Relationship Between Insulin Resistance and Endometrial Cancer: Determination of Key Genes and Pathways

Dou-Dou Ding
the People's Hospital of China Three Gorges University/the First People's Hospital of Yichang
Quan Zhou
the People's Hospital of China Three Gorges University/the First People's Hospital of Yichang
Ze He
the People's Hospital of China Three Gorges University/the First People's Hospital of Yichang
Hong-Xia He
the People's Hospital of China Three Gorges University/the First People's Hospital of Yichang
Man-Zhen Zuo (ycyy_zmz@163.com)
the People's Hospital of China Three Gorges University/the First People's Hospital of Yichang

Research Article

Keywords: bioinformatics analysis, endometrial cancer, insulin resistance, prognostic value, targeted therapy

Posted Date: September 8th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-864828/v1

Abstract

Introduction: Epidemiological studies have found that the occurrence of endometrial cancer(EC) is closely related to metabolic diseases, and insulin resistance (IR) plays an important role in the pathogenesis of endometrium, but the specific pathogenesis is still unclear. The purpose of this study is to reveal the relationship between insulin resistance and endothelial cells by gene screening technology.

Material and methods: We analyzed one endometrial carcinoma database (GSE106191) and one insulin-resistant database (GSE63992), with Gene Expression Omnibus (GEO) database and Venny online analysis tool, then, we found an add-up to 148 different genes. Functional enrichment analysis of these genes using DAVID showed that they were participated in transcription factor activity,signaling pathways and response to factors, etc. Then used cytoHubba in Cytoscape,we got 25 hub genes.

Results: The results showed that the survival time of OGT, IGSF3, TRO, NEURL2 and PIK3C2B was significantly and closely related to EC, and the percentage of gene changes of five central genes ranged from 3% to 10% of a single gene, was also related to the infiltration of seven kinds of immune cells in endometrial carcinoma.

Conclusion: The five key genes (OGT,IGSF3, PIK3C2B,TRO and NEURL2) are involved in immune infiltration in the progression of endometrial carcinoma, and there is also a certain mutation probability in gene mutation. This may be the pathogenesis of insulin resistance and endometrial cancer.

Key Message

Epidemiology shows that insulin resistance is closely related to the pathogenesis of endometrial cancer, and its specific pathogenesis may be related to the transcription and mutation of key genes.

1 | Introduction

Endometrial cancer (EC)occupies an important place among the three common malignant tumors in gynecology, accounting for one of the three malignant tumors of the female reproductive system,which is more common in postmenopausal or menopausal women[1]. In the United States, the new incidence of endometrial carcinoma ranks fourth among female malignant tumors, accounting for 7% of new cancers every year, and 10 170 patients died[2].Over the last few years, the incidence and death rates in China have been increasing year by year, and now the morbidity ranks second only to cervical cancer, with the incidence and death rates being 63.4/100,000 and 21.8/100,000 separately[3],and the onset age tends to be younger, which seriously affect women's physical and mental health and quality of life.Despite the rapid development of diagnosis and treatment technology of endometrial cancer,and patients with early diagnosis of disease are related to good prognosis,but there are still 5% ~ 15% patients with recurrence risk, who with recurrent or metastatic diseases have limited treatment options and short overall survival time, they are high mortality and poor survival rate[4]. Besides, the development of endometrial cancer is usually related to some metabolic diseases in confirmed patients.

Evidence-based medicines have confirmed that the risk of endometrial cancer is related to metabolic diseases such as overweight or obesity, insulin resistance and impaired glucose toleration. Among them, obesity and diabetes have been proved to significantly increase the incidence of endometrial cancer[5].In recent years, the relationship between endometrial cancer and insulin resistance(IR) has been paid increasing attention.

Epidemiological studies have shown that insulin resistance exists at the early stage of endometrial hyperplasia, also the increase of insulin is positively correlated with endometrial hyperplasia, atypical hyperplasia and endometrial cancer[6],however, the exact pathogenesis of insulin resistance helping increase the development of endometrial cancer has not been fully elucidated.The increase of insulin levels induced by insulin resistance promotes cell proliferation and leads to the direct or indirect change of sex hormone levels, which may be the pathogenic relationship between insulin resistance and endometrial cancer[7].And insulin sensitizer has potential anti-tumor effect. It is considered that insulin sensitizers can inhibit mitochondrial breathing and lung related chain complex 1[8], inhibit oxidative phosphorylation and reduce ATP production, thus reducing the energy supply and blocking the metabolism of tumor cells[9].However, this theory is still controversial, which indicates that insulin regulation may be a potential treatment strategy for endometrial cancer.

