HMMR Functions as a Prognostic Biomarker in Uterine Carcinosarcoma Identified by Comprehensive Bioinformatics Analysis

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Research

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Abstract

Background

Uterine carcinosarcoma (UCS) is a rare aggressive tumor with a high metastasis rate and poor prognosis. Bioinformatics analysis has been widely applied to screen and analyze genes in linkage to various types of cancer progression. This study aims to explore the molecular mechanism of UCS.

Methods

First, transcriptional different expression data between UCS and normal samples were got from the GEPIA database. Subsequently, differentially expressed genes were analyzed through the Metascape with Gene Ontology (GO) enrichment analysis and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis. Then, the STRING website and Cytoscape software were applied to construct the protein-protein interaction network. Finally, the top 30 genes obtained through the MCC algorithm were selected as hub genes, which was finally validated in TIMER, and UALCAN databases.

Results

A total of 1894 DEGs (579 up-regulated and 1315 down-regulated) were identified, GO and KEGG functional enrichment analysis were performed for the DEGs. The PPI network was constructed based on DEGs, and four clusters were excavated for further analysis and the top 30 genes were identified as hub genes. Our data showed that the expression of HMMR is significantly higher in UCS tissues compared to the paired normal tissues ($p<0.05$) and the elevated expression of HMMR is related to poor prognosis in patients with UCS ($p= 0.0031$). TPX2, AURKA, BRCA1 and BARD1 are essential for the function of HMMR. TPX2 and AURKA were found to be *significantly higher in UCS* compared to *the normal tissue* ($p<0.05$)*, and* there was a statistically *significant positive correlation* between the expression of HMMR and AURKA, TPX2, BRCA1, BARD1 *in* UCS ($p=1.08e-07$, $p=1.62e-05$, $p=2.02e-3$, $p=6.54e-6$).

Conclusions

Our study suggested that HMMR may be a potential biomarker for predicting the prognosis of UCS patients.

Background

Uterine carcinosarcoma (UCS), also known as malignant mixed mullerian tumor (MMMT), is a very aggressive and extremely rare tumor [1]. Histologically, UCS is composed of carcinomatous and sarcomatous components [2], which accounts for less than 5% of all uterine malignancies, but it is responsible for more than 16% of uterine cancer-associated deaths [3]. Although most cases can be treated surgically and the overall survival is improved [4, 5], the 5-year survival is still very poor, ranging from 33–39% [6]. Even in cases where the disease is confined to the corpus, the rate of recurrence is high [7]. Due to its complex composition and low incidence, the relevant study was limited. Exploring the

pathogenesis and identifying novel biomarkers that could predict the occurrence and prognosis of UCS are needed.

Hyaluronan mediated motility receptor (HMMR, also known as RHAMM, XRHAMM, IHABP, or CD168), one of few defined receptors for Hyaluronan, is an oncogene which can enhance tumor invasion and progression by multiple functional mechanisms that manifest in distinct subcellular locations [8]. On the cell surface, HMMR combines with CD44, and HA binding to this complex leads to the activation of downstream extracellular signal-regulated protein kinase (ERK) 1/2 signal pathway, which results in the expressions of genes that are required for invasion and migration in multiple cancers [9, 10, 11]. Intracellular HMMR is an actin and microtubule-associated protein that acts on the breast cancer 1 early onset (BRCA1)–BRCA1-associated RING domain 1 (BARD1) pathway to maintain spindle integrity [8]. Silencing or elevating HMMR expression disrupts microtubule-based processes during cell division, and results in mitotic spindle abnormalities, genome instability, and changes to the cell division axis [12]. Elevated HMMR expression was found to be associated with the progression of cancer and poor prognosis in a variety of tumor types [13, 14].

In recent years, with the rapid development of high-throughput sequencing technology, a large number of clinical, pathological, and biological data of patients with malignancies can be freely accessed from public databases, such as The Cancer Genome Atlas (TCGA) [15] and Gene Expression Omnibus (GEO) database [16]. To the best of our knowledge, bioinformatics analysis has yet to be applied to explore the biomarker in UCS. In our study, we explored genes related to the prognosis of UCS and extended the knowledge related to UCS based on various large databases for conducting the comprehensive analysis.

Methods

GEPIA

GEPIA (http://gepia.cancer-pku.cn/index.html) is a newly developed interactive web server for estimating mRNA expression data based on 9,736 tumors and 8,587 normal samples in the TCGA and Genotype-tissue Expression dataset projects [17]. In our study, transcriptional different expression data between UCS and paired normal samples were got from the GEPIA database. The difference in transcriptional expression was compared by ANOVA. P-value<0.01 and |log-fold change (LFC)| >2 were selected as the thresholds for DEGs screening.

Metascape

To better explore the biological significance of DEGs, enrichment of the functions and pathways was analyzed through Metascape (http://metascape.org), which is a free, user-friendly analysis tool for gene and pathway enrichment analysis [18]. In our study, the GO terms for biological process (BP), cellular component (CC), and molecular function (MF) categories, as well as KEGG pathways, were enriched based on Metascape. The criteria for selection were as follows: minimum overlap of 3, P-value cutoff of 0.01 and minimum enrichment of 1.5. Meeting the above standards were considered as significant

enrichment. The most statistically significant term in the cluster was chosen as the one representing the cluster.

STRING

STRING (http://string-db.org/) is a biological database, designed to construct a PPI network [19]. Analyzing the functional interactions between proteins may provide insights into the mechanisms of generation and development of diseases. We used it to assess potential PPI relationships and explore the interactions between DEGs. Confidence score > 0.7 was set as significant. Cytoscape (www.cytoscape.org/) is an open-source bioinformatics software platform for visualizing molecular interaction networks [20]. The PPI networks were visualized by Cytoscape software.

Hub gene identification and functional analysis

Molecular Complex Detection (MCODE), a plug-in in Cytoscape, was applied to identify hub modules of the PPI network. Degree cut-off of 2, node score cut-off of 0.2, k-core of 2, and max. Depth of 100 was set as the criterion. The top four significant clusters were selected and genes in the selected clusters were analyzed for functional enrichment through 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.

CytoHubba, another plug-in in Cytoscape, was used to calculate the degree of each protein node. Nodes with a higher degree of connectivity tend to be more essential in maintaining the stability of the whole network. In our study, the top 30 genes obtained through the MCC algorithm were identified as hub genes.

UALCAN

UALCAN (http://ualcan.path.uab.edu) is an interactive web resource based on TCGA and MET500 cohort data [21]. In our study, the survival curve of UCS patients with high gene expression and low/medium gene expression were compared by the log-rank test through UALCAN. $P<0.05$ was considered statistically significant.

TIMER

TIMER (https://cistrome.shinyapps.io/timer/) is a comprehensive resource for systematical analysis of immune infiltrates across diverse cancer types and their clinical impact [22]. In our study, the " Correlation module" was used to evaluate the correlation between HMMR and other important genes in UCS. "Diff Exp module" was utilized to explore HMMR expression between common cancer types and their normal adjacent tissues. $P<0.05$ was considered statistically significant.

GeneMANIA

GeneMANIA (http://genemania.org) is a database designed to finds genes that are related to a set of input genes, using a very large set of functional association data including protein and genetic

interactions, pathways, co-expression, co-localization and protein domain similarity [23]. In our study, the GeneMANIA database was used to identify protein and genetic interactions of HMMR.

Results

Identification of DEGs in UCS

We used $p < 0.01$ and $|LFC| \geq 2$ as the cutoff criteria and screened 1894 DEGs, including 579 up-regulated genes and 1315 down-regulated genes. The position of the DEGs on the chromosome is shown in Figure 1.

Functional Enrichment Analysis

Metascape was utilized to perform enrichment analysis for DEGs. Figure 2 showed the top 20 most highly enriched items of GO and KEGG in up-regulated and down-regulated DEGs. The up-regulated DEGs were mainly enriched in BP, including cell division, positive regulation of 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. For the CC, the DEGs were enriched in spindle, microtubule organizing center and extracellular matrix (Fig. 2a). Moreover, the result of KEGG pathway analysis showed that DEGs were mainly enriched in the cell cycle, p53 signaling pathway, ECM-receptor interaction, pathways in cancer and cell adhesion molecules (CAMs) (Fig. 2c). These BP terms and pathways are related to tumorigenesis and pathogenesis in multiple tumors.

In down-regulated DEGs analysis, BP analysis showed that the DEGs were mostly enriched in muscle structure development, blood vessel development, actin filament-based process, regulation of ion transport, extracellular structure organization, positive regulation of cellular component movement and cell-matrix adhesion. For the MF, the DEGs were highly enriched in glycosaminoglycan binding. For the CC, the DEGs were enriched in contractile fiber, collagen-containing extracellular matrix, actin cytoskeleton, sarcolemma, stress fiber and adherens junction (Fig. 2b). The result of KEGG pathway analysis showed that down-regulated DEGs were mainly enriched in focal adhesion, vascular smooth muscle contraction, complement and coagulation cascades, calcium signaling pathway and ras signaling pathway (Fig. 2d). The above enrichment analysis can help us further study the role of DEGs in UCS.

