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# Hyaluronan-mediated motility receptor expression functions as a prognostic biomarker in uterine carcinosarcoma based on bioinformatics analysis

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**Abstract**

**Objective:** Uterine carcinosarcoma (UCS) is a rare, aggressive tumour with a high metastasis rate and poor prognosis. This study aimed to explore potential key genes associated with the prognosis of UCS.

**Methods:** Transcriptional expression data were downloaded from the Gene Expression Profiling Interactive Analysis database and differentially expressed genes (DEGs) were subjected to Gene Ontology and Kyoto Encyclopedia of Genes and Genomes analyses using Metascape. A protein–protein interaction network was constructed using the STRING website and Cytoscape software, and the top 30 genes obtained through the Maximal Clique Centrality algorithm were selected as hub genes. These hub genes were validated by clinicopathological and sequencing data for 56 patients with UCS from The Cancer Genome Atlas database.

**Results:** A total of 1894 DEGs were identified, and the top 30 genes were considered as hub genes. Hyaluronan-mediated motility receptor (HMMR) expression was significantly higher in UCS tissues compared with normal tissues, and elevated expression of HMMR was identified as an independent prognostic factor for shorter survival in patients with UCS.

**Conclusions:** These results suggest that HMMR may be a potential biomarker for predicting the prognosis of patients with UCS.

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Hyaluronan-mediated motility receptor, receptor for hyaluronan-mediated motility, uterine carcinosarcoma, bioinformatics analysis, prognosis, gene expression

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## Introduction

Uterine carcinosarcoma (UCS), also known as malignant mixed Müllerian tumour, is an extremely rare and aggressive tumour[1] composed of carcinomatous and sarcomatous components.[2] UCS accounts for less than 5% of all uterine malignancies but more than 16% of uterine cancer-related deaths.[3] Although most cases can be treated by surgery, with improved survival,[4,5] the 5-year survival is still very poor, ranging from 33% to 39%.[6] Even if the tumour is confined to the corpus, the recurrence rate remains very high.[7] However, the complex composition and low incidence of UCS mean that relevant studies are limited, and further research into the pathogenesis of UCS and the identification of novel biomarkers to improve the prognostic prediction of UCS are needed.

Hyaluronan-mediated motility receptor (HMMR, also known as receptor for hyaluronan-mediated motility, RHAMM) is one of the few defined receptors for hyaluronan, and is also an oncogene that can enhance tumour invasion and progression.[8] HMMR on the cell surface binds to CD44 and hyaluronan to activate downstream pathways and molecules, resulting in invasion and migration in many types of cancers.[9–11] Intracellular HMMR is an actin- and microtubule-associated protein that can maintain spindle integrity,[8] and increased or decreased HMMR expression disrupts microtubule-based processes during cell division, resulting in mitotic spindle abnormalities and genome instability.[12] Increased HMMR expression was found to be associated with cancer progression and poor prognosis in a variety of tumour types.[13,14]

Recent developments in high-throughput sequencing technology have made large amounts of clinical, pathological, and biological data for tumour patients available in public databases. However, to the best of our knowledge, bioinformatics analysis has not yet been used to explore possible biomarkers in UCS. In our study, we explored genes related to the prognosis of UCS and extended our understanding of UCS based on a comprehensive analysis of large databases.

## Materials and methods

### *Ethical approval*

All data analysed in this study were retrieved from online databases, which stated that appropriate written informed consent had already been obtained. The Medical Ethics Committee of Harbin Medical University Cancer Hospital thus deemed the current study exempt from ethics approval.

### *Gene Expression Profiling Interactive Analysis (GEPIA)*

GEPIA (http://gepia.cancer-pku.cn/index.html) is a web server for estimating mRNA expression based on 9736 tumours and 8587 normal samples in The Cancer Genome Atlas (TCGA) database and

Genotype-Tissue Expression dataset projects.[15] In our study, transcriptional expression data for UCS and paired normal samples were obtained from the GEPIA database (TCGA and Genotype-Tissue Expression dataset projects). Differences in transcriptional expression between the samples was compared by analysis of variance. A P-value $<0.01$ and |log-fold change| $>2$ were selected as the thresholds for differentially expressed gene (DEG) screening.

### Metascape

We further explored the biological significance of the DEGs by analysing the enriched functions and pathways of the DEGs using Metascape (http://metascape.org).[16] We used 'custom analysis' in Metascape to explore Gene Ontology (GO) terms, including the biological process (BP), cellular component (CC), and molecular function (MF) categories, and also identified enriched Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways, based on Metascape. Significant enrichment was indicated by a minimum overlap of 3, P-value cut-off of 0.01, and minimum enrichment of 1.5. The most significant term within a cluster was chosen to represent the cluster.

### Search Tool for the Retrieval of Interacting Genes (STRING)

STRING (http://string-db.org/) is a biological database of known and predicted protein–protein interactions (PPIs), including direct (physical) and indirect (functional) associations.[17] Analysing the interactions between proteins may provide insights into the mechanisms responsible for the generation and development of diseases. We used STRING to assess potential PPI relationships and explore the interactions between DEGs to construct PPI networks for upregulated and downregulated DEGs. A confidence score $>0.7$ was set as significant, and disconnected nodes in the network were removed.

### Hub module identification and functional analysis

Cytoscape (www.cytoscape.org/) is a software platform for visualising molecular interaction networks.[18] The Cytoscape plug-in, Molecular Complex Detection (MCODE), was applied to identify the hub modules of the PPI network[19] using the following parameters: degree cut-off of 2, node score cut-off of 0.2, k-core of 2, and maximum depth of 100. The top four significant clusters were selected, and genes in the selected clusters were analysed for functional enrichment using Metascape. A subset of enriched terms was selected and rendered as a network plot to further determine the relationship among terms, where terms with a similarity of $>0.3$ were connected by edges.

### Screening hub genes

The Cytoscape plug-in cytoHubba was used to calculate the degree of each protein node,[20] given that nodes with a higher degree of connectivity tend to be more essential for maintaining the network stability. In our study, the top 30 genes obtained through the Maximal Clique Centrality (MCC) algorithm were identified as hub genes.