Although epidemiology has confirmed that insulin resistance is intimate related to the germination and progress of endometrial carcinoma, the details of the pathophysiological methods are still hard to catch.In this article, we aim to prove the potential pathophysiological relationship between endometrial cancer and insulin resistance.In addition, we also discussed the function, prognosis and potential mechanism of pivotal genes in endometrial carcinoma.

2 | Materials And Methods

2 . 1 | Acquisition of data

The gene expression files of GSE63992 and GSE106191 were downloaded from the Gene Expression Omnibus (GEO, https://www.ncbi.nlm.nih.gov/geo/) database.The GSE63992 dataset included the gene expression profiles of nine insulin-resistant invalids and nine insulin-sensitive controls[10].The GSE106191 dataset involved the gene expression profiles of sixty-four endometrial carcinoma patients and thirty-three endometrial hyperplasia controls,submitted by Sugiyama Y[11],et al. After analyzing genes with GEO2R,an online tool for GEO,then, we got all genes with difference expression.

2 . 2 | Acquisition of differentially expressed genes (DEGs)

The primary genetic databases were extracted ,and the condition of |log-FC| >0, P-value< 0.05 was carefully thought about as statistically significant for differentially expressed genes. The interactive DEGs of IR and EC were used for further analysis,in addition,we set log FC over 0 as up-regulated threshold,and below 0 as down-regulated threshold. Finally, 148 eligible difference genes were found to get analysed.

2 . 3 | Enrichment analysis and MOCOD analysis of DEGs

Gene Ontology (GO) is an ontology widely used in the field of bioinformatics. It mainly includes three branches: biological processes, molecular functions and cellular components[12]. Kyoto Encyclopedia of Genes and Genomes (KEGG) is a database integrating genome chemistry and system function information, which can link the gene catalogue obtained from the completely sequenced genome with the system function at higher level of cells, species and ecosystems. We explore the remarkably enriched GO terms and KEGG pathways of DEGs in the David online tool(https:// david.ncifcrf.gov/),which supplies research scholars with a complete and thorough set of functional note tools to comprehend the biological importance behind large amounts of gene lists[13]. The

differential genes of David online tool analysis (P-value < 0.05) were thought to be statistically significant.Then,we used another online tool,called Metascape,to determine the most significal MOCODE.

2 . 4 | Protein-Protein Interaction (PPI) network,acquisition of hub genes

Search Tool ,GeneMANIA(http://genemania.org), is a free online tool for identifying the interaction between proteins and proteins,which can build the PPI network of gene expression products,and which is a flexible web and supports 9 kinds of organisms[14].We assess the relationship of different genes, mapped differences of all genes in GeneMANIA.Then,we imported PPI network information dates into the software Cytoscape 3.8.2 to explore. And to clarify the hub gene in the PPI network by used cytoHubba, which is a software in Cytoscape.

2 . 5 | Differences of hub gene expression

UALCAN(http://ualcan.path.uab.edu/cgi-bin/ualcan-res.pl), is a nice website that incorporates different RNA data and patient medicine-based data from TCGA and provides survival analysis, used to analysis the expression difference of hub genes[15].We imported hub genes to analyze their expression in endometrial carcinoma,and we consider P-value > 0.005 is significant.

2 . 6 | The histological analysis of hub genes

The Human Protein Atlas(HPA,https://www.proteinatlas.org/), which is an open on-line tool,which uses the integration of various omics techniques, including protein omics, transcriptomics and systems biology based on mass spectrometry, antibody-based imaging and mass spectrometry-based imaging, all human protein in cells, tissues and organs can be drawn.We analysis the expression of the hub genes on HPA dataset in EC.The hub genes were analyzed by histopathological to assess differences in expression.

2 . 7 | Analysis of survival in hub genes in endometrial cancer

Survival analysis for the hub genes,which had expression differences, were also analyzed using web-based tool UALCAN (http://ualcan.path.uab.edu). For all survival analyses, log-rank P < 0.05 was believed to be statistically significant.And we considered genes,which had survival significance,as candidate genes for further analysis.