PPI network and analysis on clusters

To better understand the protein interactions among the DEGs, we performed the PPI network analysis. Via the STRING website, 1894 DEGs were screened into the PPI network complex, which contained 1510 nodes and 6586 edges (Fig. 3). Then, MCODE was used to identify modules for the network. 42 clusters were found according to the criterion described previously and the top four significant clusters were

selected (Fig. 4). Cluster 1 contained 76 nodes and 2437 edges, which got the highest score in these clusters (Fig. 4a). Cluster 2 contained 21 nodes and 210 edges (Fig. 4d). Cluster 3 contained 15 nodes and 105 edges (Fig. 4g). Cluster 4 contained 12 nodes and 66 edges (Fig. 4j). GO analysis and KEGG analysis were independently applied to each cluster.

The DEGs of 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 signaling pathway (KEGG) and DNA replication (KEGG) (Fig. 4b). The DEGs of cluster 2 were highly enriched in adenylate cyclase-modulating G protein-coupled receptor signaling 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 signaling pathway (KEGG) and neuroactive ligand-receptor interaction (KEGG) (Fig. 4e). The DEGs of cluster 3 were mostly enriched in regulation of growth (BP), negative regulation of canonical Wnt signaling pathway (BP), endoplasmic reticulum lumen (CC) and collagen-containing extracellular matrix (CC) (Fig. 4h). The DEGs of cluster 4 were highly enriched in protein polyubiquitination (BP) and ubiquitin ligase complex (CC) (Fig. 4k). Genes in cluster 3 and 4 did not enrich any KEGG pathway. Networks of GO enriched terms of the four clusters were also shown in Figure 4, where terms containing more genes tend to have a more significant p-value. The top 30 genes evaluated by the MCC algorithm of CytoHubba were identified as hug genes (Fig. 5).

Hub gene validation

All of the 30 hub genes were validated in UALCAN and we found the mRNA expression of HMMR was significantly associated with UCS patients' prognosis, whose tissues displayed a higher expression of HMMR had significantly shorter survival compared to those with lower expression (p= 0.0031) (Fig. 6a). HMMR not only has higher expression in UCS than in normal tissues (p<0.05) (Fig. 6b), but also has higher expression in multiple cancer types compared to paired normal tissues (Fig. 6c).

Predicted functions and pathways of HMMR and HMMR related genes

We explored the interaction network based on HMMR through GeneMANIA and STRING databases (Fig. 7a, b), and then the functions of HMMR and related genes were analyzed by GO and KEGG through Metascape. As were shown in Figure 7c, HMMR related genes were mainly involved in cell cycle and mitosis, which is consistent with a review on HMMR published by Christopher Alan Maxwell [8]. In the review, it described that RHAMM–centrosome–mitotic-spindle associations have the potential to affect cell transformation and tumor progression by promoting genomic instability in the cell, and TPX2, AURKA, BRCA1 and BARD1 are essential collaborators for HMMR in the process of accomplishing intracellular functions [8]. Together with the bioinformatics analyses, TPX2 and AURKA *were* found to be *significantlyhigherinUCS tissues* compared to *the normal tissues* (p<0.05) (Fig. 7d) and there were statistically *significantpositivecorrelations* between the expression of HMMR and AURKA, TPX2, BRCA1, BARD1 *in* UCS (p=1.08e-07, p=1.62e-05, p=2.02e-3, p=6.54e-6) (Fig. 7e).

Discussion

UCS is a rare aggressive tumor with a high metastasis rate and poor prognosis [24]. Histologically, it is composed of an admixture of malignant epithelial and sarcomatous elements. The sarcoma component exhibits differentiation with histologic features of leiomyosarcoma, endometrial stromal sarcoma (ESS), or fibrosarcoma, or may resemble rhabdomyosarcoma, chondrosarcoma, osteosarcoma, or other heterologous sarcomas [25, 26]. The low incidence and complex composition limit the researches. With the development of gene sequencing technology, we can have a better understanding of the mechanism of diseases based on a bioinformatic prediction, which may provide a broader perspective.

In our study, transcriptional different expression data between UCS and normal samples were got from the GEPIA database. A total of 1894 DEGs were identified, including 579 up-regulated genes and 1315 down-regulated genes. We performed GO and KEGG functional enrichment analysis for the DEGs. The PPI network analysis was constructed through the STRING website and Cytoscape software. In this step, four clusters and the 30 hub genes were excavated for further analysis. HMMR expression was related to the prognosis of patients, with a p-value of 0.0031. TPX2 and AURKA are important genes with high expression levels in UCS patients, and the expression of AURKA, TPX2, BRCA1, BARD1 are positively correlated with HMMR in UCS. All these results implied that HMMR could become a potential prognostic marker and therapeutic target for UCS.

The expression of HMMR is low in most healthy tissues but is elevated in proliferative tissues, such as the spleen, placenta, and thymus [27, 28], and a variety of cancer tissues [14]. Some studies have identified the overexpression of HMMR is associated with the early age of diagnosis, invasion, progression and poor prognosis of breast cancer [29]; with the invasion of pancreatic adenocarcinoma and prostate cancer [30, 31]; with the histological grade, invasion and metastasis of endometrial cancer [32]; and with poor prognostic factors in multiple myeloma, colorectal and gastric cancer [33, 34, 35]. In this study, HMMR expression was found to significantly inversely correlate with survival in UCS patients, which is in agreement with findings in other tumor types.

The ability of the HMMR-derived peptide vaccine to promote immune recognition and destruction of tumors by activated T cells is investigated in the pre-clinical, phase I and II trials [36, 37]. Therefore, HMMR is a promising novel cancer-associated antigen and may serve as an attractive target for cancer therapy.

There were some limitations in our study. First, although elevated expression of HMMR were prognostic factors for UCS patients, all the data analyzed in our study was derived from the bioinformatics databases, in vivo and in vitro studies are required to validate our findings. Second, due to its low incidence, there was insufficient data available in public databases, which may cause statistical inaccuracy, more datasets consist of larger sample sizes should be introduced. Third, we did not assess the potential diagnostic and therapeutic roles of HMMR in UCS, further studies are needed to explore whether HMMR could be exploited as diagnostic markers or as therapeutic targets in UCS.

Conclusions

Our study discovered that high expression of HMMR predicts poor prognosis and HMMR may be a potential prognostic biomarker for UCS patients, which provides a better understanding of molecular targets for improved therapeutic strategies in UCS.

Abbreviations

UCS: Uterine carcinosarcoma; DEGs: Differentially expressed genes; GO: Gene ontology; KEGG: Kyoto encyclopedia of genes and genomes; PPI: Protein–protein interaction; TCGA: The cancer genome atlas; GEO: Gene expression omnibus; HMMR/RHAMM: Hyaluronan mediated motility receptor; ERK: Extracellular signal regulated protein kinase; BRCA1: Breast cancer 1 early onset; BARD1: BRCA1-associated ring domain 1; BP: Biological process; CC: Cellular component; MF: Molecular function; MCODE: Molecular complex detection; ESS: Endometrial stromal sarcoma.

Declarations

Ethics approval and consent to participate

Not applicable.

As all the data were retrieved from the online databases, so it could be confirmed that all written informed consent had already been obtained.

Consent for publication

Not applicable.

Availability of data and materials

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

Competing interests

The authors declare that they have no conflict of interest.

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Authors' contributions

The conception and design of the study: Hui Sun, Jie Chen. Acquisition of data, analysis and interpretation of data: Hui Sun. Drafting the article and revising it critically for important intellectual

content: Hui Sun, Li Ma. Both authors contributed equally to this manuscript. Final approval of the version to be submitted: all the authors. The authors read and approved the final manuscript.