### Construction of a gene–microRNA (miRNA)–transcription factor (TF) regulatory network

The starBase (http://starbase.sysu.edu.cn/index.php) platform is an open-source platform for studying miRNA–non-coding RNA (ncRNA), miRNA–mRNA, ncRNA–RNA, RNA–RNA, RNA-binding protein (RBP)–ncRNA, and RBP–mRNA

interactions from CLIP-Seq, degradome-seq, and RNA–RNA interactome data.[21] We used starBase to predict miRNAs that bound to the identified hub genes based on the standard CLIP data $\geq 3$. We then selected miRNAs with the most intersections in the three databases. TF regulation networks were predicted using the Cytoscape iRegulon plug-in.[22] Gene–miRNA–TF regulatory networks were then visualised using Cytoscape software.

### *Validation of hub genes*

TCGA contains sequencing and pathological data for more than 30 types of human tumours.[23] We downloaded TCGA clinicopathological and sequencing data related to the 30 hub genes in patients with UCS from the cBioPortal website (http://www.cbioportal.org) for survival analysis.[24] We then analysed the correlations between the expression levels of the 30 genes and patient survival using Kaplan–Meier analysis and the log-rank test, to identify hub genes significantly correlated with the prognosis of UCS.

### *Statistical methods*

Expression levels of hub genes significantly correlated with prognosis were defined as high (within the 75% quartile) or low (within the 25% quartile) based on the median value. Relationships between the mRNA expression levels of significant genes and the clinicopathologic characteristics of the patients were analysed by $\chi^2$ tests, and the effects of clinicopathologic characteristics and mRNA expression on patient survival were evaluated by the Kaplan–Meier method and log-rank test. Cox regression analysis (forward logistic regression algorithm) was used to confirm the independent prognostic factors in patients with UCS, and variables with $P < 0.05$ in univariate analyses were included in multivariate analyses. Variables with a $P < 0.05$ in univariate analysis were included in multivariate Cox regression analyses of progression-free survival (PFS), disease-specific survival (DSS), and overall survival (OS). Because the forward LR algorithm was adopted in the multivariate analysis, only variables that were significant among many variables included in the multivariate analysis are displayed in the analysis results. The hazard ratios (HR) and corresponding 95% confidence intervals (CIs) were calculated for the log-rank test and Cox regression analyses. The HR was adjusted for age <70 years, low/medium HMMR expression, no history of menopausal hormone therapy, no hypertension, no diabetes, no pregnancy, minimally invasive surgical approach at diagnosis, use of adjuvant pharmaceutical treatment, use of adjuvant radiation treatment, low clinical stage, and no lymph node metastasis as the reference groups. Statistical analysis was performed using Prism 7 and SPSS (v23; IBM Corp, Armonk, NY, USA), and survival curves were generated using Prism7. $P < 0.05$ was considered statistically significant.

### *Tumour Immune Estimation Resource (TIMER)*

TIMER (https://cistrome.shinyapps.io/timer/) is a comprehensive resource for the systematic analysis of immune infiltrates of different cancer types and their clinical impact.[25] We used the 'Diff Exp module' to explore significant hub gene expression between common cancer types and their normal adjacent tissues. $P < 0.05$ was considered statistically significant.

## Results

### *Identification of DEGs in UCS*

We screened 1894 DEGs, including 579 upregulated genes and 1315 downregulated genes, using cut-off criteria of $P < 0.01$ and $|\text{log-fold change}| \geq 2$.

## Functional enrichment analysis

We performed enrichment analysis of the DEGs using Metascape. Figure 1 shows the top 20 most highly enriched GO and KEGG terms associated with the upregulated and downregulated DEGs. The upregulated DEGs were mainly enriched in BPs, including cell division, positive regulation of the cell cycle, attachment of spindle microtubules to kinetochore, DNA conformation change, metaphase plate congression, epithelial cell differentiation, regulation of cyclin-dependent protein serine/threonine kinase activity, and DNA replication. MF analysis showed that the DEGs were significantly enriched in kinase binding. In terms of CCs, the DEGs were enriched in spindle, microtubule-organising centre, and extracellular matrix (Figure 1a). KEGG pathway analysis showed that the DEGs were mainly enriched in the cell cycle, p53 signalling pathway, pathways in cancer, and cell adhesion molecules (Figure 1c).

BP analysis showed that the downregulated DEGs were mostly enriched in muscle structure development, blood vessel development, actin filament-based process, regulation of ion transport, extracellular structure organisation, positive regulation of cellular component movement, and cell-matrix adhesion. In the MF category, the downregulated DEGs were highly enriched in glycosaminoglycan binding, and in the CC category, they were enriched in contractile fibres, collagen-containing extracellular matrix, actin cytoskeleton, sarcolemma, stress fibres, and adherens junctions (Figure 1b). KEGG pathway analysis showed that the downregulated DEGs were mainly enriched in focal adhesion, vascular smooth muscle contraction, complement and coagulation cascades, the calcium signalling pathway, and the Ras signalling pathway (Figure 1d).

## PPI network and analysis of clusters

We performed PPI network analyses for the upregulated and downregulated DEGs to clarify the protein interactions among the DEGs. Using the STRING website, 579 upregulated DEGs were included in the

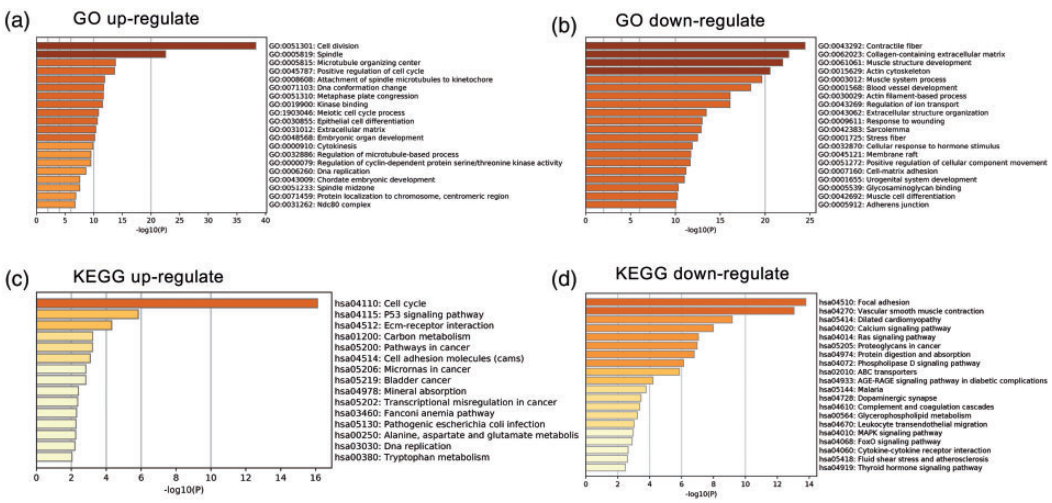


**Figure 1.** Functional enrichment analysis of differentially expressed genes (DEGs), coloured by P-values. (a) Gene Ontology (GO) analysis of upregulated and (b) downregulated DEGs. (c) Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis of upregulated and (d) downregulated DEGs.