2 . 8 | Genetic alteration analysis of candidate genes

Gene change-related frequency in EC was performed using CBioPortal for Cancer Genomics,CBioPortal (http://www.cbioportal.org/)is associated with the Memorial Sloan Kettering Cancer Center and provides information related to the integrative analysis of complex cancer genomics and clinical profiles from 105 cancer win the TCGA pipeline[16].We used CBioPortal to analyse Genetic alterations of candidate genes.

2 . 9 | Tumor-Infiltrating Immune Cells analysis of candidate genes

Comprehensive analysis of the relationship between the expression of candidate genes and tumor-infiltrating immune using TIMER(https://cistrome.shinyapps.io/timer/),a web server can render services for complete and thorough analysis of tumor-infiltrating immune cells[17].

3 | Results

3 . 1 | Identification of differential genes in IR and EC

In the GSE63992 data set, we got 594 up-regulated and 526 down-regulated genes to be identified,which are compared with IR patients and normal control group(**Figure 1a,c**). And in the dataset GSE106191, 3111 up-regulated and 2590 down-regulated genes were obtained in EC group compared with normal control group(**Figure 1b ,d**). As shown in Figure 1e and Table 1,there were included 81 intersectional upregulated genes and 67 intersectional downregulated genes in IR and EC.

3 . 2 | The gene ontology analysis and pathway of differential genes

We used David online tool to analysis the DEGs in detail,the conclusions of GO analysis indicated that the up-regulated gene changes in biological processes (BP) included significant increasement of several metabolic processes (regulation of DNA-binding transcription factor activity, pattern recognition receptor signaling pathway etc.),as the down-regulated genes were predominantly concentrated in response to glucose,regulation of myeloid leukocyte differentiation and endocytosis(**Figure 2a.** and **Table 2**).The molecular function (MF) enrichment analysis indicated the function of the genes mainly concentrated on significant enrichment of binding-related function (protein binding, transcription factor activity binding, and transcription factor binding),as shown in **Figure 2b**. and **Table 2**. The changes of cellular component (CC) included significant enrichment of genes related to the cytoplasm and nucleoplasm(**Figure 2c.** and **Table 2**).And the changes in KEGG pathway were significantly enriched in NF-kappa B signaling pathway , small cell lung cancer and Epstein-Barr virus infection(**Table 3** and **Figure 2d**).

3 . 3 | Comprehensive analysis of PPI Network

According to the information on GeneMANIA database.There were 148 DEGs to be found , including 81 up- and 67 down-regulated genes in the overall number of 6821 genes , and the PPI network was built with 1364 edges (**Figure 3a**). We used the Metascape (http://metascape.org),which is a gratuitous online tool for gene annotation and analysis,to further analyze the PPI network, and selected the top three most significant modules(**Figure 3b-d**).We also carefully studied the function annotation of the genes in the modules, separately. According to the results of enrichment analysis, the gene functions of these three modules were mainly related to the cell cycle process,actin cytoskeleton organization,ligand binding and protein polyubiquitination(**Figure 3e**).

Cytoscape is an open source software platform for inquiring about complex networks and assembling these with any type of attribute data.Cytoscape was used to find hub genes.The default limits were as follows: a degree cut-off ≥2, node score cutoff ≥0.2, K-score ≥2 and max depth of 100 for selecting hub genes.Twenty-five hub genes related to EC and insulin resistance were analyzed by multiple algorithms (including EcCentricity,Closeness,Radiality and MNC),and there were used for further analysis.The hub genes were PTEN, YY1, KLF5, RARA, ING3, HES1, BRD4, OGT, E2F2, IGSF3, FBXW7, LYN, PIK3C3, TRAF3, RAD21, PTPN1, PIK3C2B, DDX58, GJB3, TRO, NEURL2, MYL12A, IRAK2, TGFBRAP1, RYBP,respectively.For the connection between EC and IR, the results were revealed in **Table 4**.