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Figures

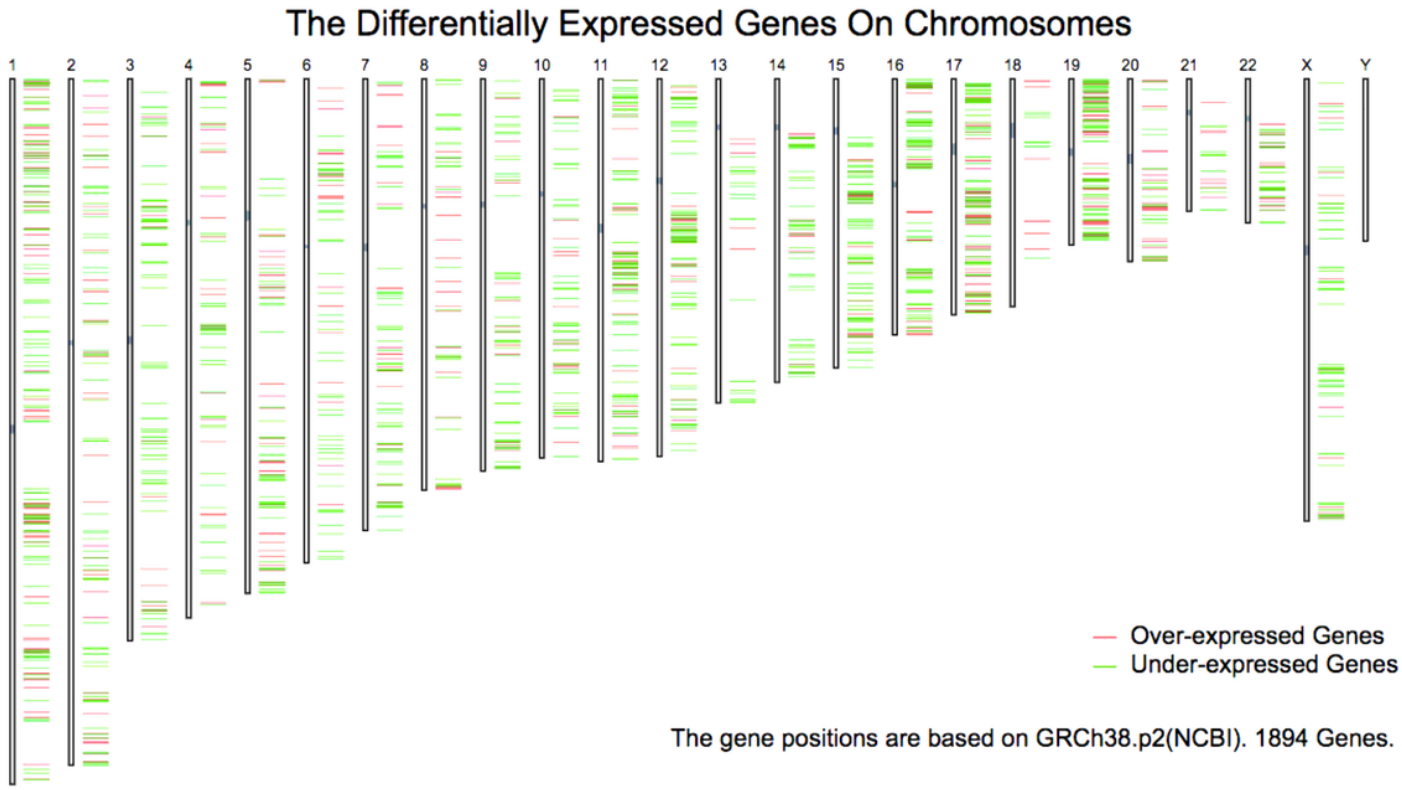


Figure 1

The DEGs on chromosomes.

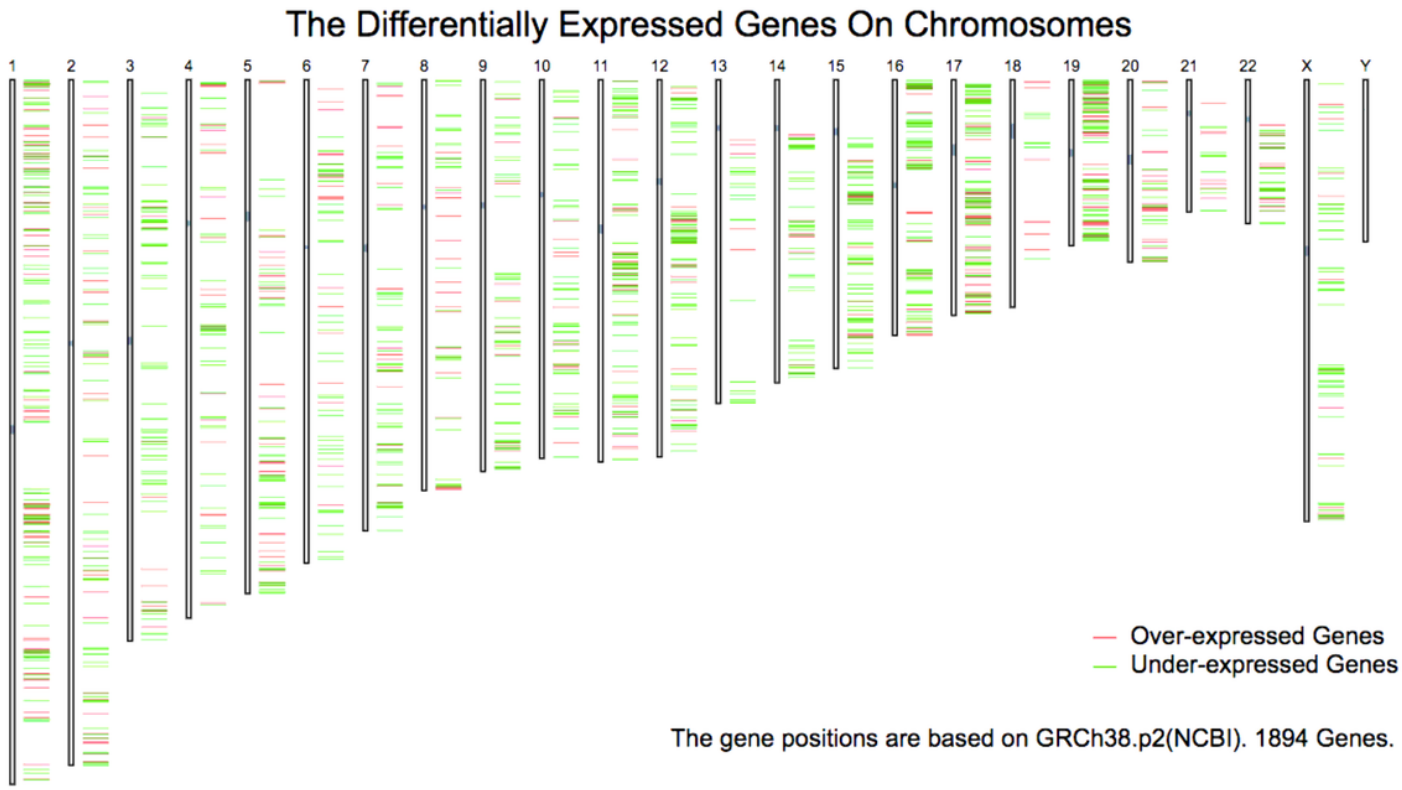


Figure 1

The DEGs on chromosomes.