PPI network complex, which contained 496 nodes and 4114 edges (Figure 2), while 1315 downregulated DEGs were included in the PPI network complex, containing 1015 nodes and 1476 edges (Figure 3).

We used MCODE to identify the modules in the network. Forty-two clusters were found according to the above criteria, and the top four significant clusters were selected (Figure 4): cluster 1, with the highest score, contained 76 nodes and 2437 edges (Figure 4a); cluster 2 contained 21 nodes and 210 edges (Figure 4d); cluster 3 contained 15 nodes and 105 edges (Figure 4g); and cluster 4 contained 12 nodes and 66 edges (Figure 4j). GO and KEGG analyses were performed independently for each cluster. The DEGs in cluster 1 were mostly enriched in cell division (BP), regulation of chromosome segregation (BP), positive regulation of cell cycle (BP), meiotic cell cycle (BP), kinase binding (MF), chromosomal region (CC), spindle (CC), centrosome (CC), cell cycle, p53 signalling pathway (KEGG), and DNA replication (KEGG) (Figure 4b). The DEGs in cluster 2 were highly enriched in adenylate cyclase-modulating G protein-coupled receptor signalling pathway (BP), cellular calcium ion homeostasis (BP), positive regulation of response to external stimulus (BP), G protein-coupled receptor binding (MF), G protein-coupled peptide receptor activity (MF), chemokine signalling pathway (KEGG), and neuroactive ligand-receptor interaction (KEGG) (Figure 4e). DEGs in cluster 3 were mostly enriched in regulation of growth (BP), negative

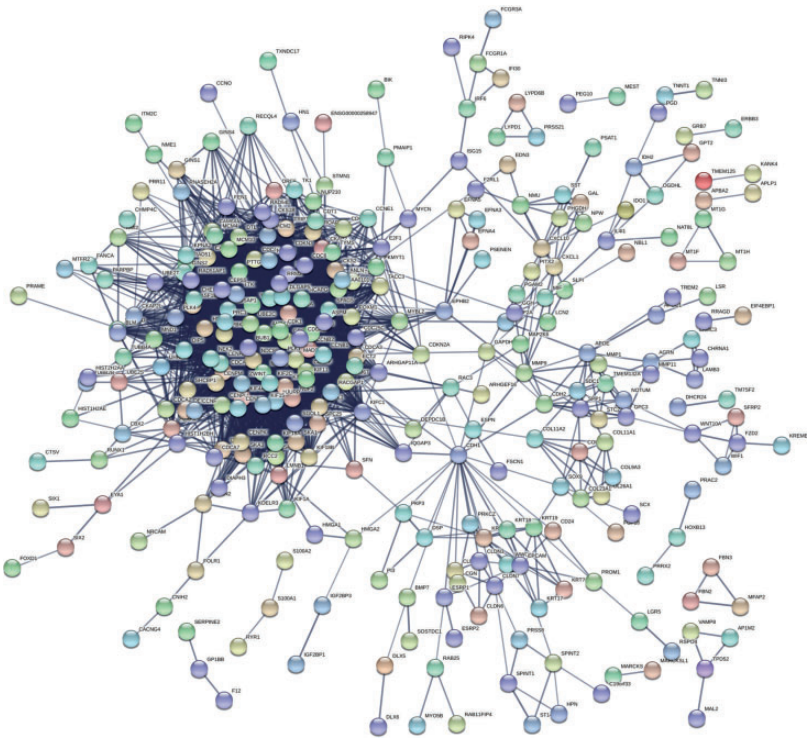

**Figure 2.** Protein–protein interaction network of upregulated genes based on the Search Tool for the Retrieval of Interacting Genes. A total of 579 differentially expressed genes were included in the network complex, which contained 496 nodes and 4,114 edges.

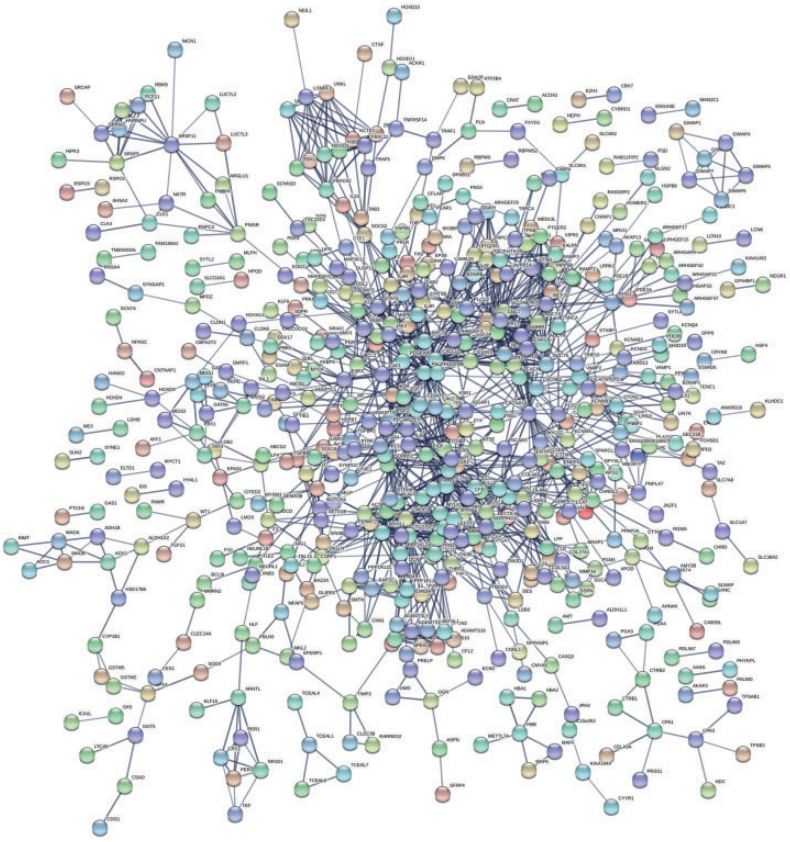

**Figure 3.** Protein–protein interaction network of downregulated genes based on the Search Tool for the Retrieval of Interacting Genes. A total of 1315 differentially expressed genes were included in the network complex, which contained 1015 nodes and 1476 edges.

regulation of canonical Wnt signalling pathway (BP), endoplasmic reticulum lumen (CC), and collagen-containing extracellular matrix (CC) (Figure 4h). The DEGs in cluster 4 were highly enriched in protein polyubiquitination (BP) and ubiquitin ligase complex (CC) (Figure 4k). The genes in clusters 3 and 4 were not enriched in any KEGG pathways. The GO enriched term networks for the four clusters are also shown in Figure 4c, f, i, l, where terms containing more genes tended to have a more significant P-values.