3 . 4 | Expression difference of hub genes in EC

We compared the expression difference of the twenty-five hub genes between EC and normal tissues by using the UALCAN dataset.We discovered that the expression levels of KLF5,BRD4,E2F2,LYN, HES1,YY1,IGSF3,TRAF3and GJB3 were much higher in EC tissues than in normal tissues, and the expression levels of TRO, NEURL2, PTEN,

RARA, RYBP, OGT, FBXW7, PIK3C3, PIK3C2B, ING3 and DDX58 were clearly lower in EC tissues than in normal tissues,whereas the expression levels of MYL12A, RAD21,TGFBRAP1,IRAK2and PTPN1 were not significantly different between EC and normal tissues,as shown in **Figure 4.**

3 . 5 | The histological analysis of hub genes

We compared the twenty-five hub genes between EC and healthy tissues by using the HPA dataset to analysis the expression,**Figure 5**.The results were the same as UALCAN, the expression levels of KLF5,BRD4,E2F2,LYN,YY1,IGSF3 and TRAF3 were higher in EC tissues than in regular tissues,and the expression levels of TRO, NEURL2, PTEN, RYBP, OGT, FBXW7, PIK3C3, PIK3C2B, ING3 and DDX58 were lower in EC tissues than in normal tissues,whereas the expression levels of MYL12A, RAD21,TGFBRAP1,IRAK2and PTPN1 were not much different between EC and normal tissues.But we did not find RARA,GJB3 and HES1in HPA.

3 . 6 | Survival analysis of the hub genes based on the TCGA dataset

Further analysis was conducted on the above the hub genes,which had expression difference. The Ualcan web tool showed the survival curve of these genes,and the survival curve showed that the survival time of OGT,IGSF3,TRO,NEURL2and PIK3C2B is significantly strongly related to EC ($P < 0.05$), as shown in **Figure 6**.And we think about genes, which had survival significant,as candidate genes.

3 . 7 | Genetic alteration analysis in patients with EC

We analyzed the genetic alterations of OGT, IGSF3,TRO,NEURL2 and PIK3C2B in EC by using cBioPortal online tool. A total of 147 patients from four datasets of endometrium cancer were carefully studied. Among the four datasets analyzed, the gene set pathway was changed in 237 (24.6%) of the queried samples, and alterations ranging from 25.52% (135/529) to 13.42% (73/544) were found for the gene sets submitted for analysis(**Figure 7a**).The percentages of genetic alterations in the five hub genes for EC varied from 3% to 10% for individual genes (OGT, 5%; IGSF3, 4%; TRO, 7%;NEURL2,3% and PIK3C2B, 10%).For OGT, most of the changes are missense mutation, IGSF3 is missense mutation and amplification, TRO is missense mutation, truncation mutation, amplification and deep deletion,NEURL2 is mainly amplification, and PIK3C2B is missense mutation and amplification(**Figure 7b**). The results of Kaplan–Meier plotter and log-rank test pointed to no significant difference in overall survival (OS) and disease-free survival (DFS) between the cases with changes in one of the query genes and those without alterations in any query genes (P-values, 0.444 and 0.215, respectively; **Figure 7c,d**)

3 . 8 | Analysis of Tumor-Infiltrating Immune Cells using TIMER tool

Comprehensive analysis of the relationship between the expression of five hub genes and tumor-infiltrating Immune using TIMER.The results showed that OGT, IGSF3,TRO,NEURL2 and PIK3C2B are related to the infiltration of seven kinds of immune cells in endometrial carcinoma.OGT is related to the infiltration of CD8+ T cells and neutrophils(**Figure 8a**), IGSF3 is related to the infiltration of B cells and CD4+ T cells(**Figure 8b**),TRO is related to the infiltration of CD8+ T cells and CD4+ T cells(**Figure 8c**),NEURL2 is related to the infiltration of CD8+ T cells(**Figure 8d)**,and PIK3C2B is related to the infiltration of CD4+ T cells, neutrophils and dendritic cells(**Figure 8e**).

4 | Discussion

In our study, we explore the risk factors for EC.We received 64 EC samples and 33 normal samples from GSE106191 in GEO database, and 9 insulin-resistant samples and 9 insulin-sensitive samples in GSE63992 dataset.At last, we got 148 different genes, 81 up- and 67 down-regulated genes. The results of GO analysis showed that DEGs were mainly related to DNA-binding transcription factor activity, pattern recognition receptor signaling pathway and response to glucose.The results of KEGG pathways were significantly enriched in NF-kappa B signaling pathway, small cell lung cancer and Epstein-Barr virus infection.Moreover the twenty-five hub genes were found in DEGs,which are: PIK3C2B, OGT, PTEN, YY1, KLF5, RARA, ING3, HES1,BRD4,TRO,E2F2,IGSF3,FBXW7,LYN,GJB3,PIK3C3,TRAF3,RYBP,IRAK2,NEURL2,RAD21,PTPN1,DDX58,MYL12A and TGFBRAP1.Accord- ing to the survival analysis of hub genes,five out of the twenty-five hub genes, namely, OGT,IGSF3, PIK3C2B,TRO and NEURL2 are significantly correlated with EC.