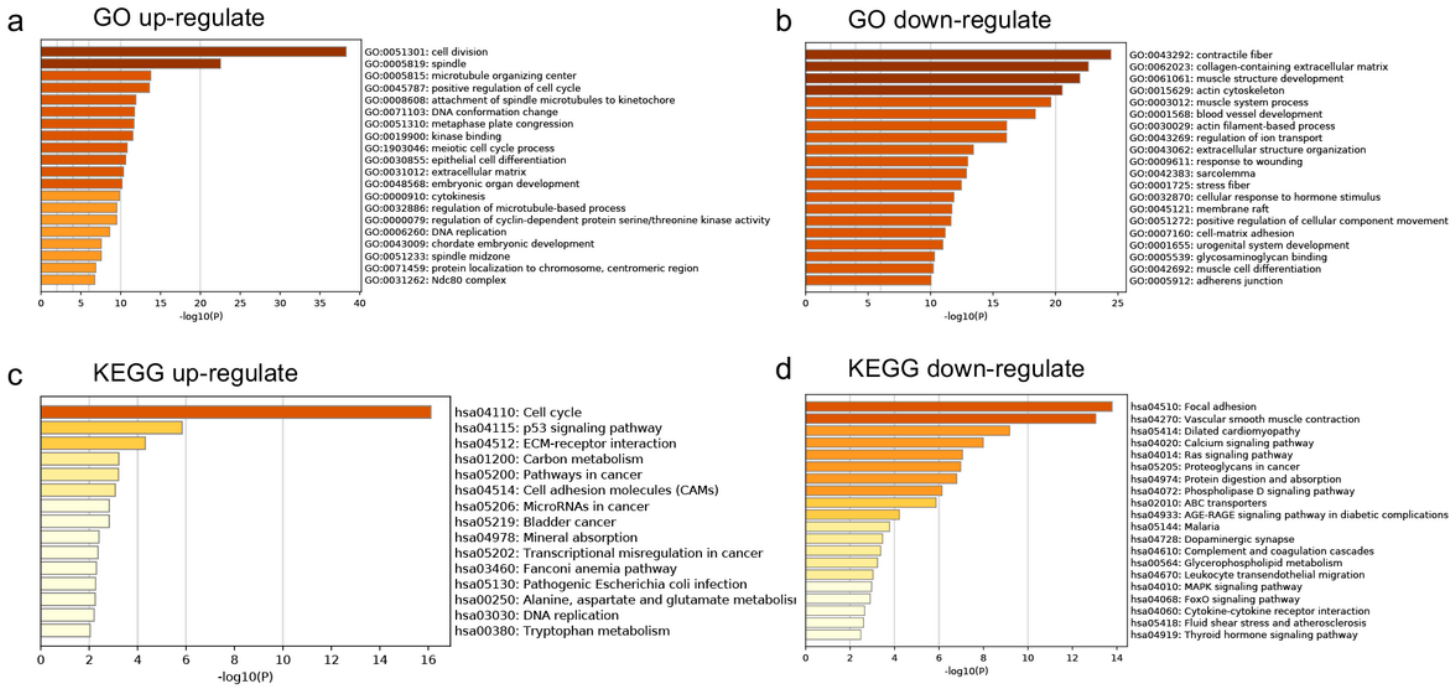
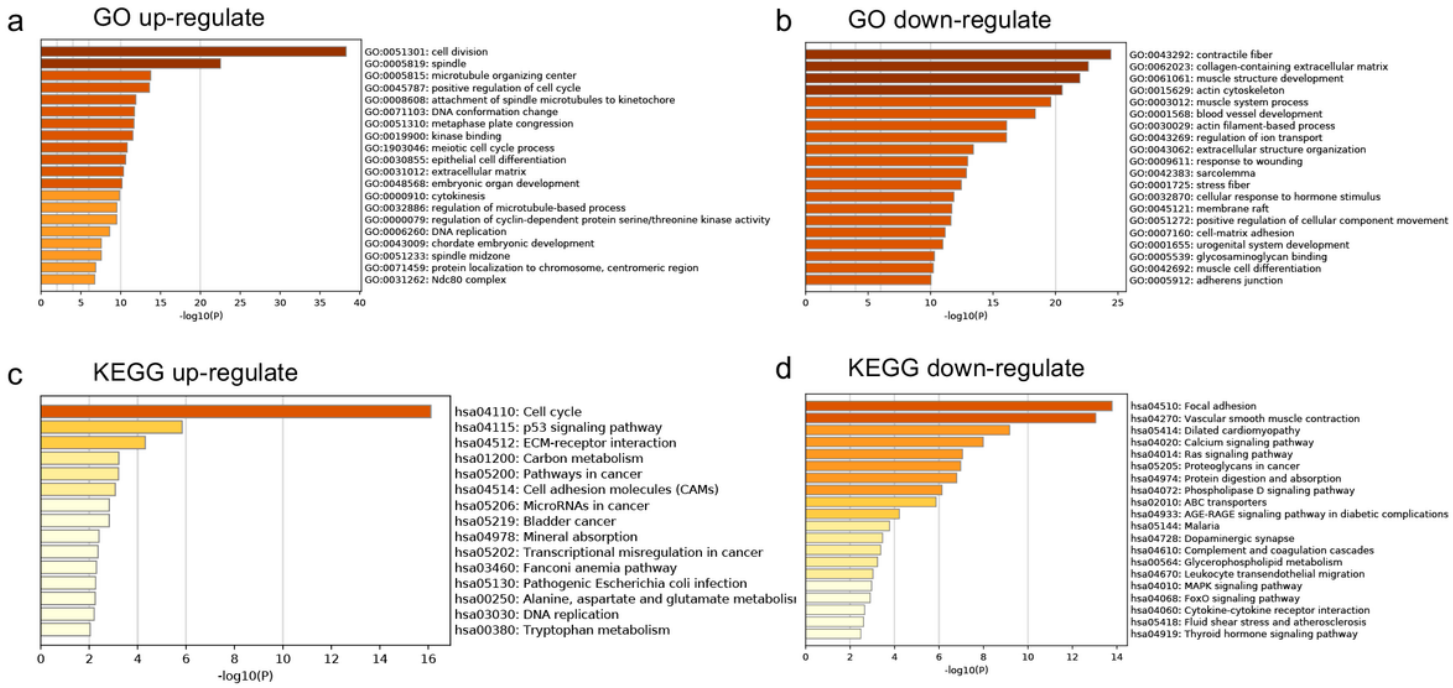


Figure 2

Functional enrichment analysis on the DEGs. a GO analysis on the up-regulated DEGs colored by p-values. b GO analysis on the down-regulated DEGs. c KEGG analysis on the up-regulated DEGs. d KEGG analysis on the down-regulated DEGs.



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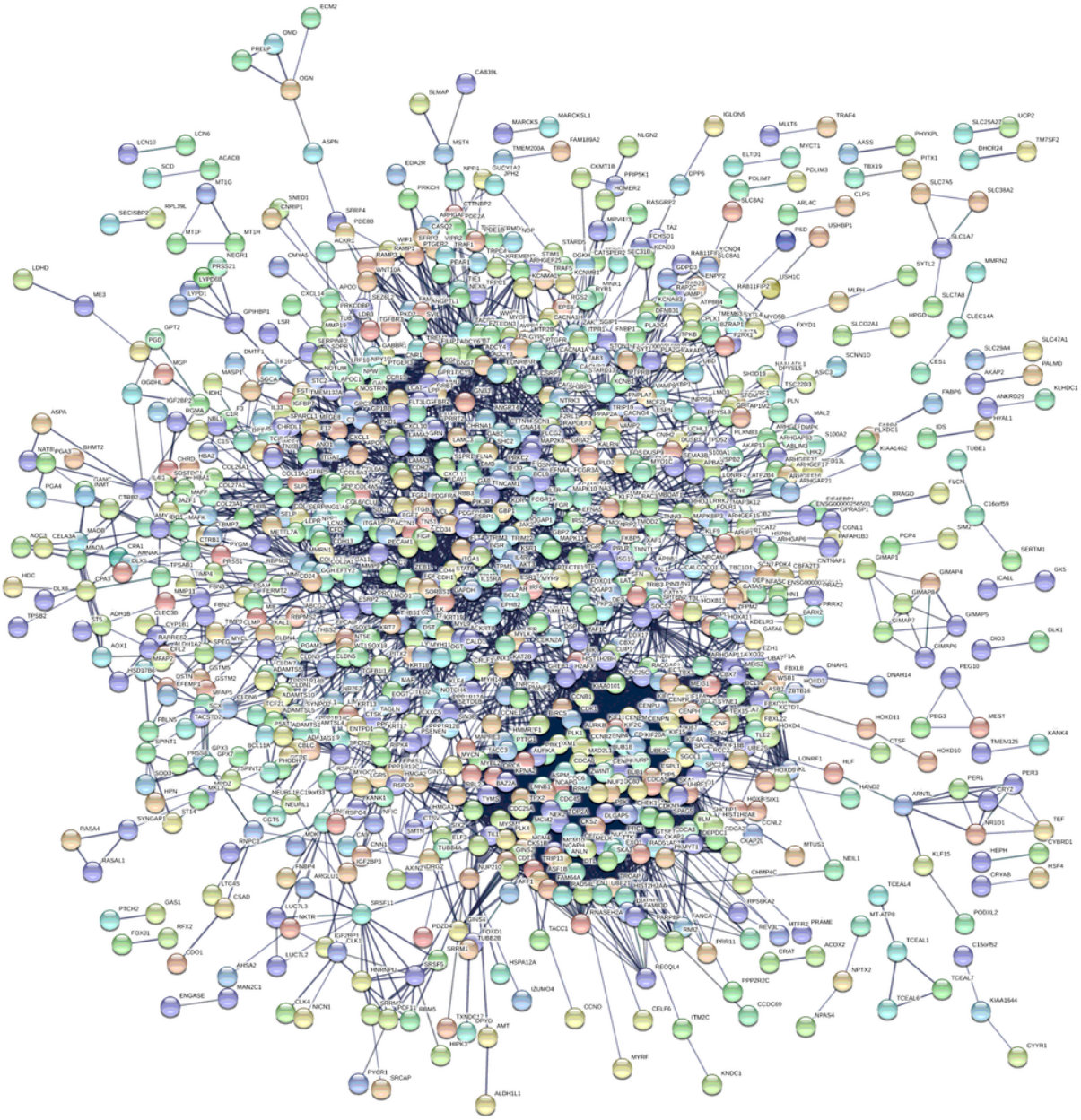


Figure 3

PPI network construction based on the STRING website. 1894 DEGs were filtered into the DEGs PPI network complex that contained 1510 nodes and 6586 edges.