### *Identification of hub genes and construction of a gene–miRNA–TF regulatory network*

The top 30 genes evaluated by the MCC algorithm in cytoHubba were identified as hub genes, and the higher-ranking genes are represented by a redder colour in Figure 5a. A total of 184 miRNAs could bind to the hub genes, as predicted by starBase. Three hub genes, CDC45, CCNB2, and NDC80, did not bind miRNAs. Seventy-two TFs were identified by iRegulon.

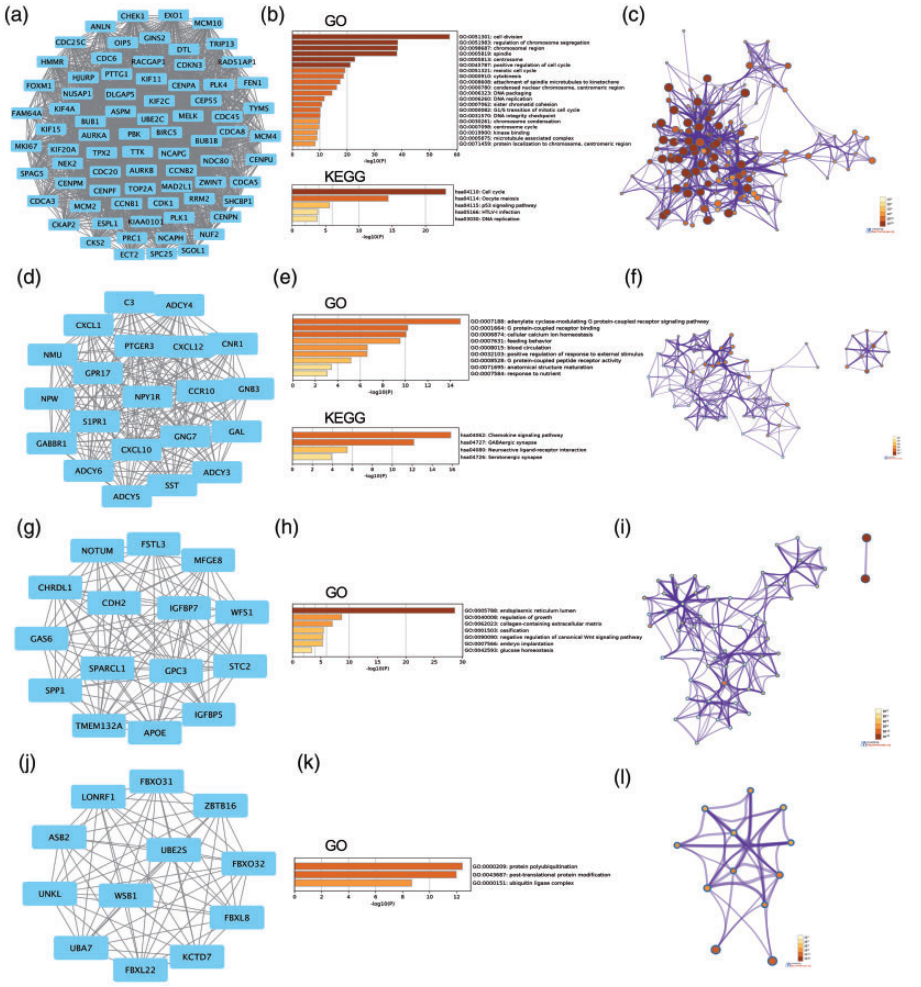


**Figure 4.** Cluster analysis of the protein–protein interaction network. (a) Cluster 1 consisted of 76 nodes and 2437 edges and had the highest score among the clusters; (b) Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) analyses of cluster 1, coloured by P-values; (c) network of GO-enriched terms in cluster 1, coloured by P-value, with terms containing more genes tending to have more significant P-values. (d) Cluster 2 consisted of 21 nodes and 210 edges; (e) GO and KEGG analyses of cluster 2; (f) network of GO-enriched terms in cluster 2. (g) Cluster 3 consisted of 15 nodes and 105 edges; (h) GO analysis of cluster 3; (i) network of GO-enriched terms in cluster 3. (j) Cluster 4 consisted of 12 nodes and 66 edges; (k) GO analysis of cluster 4; (l) network of GO-enriched terms in cluster 4.

A gene–miRNA–TF regulatory network was established (Figure 5b).

## *Hub gene validation*

We downloaded the clinicopathologic and sequencing data related to the 30 hub genes for 57 UCS tissues and 78 normal tissues from the cBioPortal website for survival analysis. One patient was excluded because of a lack of follow-up data, and the clinicopathological data for 56 patients were included in our analysis. HMMR mRNA expression was significantly