The functions of each hub gene were different.OGT exists in healthy endometrium tissues and endometrium cancer tissues. Low expression of OGT was associated with prognosis in the endometrium tumor (P = 1.6616E-06).OGT were reported that it is overactivated in many kinds of cancers and is involved in the advanced progression of cancer[18],the increase of O-GlcNAc modification of cell protein is a common feature of tumor cells, and the glycosylation level of O-GlcNAc in tumor cells with higher deterioration degree is higher[19],and stably interacts with and modifies all three TET proteins,and help produce TET to form O-GlcNAcylated TET and can also interact with TET to form a complex with the ability to further modify chromatin which participated in controlling embryonic development and cancer progression[20],OGT is highly dependent on glucose and this modification generally becomes more abundant in various metabolic pathways in cancer cells[21].OGT controls ovarian cancer cell motility through the RhoA/ROCK signal pathway[22].However, studies suggest that TET is related to the onset and progression of endometrium cancer[23].In another study demonstrated,OGT plays a very important role for maintenance and stress response of HSCs through OGT-PINK1 axis[24],however,PINK1 can lead to mitochondrial dysfunction by inhibiting a number of mitochondrial genes in 30% of endometrial cancers[25].

IGSF3 (Immunoglobulin Superfamily Member 3) is a protein coding gene. The protein encoded by this gene is an immunoglobulin-like membrane protein containing several V-type Ig-like domains. IGSF3 can active the the NF-κB signaling pathway in hepatocellular carcinoma progression[26].The presence of tumor necrosis within primary tumors is result to hypoxia, angiogenesis and inflammation responses,the majority of them associated with NF-kB[27].We have detected abnormal expression of NF-κB signaling pathway in various cancer types[28], and it is generally believed that NF-κB should be a valuable pharmaceutical target[29].In another medical literature said that NF-κB RelB protein is highly expressed in EC samples and cell lines, and mainly increased in endometrioid adenocarcinoma[30].So,IGSF3 may represent a considerable molecular target for EC.

PIK3C2B is strongly related to tumour stage and vessel invasion, which was closely and positively connected with prognosis in colon cancer[31].In addition, PIK3C2B can regulate different cell functions, such as cell proliferation, invasion and cancer progression[32].PIK3C2B can control cell invasion partly by regulating the MEK/ERK pathway and partly by activating additional signalling pathways that modulate prostate cancer cell migration[32].And in a previous study,PIK3C2B had impacts on prognostic OS of ovarian cancer(OC), resulting in a reduction in the median survival time of OC by 7 to 10 months[33].Then PIK3C2B participates in PI3K-Akt signaling pathway,and PI3K network belongs to the most common events in human cancers[33],Philip et al. reported that inhibition of PI3K/Akt/mTOR signaling increased the sensitivity of endometrial carcinoma cells to some targeted

therapies[34],so blocking PIK3C2B may be an immunotherapeutic pathway for the treatment of endometrial carcinoma.

TRO gene encodes a membrane protein that mediates cell adhesion between trophoblastic cells and the epithelial cells of the endometrium,it can promote the proliferation of embryonic cells and the invasion of trophoblast cells into endometrium.It is worth noting that the structure of TRO is exactly the same as that of MAGE-D3 carboxy-terminal functional region in MAGE tumor gene family, suggesting that TRO may have special biological functions[35].The data from TCGA are discussed by bioinformatics method, and it is found that TRO may play a negative role in the prognosis of 13 cancer types, such as breast cancer, head and neck squamous cell carcinoma and non-small cell lung cancer. They believe that down-regulation of TRO affects the growth of cancer cells[36].However, the invasion, metastasis and rapid proliferation of malignant tumors are just like the adhesion between trophoblast cells and endometrial cells, invasion into endometrial matrix and subsequent rapid proliferation during embryo development and implantation.In recent years, the mechanism of this gene in tumors is seldom studied, which needs further exploration.