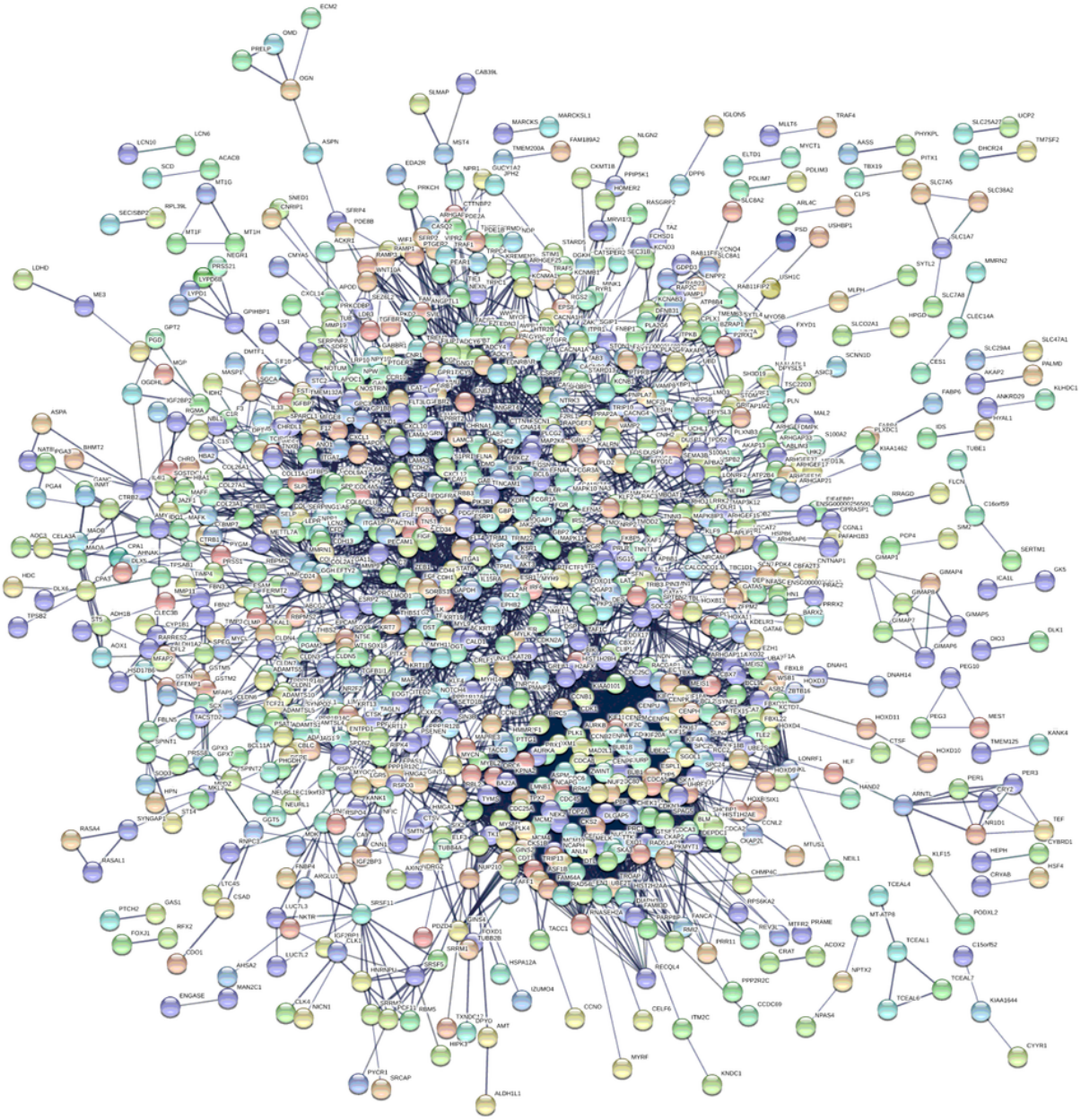


Figure 3

PPI network construction based on the STRING website. 1894 DEGs were filtered into the DEGs PPI network complex that contained 1510 nodes and 6586 edges.

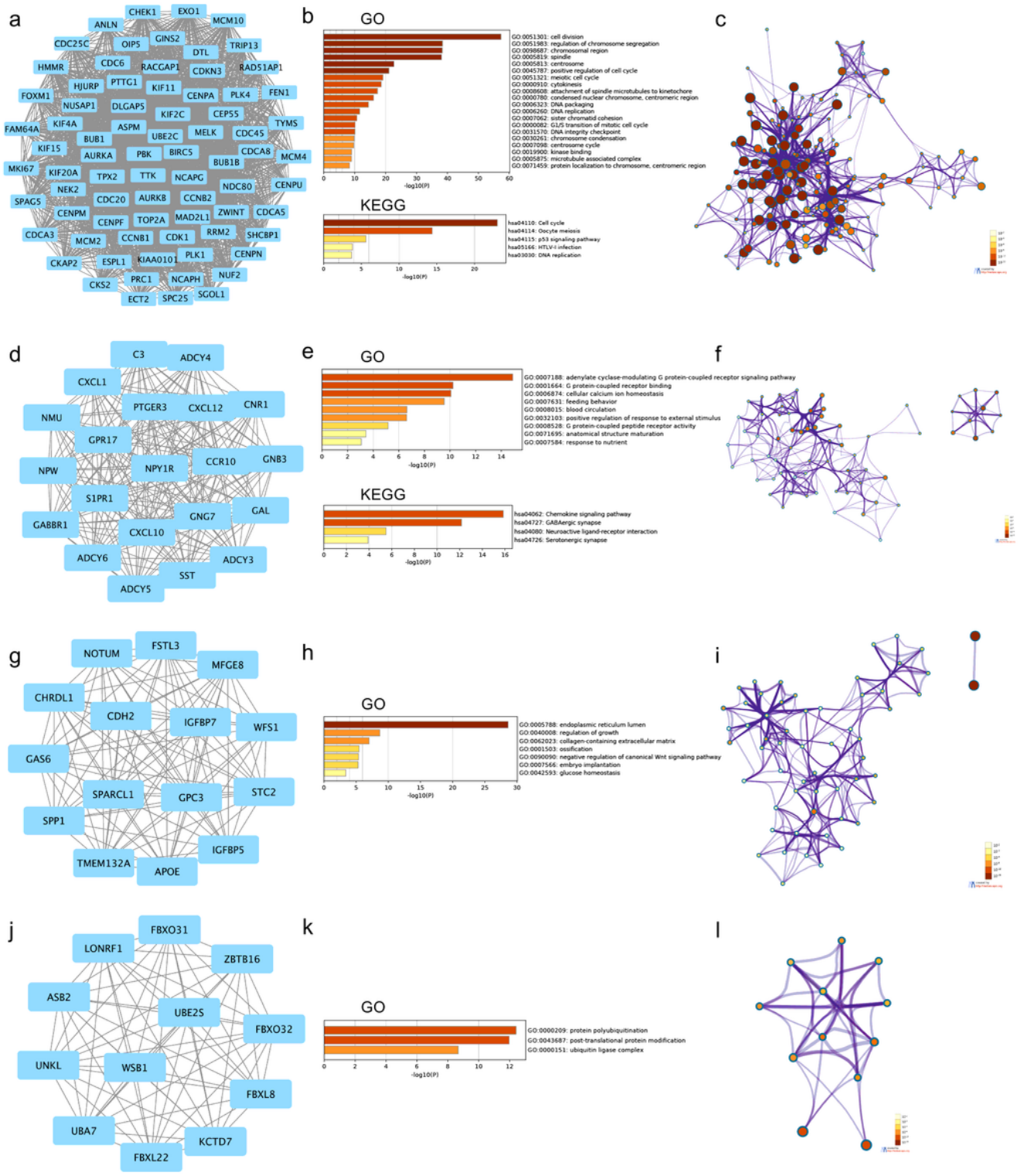
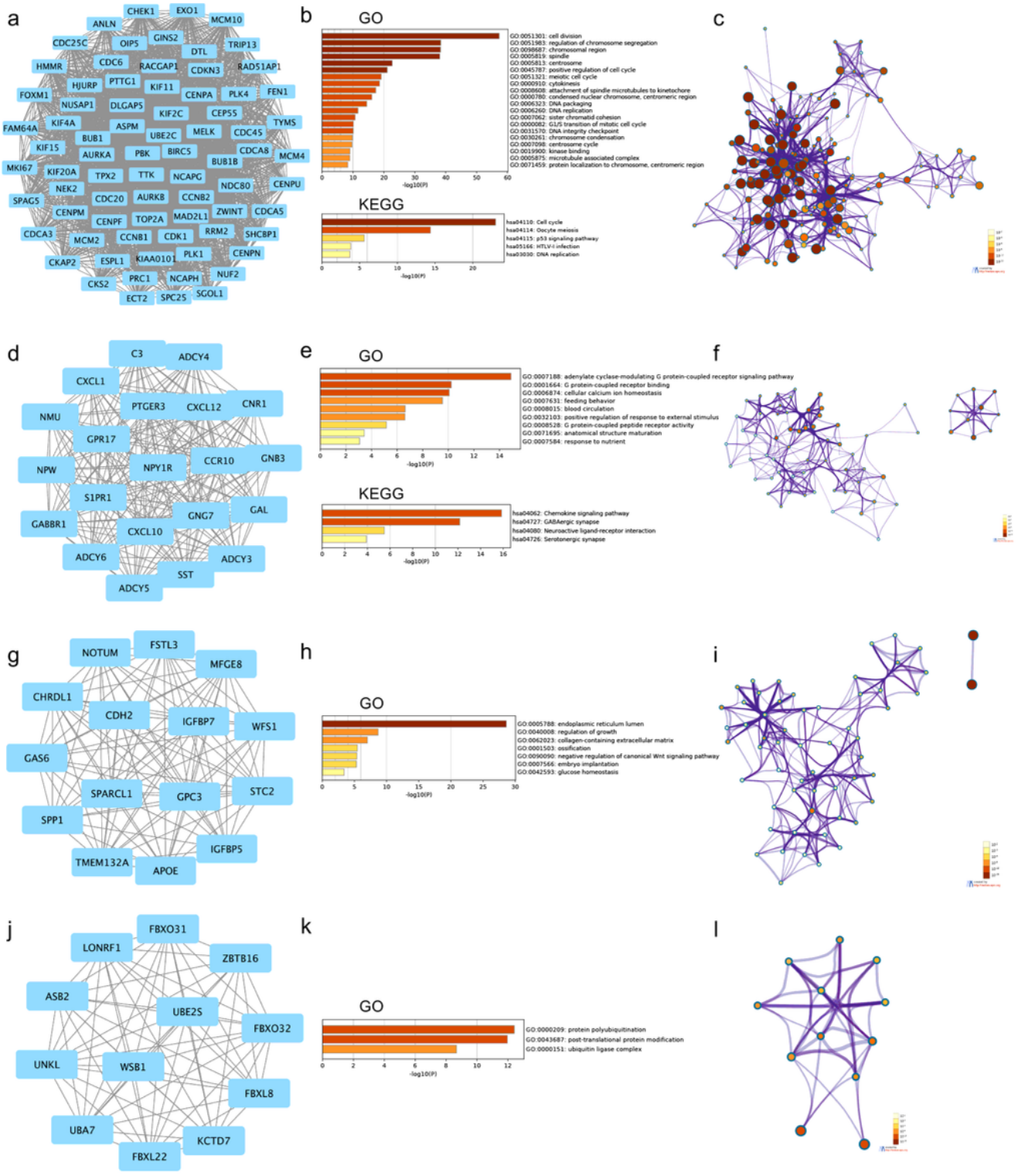


