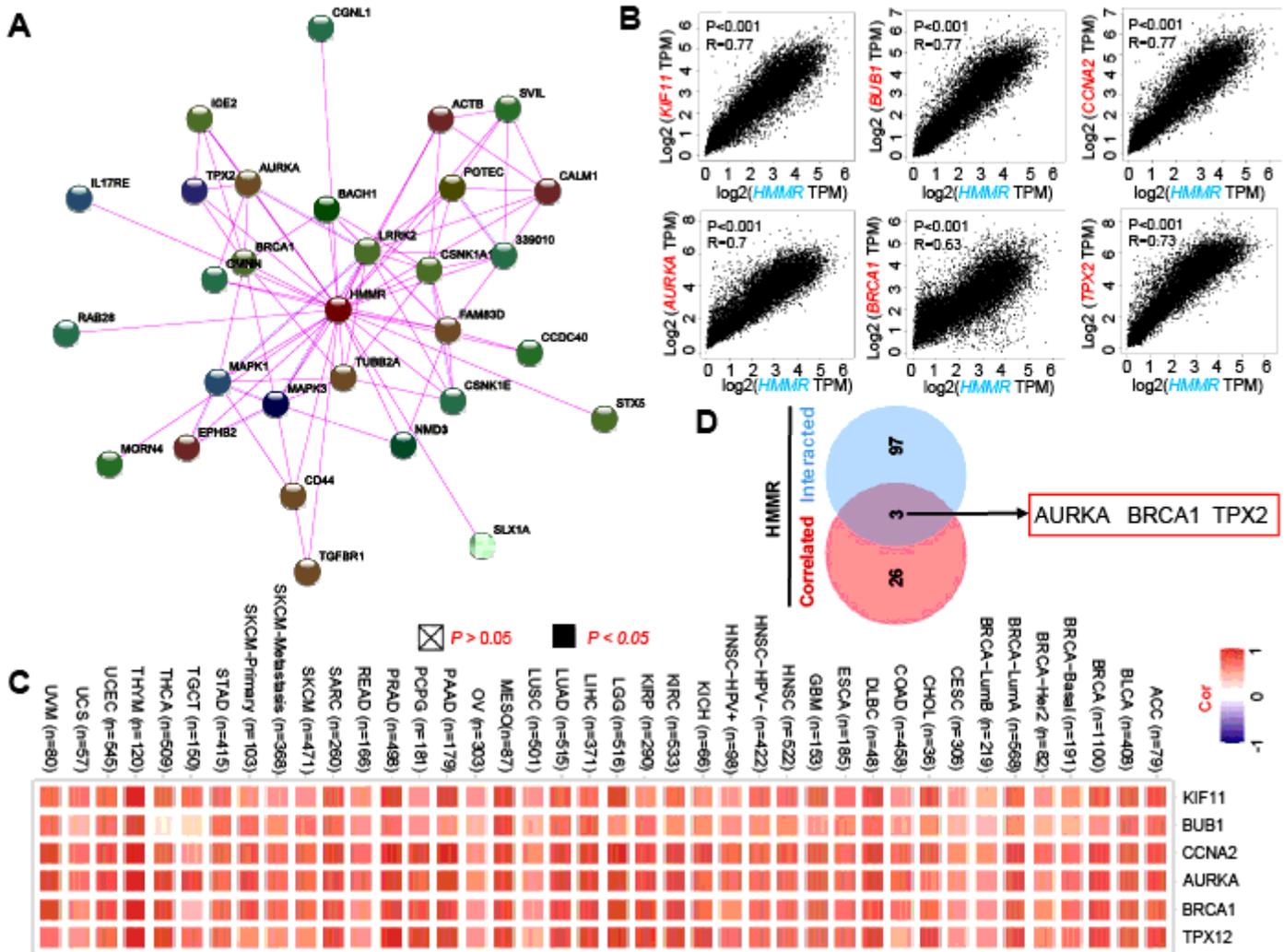


Figure 4

HMMR expression is up-regulated in PDAC cells and promotes PDAC cells proliferation and invasion. HMMR expression is up-regulated in human PDAC cell lines analyzed by real-time PCR (A) and western blotting (B). Proliferation of Capan-2-shHMMR cells and control cells was examined by colony formation assays (C). Wound-healing assay were subjected to detect the migration capacity of HMMR-interfered cells (D). Transwell invasion assay were subjected to detect the migration and invasion capacity of HMMR-interfered cells (E). * $P < 0.05$; ** $P < 0.01$ and *** $P < 0.001$ based on the Student t test. Error bars, standard deviation.