NEURL2 is an asymmetric division gene for normal stem cells,which is very important to maintain the stem cells of normal stem cells and tumor stem cells[37].Neurl2 can participate in the development of lung cancer by mediating the activity of twist 2.Moreover, the analysis of TCGA shows that the expression of twist 2 is positively correlated with the development of lung adenocarcinoma, while NEURL2 is negatively correlated with the development of lung adenocarcinoma.However, the relationship between Neurl2 and endometrial cancer is still unclear, because it is related to the prognosis of endometrial cancer patients, and it can be used as the research direction of follow-up treatment.

IR is a kind of biological effect reduction characterized by reduced glucose uptake and inhibition of glucose output by adipose tissue and muscle,the main manifestations are that the sensitivity of liver, muscle, fat and other target tissues to insulin decreases and the uptake and utilization of glucose decreases[38].However, with the prolongation of IR, the normal blood glucose level of the body cannot be maintained, resulting in abnormal blood glucose metabolism and other metabolic disorders, which in turn increases the risk of various diseases.And IR may play a crucial role in the development and metastasis of tumors,the increase of IGF-1 levels and hyperinsulinemia in obese and overweight people promote the transformation of endometrial tumors[39].IR can affect the level of sex hormones in EC patients,the increase of insulin level can inhibit the synthesis of sex hormones in liver and promote the increase of free estrogen and male hormones, while the increase of estrogen level can promote endometrial hyperplasia. Under the long-term stimulation of estrogen, the endometrium changes from hyperplasia to canceration, which increases the risk of postoperative recurrence and death.Insulin not only plays a crucial role in metabolism, but also can inhibit apoptosis and promote cell proliferation. Insulin-like growth factor is abundantly expressed in the intimal tissue of EC patients, which activates the downstream signal pathway, induces intimal hyperplasia and promotes the growth of EC cells.IR serves a crucial role in the development and progression of endometrial tumors.

For the sake of exploring the potential mechanism of insulin resistance on the pathogenesi of endometrial cancer, we analyzed the enrichment, expression, mutation and immune infiltration of related genes. The results showed that five key genes(OGT,IGSF3, PIK3C2B,TRO and NEURL2) mainly affected the prognosis of endometrial cancer patients. In short, the interaction between five key genes may become a new anti-tumor treatment strategy.

5 | Conclusion

In this study, the relationship between insulin resistance and endometrial cancer was systematically analyzed, and these results indicate that OGT,IGSF3, PIK3C2B,TRO and NEURL2 may play a pivotal role in the occurrence and development of endometrial cancerous tissue, which provides a unique perspective for the therapeutic regimen of endometrial cancer and may be a new therapeutic target for endometrial cancer.

Abbreviations

EC,endometrial cancer;IR,insulin resistance;DEGS,differentially expressed genes;PPI,Protein-Protein Interaction.

Declarations

ACKNOWLEDGMENTS

The authors would like to thank all the authors who shared the public database data in the research.

CONFLICT OF INTEREST

None.

AUTHOR CONTRIBUTIONS

D.D.D.:Conceptualization, Methodology, Writing - original draft. Q.Z.:Supervision, Validation. Z.H. and H.X.H.:Data curation, Visualization, Investigation. M.Z.Z.:Supervision, Writing - review & editing.

DATA AVAILABILITY STATEMENTT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

DATA SHARING AND DATA ACCESSIBILITY

GEO database publicly provides all the data supporting the findings of this study , which was at https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc at =GSE106191 and GSE63992.

Funding:

This work was supported by the Hubei Provincial Health Commission Joint Fund Key Project, China (Grant No. WJ2019H500).

References

1. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. 2018;68(6):394-424.
2. Buhtoiarova TN, Brenner CA, Singh M. Endometrial Carcinoma: Role of Current and Emerging Biomarkers in Resolving Persistent Clinical Dilemmas. Am J Clin Pathol. 2016;145(1):8-21.