Figure 4

Cluster analysis of the PPI network. a Cluster 1 consists of 76 nodes and 2437 edges and has the highest score in those clusters. b GO and KEGG analysis on Cluster 1, colored by p-values. c The Network of GO enriched terms of Cluster 1 colored by p-value, where terms containing more genes tend to have a more significant p-value. d Cluster 2 consists of 21 nodes and 210 edges. e GO and KEGG analysis on Cluster 2. f Network of GO enriched terms of Cluster 2. g Cluster 3 consists of 15 nodes and 105 edges. h GO

analysis on Cluster 3. i Network of GO enriched terms of Cluster 3. j Cluster 4 consists of 12 nodes and 66 edges. k GO analysis on Cluster 4. l Network of GO enriched terms of Cluster 4.



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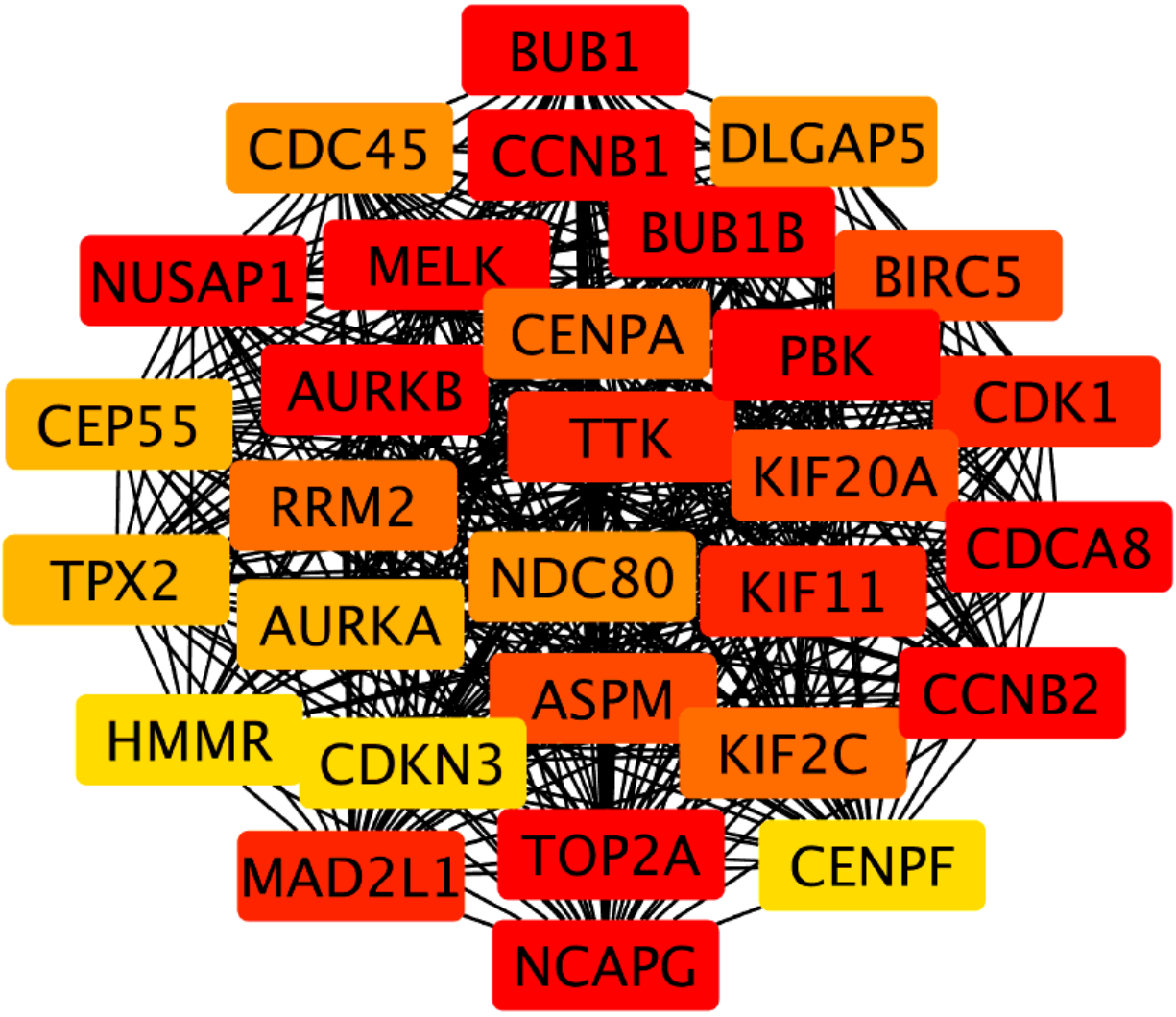
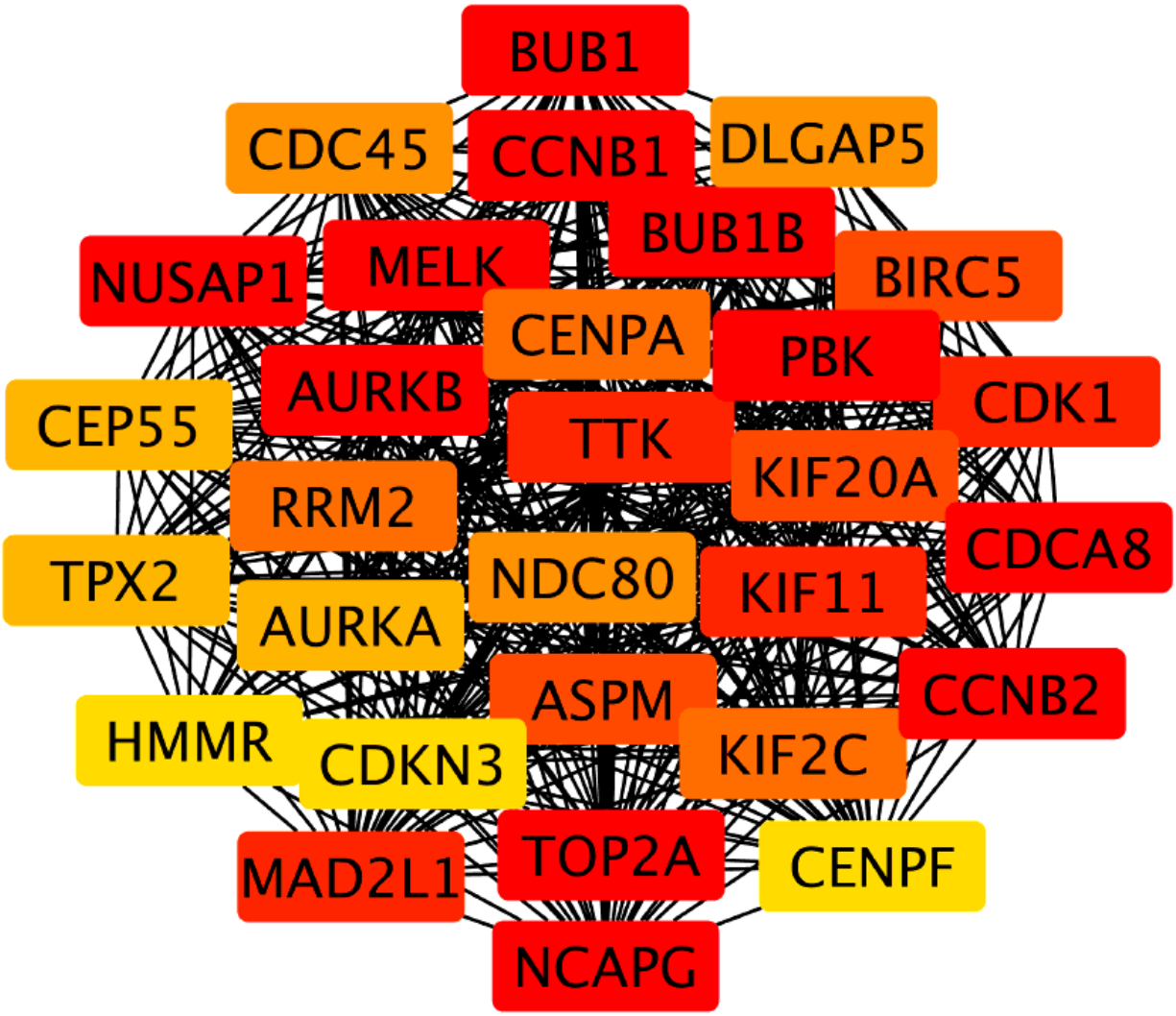