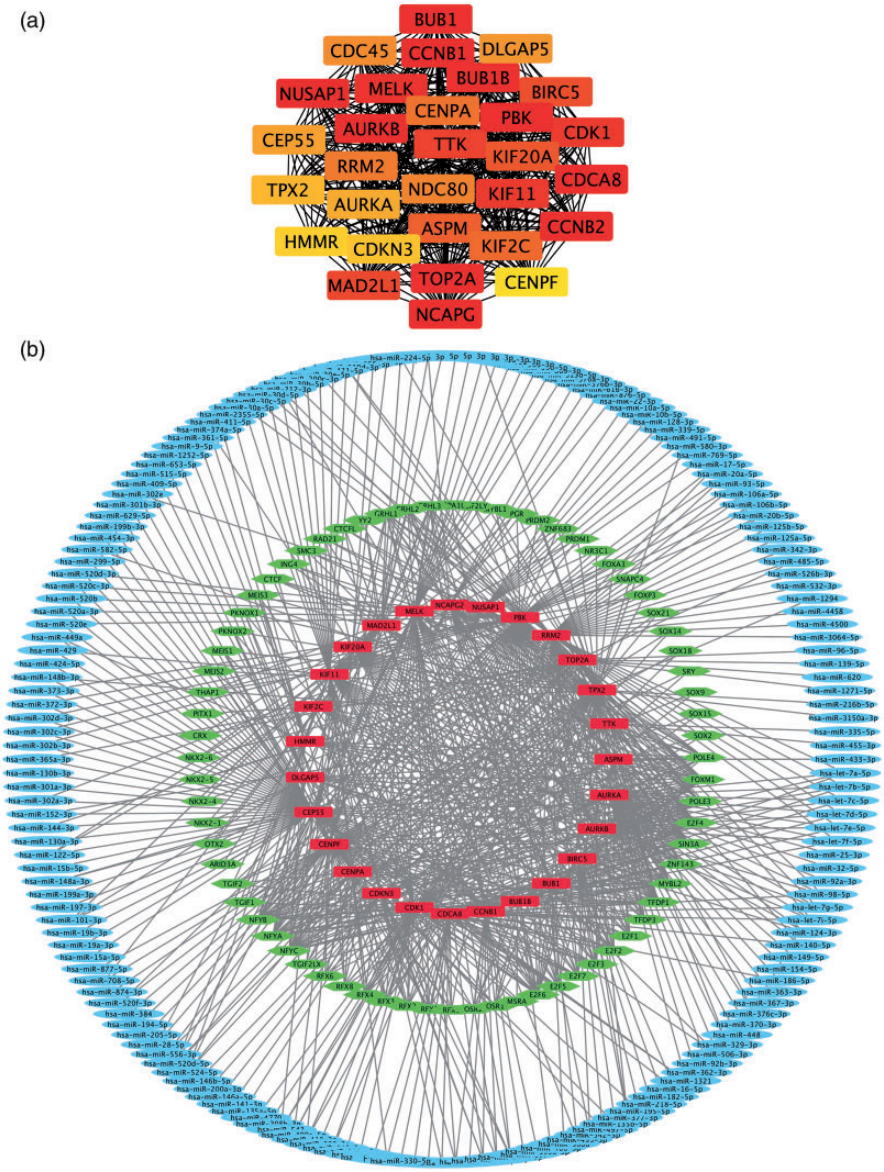


**Figure 5.** The top 30 hub genes and transcriptional regulatory network of hub genes. (a) The top 30 hub genes identified by cytoHubba. Genes with higher ranks represented by redder colour. (b) Transcriptional regulatory network of hub genes, microRNAs (miRNAs), and transcription factors. Hub genes indicated in red, miRNAs in blue, and transcription factors in green.

associated with prognosis of patients with UCS. Patients with higher HMMR expression had significantly shorter PFS, DSS, and OS than those with lower expression (Figure 6a–c). The other 29 hub genes were not significantly correlated with the prognosis of patients with UCS. HMMR expression was higher in UCS samples

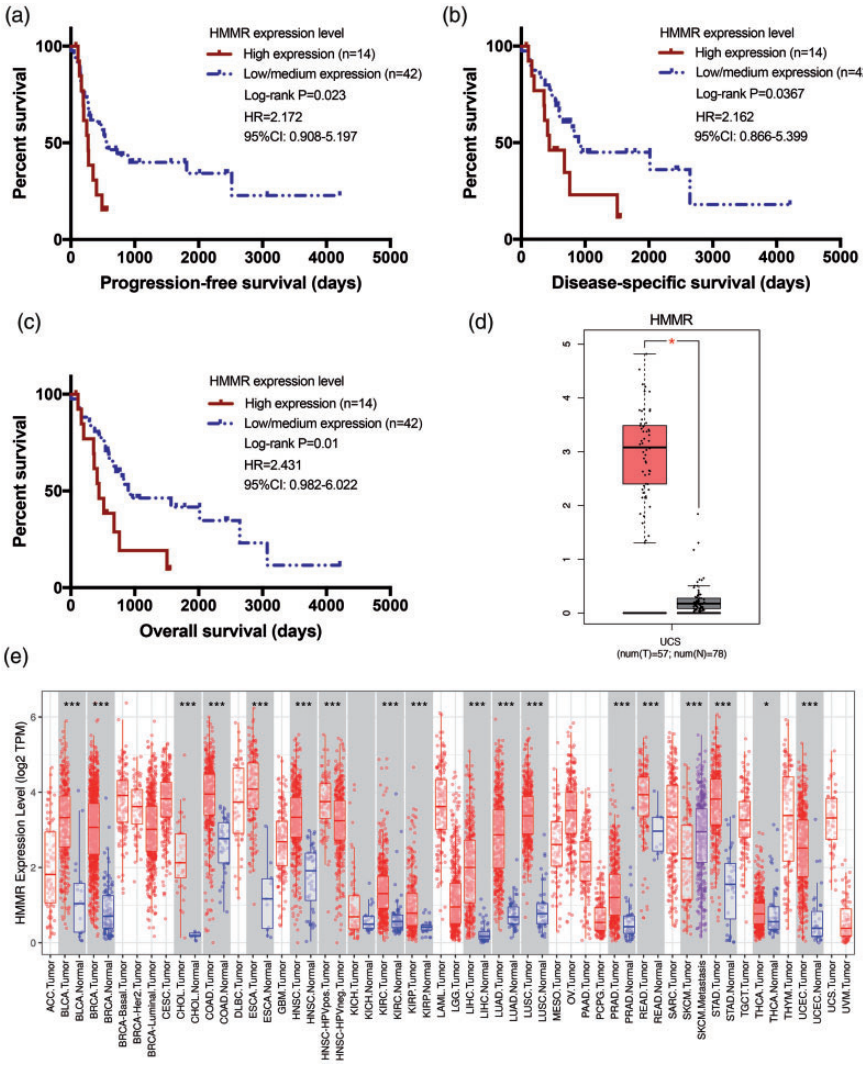


**Figure 6.** Hyaluronan-mediated motility receptor (HMMR) expression and survival analysis. HMMR expression was defined as high (within the 75% quartile) or low (within the 25% quartile), based on the median value. (a) Kaplan–Meier survival analysis showed that higher HMMR mRNA expression was significantly associated with poor progression-free survival ($P = 0.023$), (b) poor disease-specific survival ($P = 0.0367$), and (c) poor overall survival ($P = 0.01$) in patients with uterine carcinosarcoma (UCS). (d) Box plots derived from gene expression data from Gene Expression Profiling Interactive Analysis comparing expression of HMMR in UCS and normal tissues ($P < 0.05$). (e) Differential expression of HMMR in different types of cancers compared with corresponding normal adjacent tissues. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ HR, hazard ratio; CI, confidence interval.

than in normal tissues ($P < 0.05$) (Figure 6d), and was also higher in multiple cancer types than in paired normal tissues (Figure 6e).