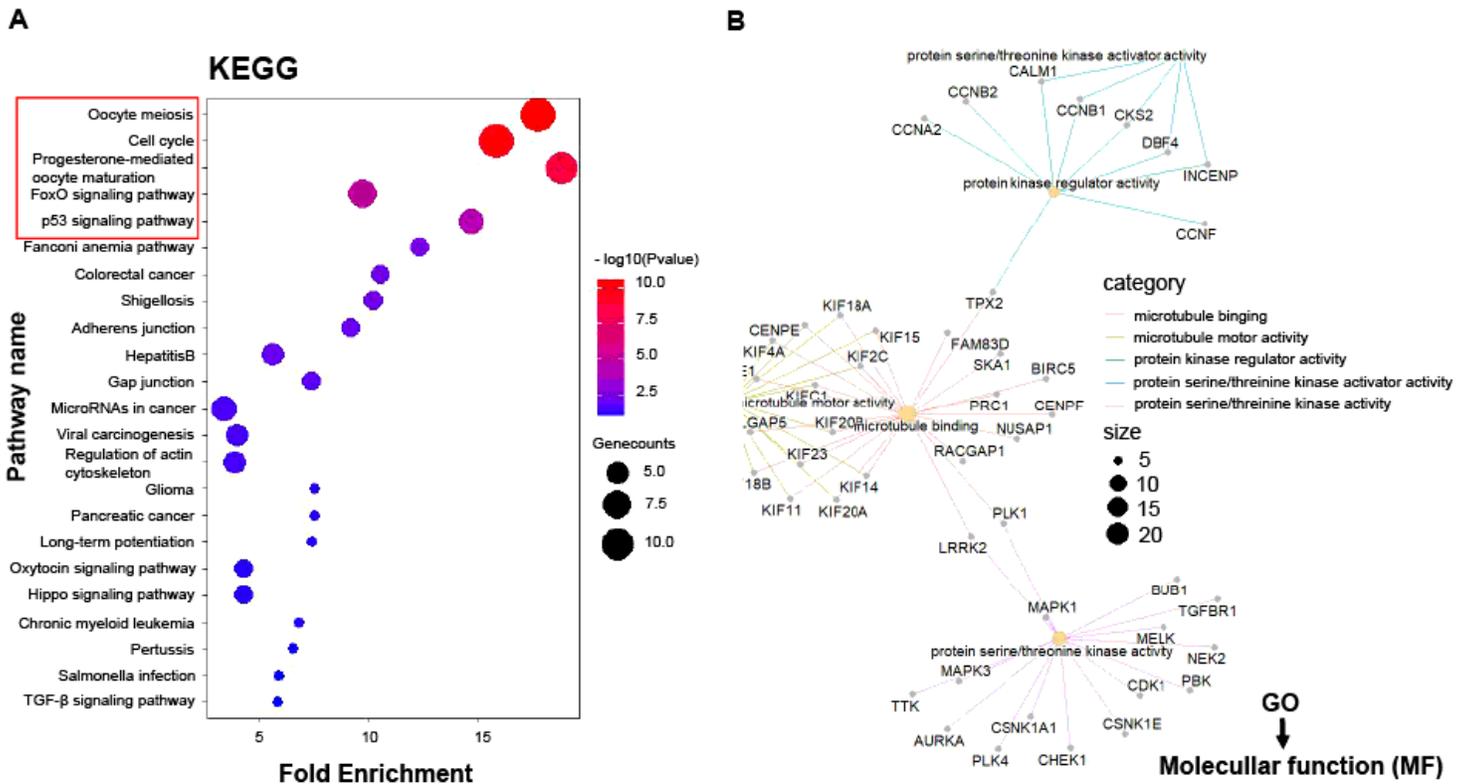


Figure 6

HMMR-related gene enrichment analysis. Based on the HMMR-binding and interacted genes, KEGG pathway analysis was performed (A). The cnetplot for the molecular function data in GO analysis is also shown (B).

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