Figure 5

The top 30 hug genes identified by Cytohubba. The more forward ranking is represented by a redder color.



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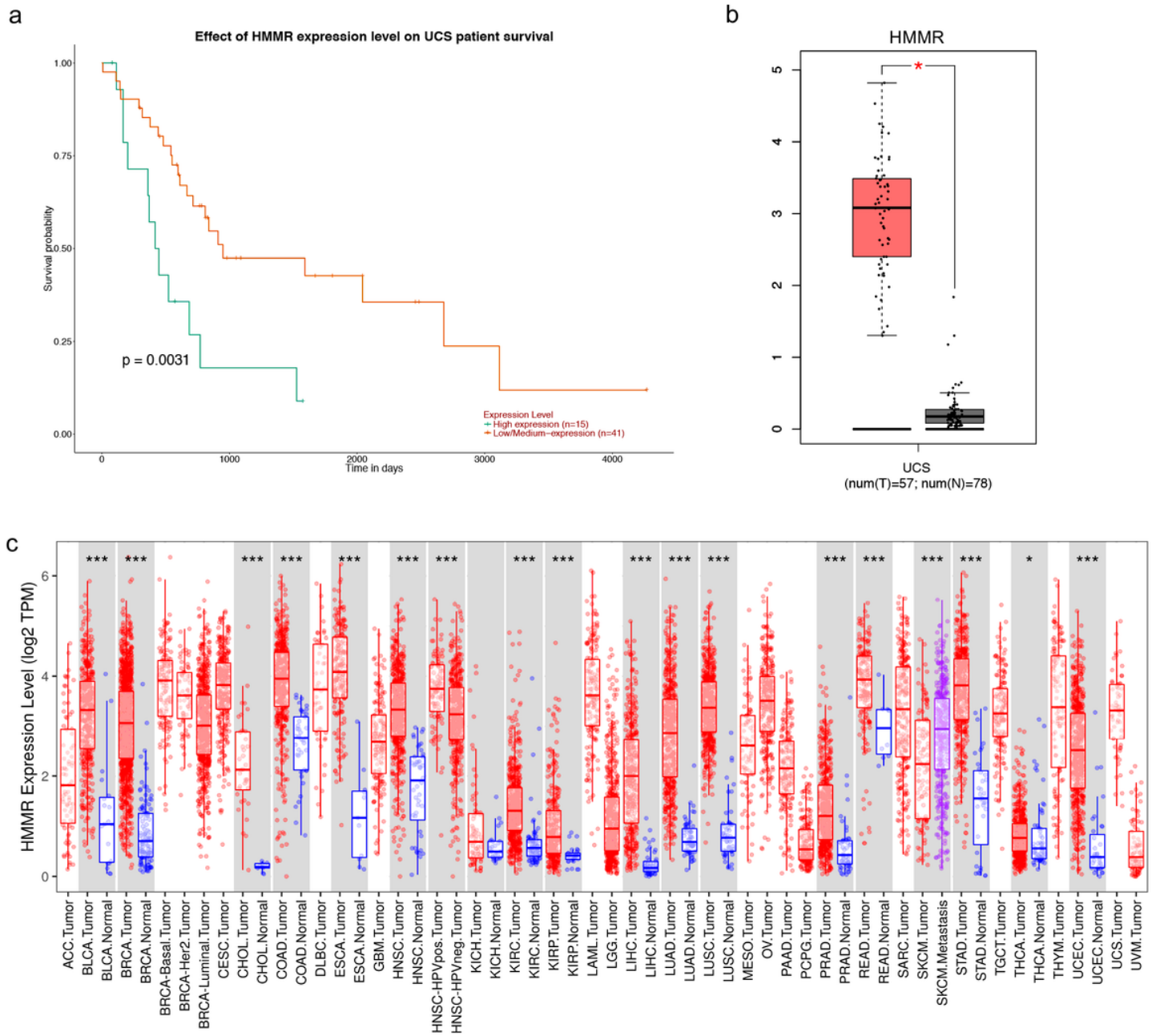
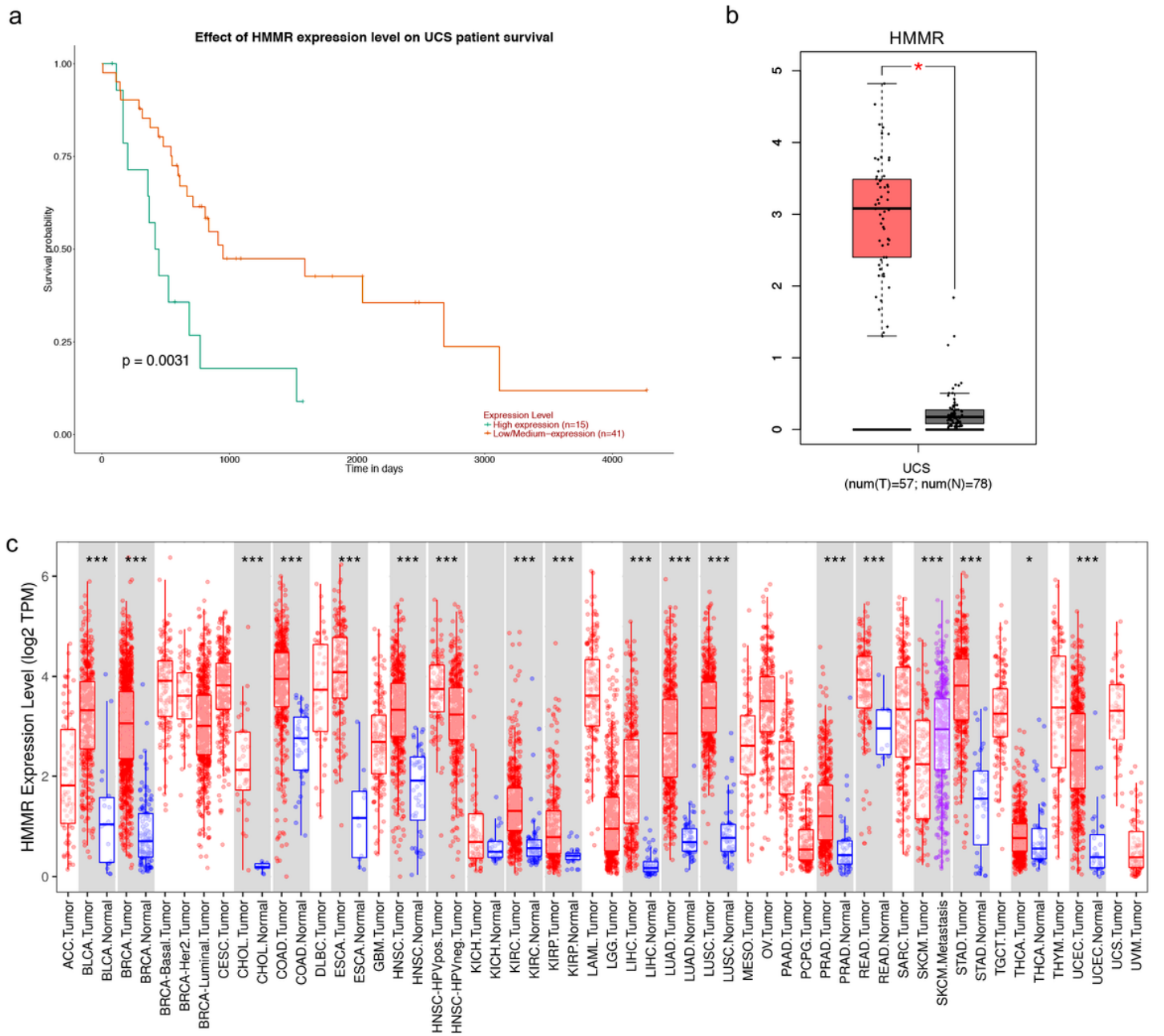


Figure 6

The expression of HMMR and survival analysis. a Higher mRNA expression of HMMR was significantly associated with the poor prognosis of UCS patients (p=0.0031). b Box plots derived from gene expression data of GEPIA comparing the expression of HMMR in UCS tissues and normal tissues(p<0.05). c Differential expression of the HMMR in different types of cancers compared to corresponding normal adjacent tissues by TIMER analysis (*p < 0.05, **p < 0.01 and ***p < 0.001).