## *Independent prognostic value of HMMR mRNA expression in patients with UCS*

There were no significant differences in HMMR expression in terms of age, menopausal status, history of menopausal hormone therapy, hypertension, diabetes, pregnancy, race, surgical approach at diagnosis, adjuvant treatment, clinical stage, or lymph node metastasis. The relationships between HMMR expression and the clinicopathological characteristics of the 56 patients with UCS are summarised in Table 1.

Survival differences were evaluated using Kaplan–Meier analysis. High clinical stage, hypertension, high HMMR mRNA expression, no adjuvant pharmaceutical treatment, and no adjuvant radiation treatment were significantly related to shorter PFS in patients with UCS ($P < 0.05$). High clinical stage, high HMMR mRNA expression, no adjuvant pharmaceutical treatment, and no adjuvant radiation treatment were significantly related to shorter DSS and OS in patients with UCS ($P < 0.05$) (Table 2).

Multivariate Cox regression analysis of PFS including variables with $P < 0.05$ in univariate analyses, including HMMR expression, hypertension, adjuvant pharmaceutical treatment, adjuvant radiation treatment, and clinical stage, identified high clinical stage as independently associated with significantly shorter PFS in patients with UCS (Table 3).

HMMR expression, adjuvant pharmaceutical treatment, adjuvant radiation

**Table 1.** Clinicopathologic characteristics of 56 patients with uterine carcinosarcoma.

| | | HMMR expression | | | |
|---|---|---|---|---|---|
| Variable | Total | High (n,%) | Medium/low (n,%) | $\chi^2$ | P-value |
| Number | 56 | 14 | 42 | | |
| Age, years | | | | 0 | 1 |
| >70 | 24 | 6 (42.9%) | 18 (42.9%) | | |
| <70 | 32 | 8 (57.1%) | 24 (57.1%) | | |
| Menopausal status | | | | 2.911 | 0.088 |
| Post-menopause | 53 | 13 (92.9%) | 40 (95.2%) | | |
| Pre-menopause | 1 | 1 (7.1%) | 0 (0.0%) | | |
| Unknown | 2 | | | | |
| Menopausal hormone therapy | | | | 0.05 | 0.823 |
| Yes | 7 | 1 (7.1%) | 6 (14.3%) | | |
| No | 28 | 5 (35.7%) | 23 (54.8%) | | |
| Unknown | 21 | | | | |
| Hypertension | | | | 0.506 | 0.477 |
| Yes | 28 | 5 (35.7%) | 23 (54.8%) | | |
| No | 23 | 6 (42.9%) | 17 (40.5%) | | |
| Unknown | 5 | | | | |
| Diabetes | | | | 3.835 | 0.05 |
| Yes | 6 | 3 (21.4%) | 3 (7.1%) | | |
| No | 44 | 7 (50.0%) | 37 (88.1%) | | |

(continued)

**Table 1.** Continued.

| Variable | Total | HMMR expression | | $\chi^2$ | P-value |
|---|---|---|---|---|---|
| | | High (n,%) | Medium/low (n,%) | | |
| Unknown | 6 | | | | |
| Pregnancy | | | | 1.117 | 0.291 |
| 0 | 4 | 0 (0.0%) | 4 (9.5%) | | |
| >0 | 45 | 10 (71.4%) | 35 (83.3%) | | |
| Unknown | 7 | | | | |
| Race | | | | 0.982 | 0.612 |
| White | 44 | 11 (78.6%) | 33 (78.6%) | | |
| Black or African American | 9 | 3 (21.4%) | 6 (14.3%) | | |
| Asian | 2 | 0 (0.0%) | 2 (4.8%) | | |
| Unknown | 1 | | | | |
| Surgical approach at diagnosis | | | | 2.771 | 0.096 |
| Minimally Invasive | 31 | 5 (35.7%) | 26 (61.9%) | | |
| Open | 19 | 7 (50.0%) | 12 (28.6%) | | |
| Unknown | 6 | | | | |
| Adjuvant pharmaceutical treatment | | | | 1.015 | 0.314 |
| Yes | 36 | 8 (57.1%) | 28 (66.7%) | | |
| No | 17 | 6 (42.9%) | 11 (26.2%) | | |
| Unknown | 3 | | | | |
| Adjuvant radiation treatment | | | | 0.142 | 0.706 |
| Yes | 25 | 6 (42.9%) | 19 (45.2%) | | |
| No | 28 | 8 (57.1%) | 20 (47.6%) | | |
| Unknown | 3 | | | | |
| Clinical stage | | | | 1.663 | 0.645 |
| I | 21 | 4 (28.6%) | 17 (40.5%) | | |
| II | 5 | 1 (7.1%) | 4 (9.5%) | | |
| III | 20 | 7 (50.0%) | 13 (31.0%) | | |
| IV | 10 | 2 (14.3%) | 8 (19.0%) | | |
| Lymph node metastasis | | | | 0.161 | 0.688 |
| Positive | 17 | 4 (28.6%) | 13 (31.0%) | | |
| Negative | 27 | 5 (35.7%) | 22 (52.4%) | | |
| Unknown | 12 | | | | |

HMMR, hyaluronan-mediated motility receptor.

treatment, and clinical stage were included in the multivariate analyses of DSS and OS. No adjuvant pharmaceutical treatment and high clinical stage were independently associated with significantly shorter DSS (Table 4), and high HMMR expression, no adjuvant pharmaceutical treatment, and no adjuvant radiation treatment were independent prognostic factors for shorter OS in patients with UCS (Table 5).

## Discussion

UCS is a rare, aggressive tumour with a high metastasis rate and poor prognosis.[26] Histologically, it is composed of a mixture of malignant epithelial and sarcomatous elements, with the sarcoma component showing different histologic features.[27,28] The low incidence and complex composition of UCS have resulted in limited research; however, recent developments in

**Table 2.** Kaplan–Meier analysis of survival in 56 patients with uterine carcinosarcoma.

| | Progression-free survival | | | | Disease-specific survival | | | | Overall survival | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Variable | Log-rank $\chi^2$ | HR | 95%CI | P-value | Log-rank $\chi^2$ | HR | 95%CI | P-value | Log-rank $\chi^2$ | HR | 95%CI | P-value |
| Age, years (<70/≥70) | 3.013 | 1.753 | 0.908–3.382 | 0.083 | 1.312 | 1.493 | 0.736–3.029 | 0.252 | 1.637 | 1.531 | 0.780–3.004 | 0.201 |
| HMMR expression (low or medium/high) | 5.167 | 2.172 | 0.908–5.197 | 0.023 | 4.365 | 2.162 | 0.866–5.399 | 0.037 | 6.629 | 2.431 | 0.982–6.022 | 0.01 |
| Menopausal hormone therapy (no/yes) | 2.696 | 0.315 | 0.116–0.856 | 0.101 | 2.176 | 0.294 | 0.075–0.827 | 0.14 | 1.358 | 0.4917 | 0.180–1.344 | 0.244 |
| Hypertension (no/yes) | 4.306 | 2.07 | 1.044–4.105 | 0.038 | 2.389 | 1.827 | 0.859–3.888 | 0.122 | 2.536 | 1.768 | 0.874–3.579 | 0.111 |
| Diabetes (no/yes) | 1.425 | 1.767 | 0.542–5.763 | 0.233 | 1.164 | 1.691 | 0.526–5.443 | 0.281 | 2.498 | 2.014 | 0.643–6.307 | 0.114 |
| Pregnancy (0/>=1) | 0.019 | 0.905 | 0.203–4.034 | 0.891 | 0.293 | 0.677 | 0.123–3.738 | 0.588 | 2.148 | 0.426 | 0.076–2.385 | 0.143 |
| Surgical approach at diagnosis (minimally invasive/open) | 0 | 1.001 | 0.493–2.035 | 0.997 | 0.025 | 1.065 | 0.484–2.341 | 0.873 | 0.193 | 1.171 | 0.560–2.450 | 0.661 |
| Adjuvant pharmaceutical treatment (yes/no) | 4.023 | 1.95 | 0.892–4.266 | 0.045 | 5.667 | 2.231 | 0.982–5.069 | 0.017 | 6.397 | 2.295 | 1.037–5.080 | 0.011 |
| Adjuvant radiation treatment (yes/no) | 4.49 | 1.98 | 1.013–3.873 | 0.034 | 4.102 | 2.038 | 0.979–4.242 | 0.043 | 5.872 | 2.234 | 1.121–4.450 | 0.015 |
| Clinical stage (I,II/III,IV) | 4.861 | 2.083 | 1.092–3.973 | 0.027 | 7.022 | 2.72 | 1.344–5.505 | 0.008 | 6.453 | 2.363 | 1.214–4.600 | 0.011 |
| Lymph node metastasis (no/yes) | 1.295 | 1.554 | 0.696–3.469 | 0.255 | 1.724 | 1.732 | 0.727–4.123 | 0.189 | 1.882 | 1.702 | 0.749–3.869 | 0.17 |

HR, hazard ratio; CI, confidence interval; HMMR, hyaluronan-mediated motility receptor.

**Table 3.** Univariate and multivariate Cox regression analyses of progression-free survival in 56 patients with uterine carcinosarcoma.

| | Univariate analysis | | | | | Multivariate analysis | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Variable | B value | SE | Wald | HR (95%CI) | P-value | B value | SE | Wald | HR (95%CI) | P-value |
| Age | 0.568 | 0.332 | 2.932 | 1.765 (0.921–3.382) | 0.087 | | | | | |
| HMMR expression | 0.838 | 0.379 | 4.883 | 2.311 (1.099–4.86) | 0.027 | | | | | NS |
| Menopausal hormone therapy | −1.16 | 0.747 | 2.415 | 0.313 (0.073–1.354) | 0.12 | | | | | |
| Hypertension | 0.742 | 0.365 | 4.124 | 2.099 (1.026–4.295) | 0.042 | | | | | NS |
| Diabetes | 0.578 | 0.492 | 1.383 | 1.783 (0.68–4.675) | 0.24 | | | | | |
| Pregnancy | −0.101 | 0.734 | 0.019 | 0.904 (0.215–3.808) | 0.891 | | | | | |
| Surgical approach at diagnosis | 0.001 | 0.366 | 0 | 1.001 (0.489–2.051) | 0.997 | | | | | |
| Adjuvant pharmaceutical treatment | 0.696 | 0.354 | 3.873 | 2.006 (1.003–4.013) | 0.049 | | | | | NS |
| Adjuvant radiation treatment | 0.714 | 0.344 | 4.316 | 2.042 (1.041–4.003) | 0.038 | | | | | NS |
| Clinical stage | 0.751 | 0.348 | 4.646 | 2.118 (1.07–4.192) | 0.031 | 0.766 | 0.367 | 4.347 | 2.151 (1.047–4.42) | 0.037 |
| Lymph node metastasis | 0.448 | 0.397 | 1.275 | 1.565 (0.719–3.406) | 0.259 | | | | | |

SE, standard error; HR, hazard ratio; CI, confidence interval; NS, not significant; HMMR, hyaluronan-mediated motility receptor.