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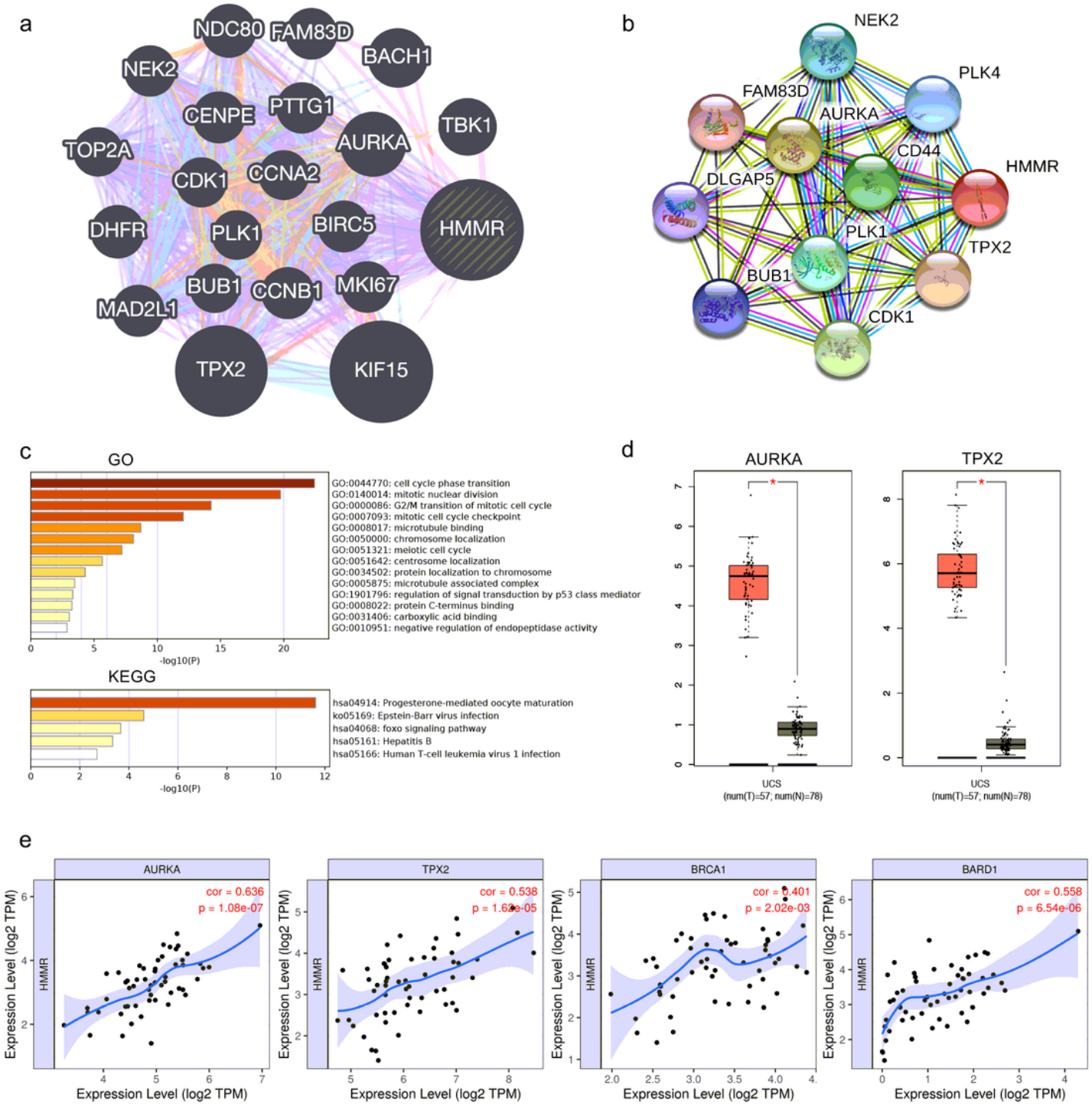
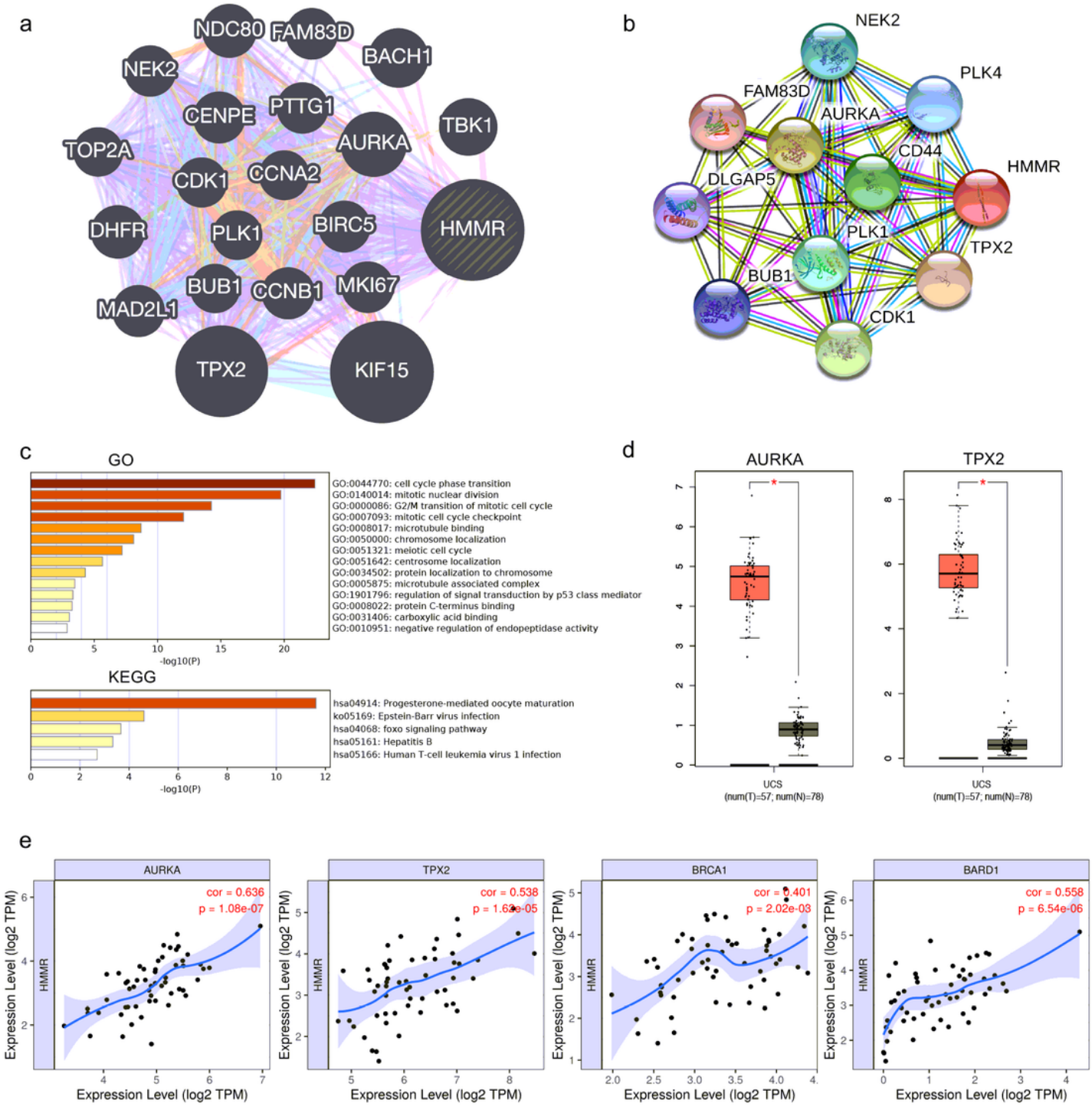


Figure 7

Co-expression and interaction analysis of HMMR and HMMR related genes. a Interaction network of HMMR based on the GeneMANIA database. b Interaction network of HMMR based on the STRING website. c GO and KEGG analysis on HMMR and related genes, colored by p-values. d mRNA expressions of AURKA and TPX2 were found to be over-expressed in UCS tissues compared to normal

tissues(p<0.05). e Correlation between HMMR and AURKA, TPX2, BRCA1, BARD1 in UCS based on TIMER database (p=1.08e-07, p=1.62e-05, p=2.02e-3, p=6.54e-6).



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