**Table 4.** Univariate and multivariate Cox regression analyses of disease-specific survival in 56 patients with uterine carcinosarcoma.

| | Univariate analysis | | | | | Multivariate analysis | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Variable | B value | SE | Wald | HR (95%CI) | P-value | B value | SE | Wald | HR (95%CI) | P-value |
| Age | 0.417 | 0.367 | 1.291 | 1.518 (0.739–3.116) | 0.256 | | | | | |
| HMMR expression | 0.803 | 0.395 | 4.139 | 2.233 (1.03–4.843) | 0.042 | | | | | NS |
| Menopausal hormone therapy | −1.41 | 1.036 | 1.85 | 0.244 (0.032–1.862) | 0.174 | | | | | |
| Hypertension | 0.635 | 0.418 | 2.312 | 1.887 (0.832–4.277) | 0.128 | | | | | |
| Diabetes | 0.537 | 0.504 | 1.134 | 1.71 (0.637–4.593) | 0.287 | | | | | |
| Pregnancy | −0.401 | 0.747 | 0.288 | 0.67 (0.155–2.895) | 0.591 | | | | | |
| Surgical approach at diagnosis | 0.064 | 0.404 | 0.025 | 1.066 (0.483–2.354) | 0.874 | | | | | |
| Adjuvant pharmaceutical treatment | 0.879 | 0.381 | 5.323 | 2.408 (1.141–5.082) | 0.021 | 0.745 | 0.385 | 3.752 | 2.106 (0.991–4.475) | 0.053 |
| Adjuvant radiation treatment | 0.753 | 0.379 | 3.935 | 2.123 (1.009–4.465) | 0.047 | | | | | NS |
| Clinical stage | 1.01 | 0.397 | 6.46 | 2.745 (1.26–5.979) | 0.011 | 0.905 | 0.404 | 5.018 | 2.471 (1.12–5.453) | 0.025 |
| Lymph node metastasis | 0.556 | 0.429 | 1.677 | 1.743 (0.752–4.043) | 0.195 | | | | | |

SE, standard error; HR, hazard ratio; CI, confidence interval; NS, not significant; HMMR, hyaluronan-mediated motility receptor.

**Table 5.** Univariate and multivariate Cox regression analyses of overall survival in 56 patients with uterine carcinosarcoma.

| | Univariate analysis | | | | | Multivariate analysis | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Variable | B value | SE | Wald | HR (95%CI) | P-value | B value | SE | Wald | HR (95%CI) | P-value |
| Age | 0.432 | 0.341 | 1.611 | 1.541 (0.79–3.004) | 0.204 | | | | | |
| HMMR expression | 0.944 | 0.38 | 6.168 | 2.571 (1.22–5.415) | 0.013 | 0.922 | 0.386 | 5.702 | 2.515 (1.18–5.36) | 0.017 |
| Menopausal hormone therapy | −0.724 | 0.635 | 1.303 | 0.485 (0.14–1.681) | 0.254 | | | | | |
| Hypertension | 0.589 | 0.375 | 2.467 | 1.802 (0.864–3.759) | 0.116 | | | | | |
| Diabetes | 0.717 | 0.464 | 2.391 | 2.049 (0.825–5.088) | 0.122 | | | | | |
| Pregnancy | −0.887 | 0.626 | 2.009 | 0.412 (0.121–1.404) | 0.156 | | | | | |
| Surgical approach at diagnosis | 0.166 | 0.378 | 0.192 | 1.18 (0.563–2.475) | 0.661 | | | | | |
| Adjuvant pharmaceutical treatment | 0.88 | 0.358 | 6.045 | 2.410 (1.195–4.859) | 0.014 | 0.817 | 0.365 | 5.008 | 2.264 (1.107–4.633) | 0.025 |
| Adjuvant radiation treatment | 0.843 | 0.357 | 5.571 | 2.324 (1.154–4.681) | 0.018 | 0.716 | 0.357 | 4.026 | 2.046 (1.017–4.119) | 0.045 |
| Clinical stage | 0.908 | 0.369 | 6.039 | 2.478 (1.202–5.111) | 0.014 | | | | | NS |
| Lymph node metastasis | 0.558 | 0.412 | 1.832 | 1.746 (0.779–3.916) | 0.176 | | | | | |

SE, standard error; HR, hazard ratio; CI, confidence interval; NS, not significant, HMMR, hyaluronan-mediated motility receptor.

gene sequencing technology have allowed us to gain a better understanding of the mechanisms of diseases based on a bioinformatics predictions, and may provide a broader perspective.

In the current study, we obtained transcriptional expression data for UCS and normal samples from the GEPIA database, and identified 1894 DEGs, including 579 upregulated and 1315 downregulated genes. We performed GO and KEGG functional enrichment analyses for the DEGs, and created a PPI network using the STRING website and Cytoscape software. Four clusters and 30 hub genes were identified for further analysis; however, HMMR was the only hub gene found to be significantly correlated with the prognosis of UCS, and statistical analysis of clinicopathological and HMMR expression data confirmed HMMR as an independent prognostic factor in patients with UCS. These results implied that HMMR may be a potential prognostic biomarker for UCS. To the best of our knowledge, this represents the first complete analysis and prediction of potential biomarkers for UCS using a bioinformatics approach, and provides the first evidence for the possible prognostic significance of HMMR in patients with UCS.

HMMR expression is low in most healthy tissues but is increased in proliferative tissues, such as the spleen and thymus,[29,30] and in a variety of cancer tissues.[14] HMMR overexpression has been related to invasion, progression, and a poor prognosis in breast cancer,[31] invasion of pancreatic adenocarcinoma and prostate cancer,[32,33] invasion and metastasis of endometrial cancer,[34] and a poor prognosis in multiple myeloma, colorectal cancer, and gastric cancer.[35–37] In this study, HMMR expression was significantly inversely correlated with survival in patients with UCS, in accord with these findings in other tumour types.

Previous preclinical phase I and II trials have assessed the ability of an HMMR-derived peptide vaccine to promote immune recognition and destruction of tumours by activated T cells.[38,39] HMMR is thus a promising cancer-related antigen that may serve as an attractive target for the treatment of cancer.

This study had some limitations. First, all the data analysed in our study were derived from bioinformatics databases, and the results are therefore preliminary. Further *in vivo* and *in vitro* experiments are thus needed to verify the present results. Second, the low incidence of UCS means there is a lack of data in public databases, and the resulting small sample size may lead to imprecise estimates of performance. Further studies with larger sample sizes are therefore needed. Third, we did not assess the potential diagnostic and therapeutic roles of HMMR in UCS, and further studies exploring the use of HMMR as a diagnostic marker or therapeutic target in UCS are needed.

## Conclusion

The current study revealed that high HMMR expression predicts a poor prognosis in patients with UCS, and suggests that HMMR may be a potential prognostic biomarker for UCS. These results thus provide a better understanding of molecular targets for improving therapeutic strategies in UCS.

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### Data availability

The datasets supporting the conclusion of this article are included in the article.

## Declaration of conflicting interest

The authors declare that there is no conflict of interest.

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