3. Wang Q, Zhang F, Gao H, Xu Y. Successful treatment of a patient with brain metastases from endometrial cancer using Niraparib: a case report. Ann Palliat Med. 2021;10(1):818-27.
4. Moritake T, Kojima J, Kubota K, Terauchi F, Isaka K, Nishi H. Kruppel-like factor 5 is upregulated and induces cell proliferation in endometrial cancer. Oncol Lett. 2021;21(6):484.
5. Stavropoulos A, Varras M, Philippou A, Vasilakaki T, Varra VK, Varra FN, et al. Immunohistochemical expression of insulin-like growth factor-1Ec in primary endometrial carcinoma: Association with PTEN, p53 and survivin expression. Oncol Lett. 2020;20(6):395.
6. Shan W, Ning C, Luo X, Zhou Q, Gu C, Zhang Z, et al. Hyperinsulinemia is associated with endometrial hyperplasia and disordered proliferative endometrium: a prospective cross-sectional study. Gynecol Oncol. 2014;132(3):606-10.
7. Mu N, Zhu Y, Wang Y, Zhang H, Xue F. Insulin resistance: a significant risk factor of endometrial cancer. Gynecol Oncol. 2012;125(3):751-7.
8. El-Mir MY, Nogueira V, Fontaine E, Averet N, Rigoulet M, Leverve X. Dimethylbiguanide inhibits cell respiration via an indirect effect targeted on the respiratory chain complex I J Biol Chem. 2020;275(1):223-8.
9. Pernicova I, Korbonits M. Metformin–mode of action and clinical implications for diabetes and cancer. Nat Rev Endocrinol. 2014;10(3):143-56.
10. Veilleux A, Mayeur S, Berube JC, Beaulieu JF, Tremblay E, Hould FS, et al. Altered intestinal functions and increased local inflammation in insulin-resistant obese subjects: a gene-expression profile analysis. BMC Gastroenterol. 2015;15:119.
11. Sugiyama Y, Gotoh O, Fukui N, Tanaka N, Hasumi K, Takazawa Y, et al. Two Distinct Tumorigenic Processes in Endometrial Endometrioid Adenocarcinoma. Am J Pathol. 2020;190(1):234-51.
12. Sun Y, Xiaoyan H, Yun L, Chaoqun L, Jialing W, Liu Y, et al. Identification of Key Candidate Genes and Pathways for Relationship between Ovarian Cancer and Diabetes Mellitus Using Bioinformatical Analysis. Asian Pac J Cancer Prev. 2019;20(1):145-55.
13. Huang da W, Sherman BT, Lempicki RA. Systematic and integrative analysis of large gene lists using DAVID bioinformatics resources. Nat Protoc. 2009;4(1):44-57.
14. Cerami E, Gao J, Dogrusoz U, Gross BE, Sumer SO, Aksoy BA, et al. The cBio cancer genomics portal: an open platform for exploring multidimensional cancer genomics data. Cancer Discov. 2012;2(5):401-4.
15. Chandrashekar DS, Bashel B, Balasubramanya SAH, Creighton CJ, Ponce-Rodriguez I, Chakravarthi B, et al. UALCAN: A Portal for Facilitating Tumor Subgroup Gene Expression and Survival Analyses. Neoplasia. 2017;19(8):649-58.
16. Zhou Q, Zhang F, He Z, Zuo MZ. E2F2/5/8 Serve as Potential Prognostic Biomarkers and Targets for Human Ovarian Cancer. Front Oncol. 2019;9:161.
17. Li T, Fu J, Zeng Z, Cohen D, Li J, Chen Q, et al. TIMER2.0 for analysis of tumor-infiltrating immune cells. Nucleic Acids Res. 2020;48(W1):W509-W14.
18. Chen Y, Liu J, Zhang W, Kadier A, Wang R, Zhang H, et al. O-GlcNAcylation Enhances NUSAP1 Stability and Promotes Bladder Cancer Aggressiveness. Onco Targets Ther. 2021;14:445-54.
19. Ma Z, Vosseller K. Cancer metabolism and elevated O-GlcNAc in oncogenic signaling. J Biol Chem. 2014;289(50):34457-65.

20. Hrit J, Goodrich L, Li C, Wang BA, Nie J, Cui X, et al. OGT binds a conserved C-terminal domain of TET1 to regulate TET1 activity and function in development. Elife. 2018;7.
21. Lee H, Oh Y, Jeon YJ, Lee SY, Kim H, Lee HJ, et al. DR4-Ser424 O-GlcNAcylation Promotes Sensitization of TRAIL-Tolerant Persisters and TRAIL-Resistant Cancer Cells to Death. Cancer Res. 2019;79(11):2839-52.
22. Niu Y, Xia Y, Wang J, Shi X. O-GlcNAcylation promotes migration and invasion in human ovarian cancer cells via the RhoA/ROCK/MLC pathway. Mol Med Rep. 2017;15(4):2083-9.
23. Ciesielski P, Jozwiak P, Wojcik-Krowiranda K, Forma E, Cwonda L, Szczepaniec S, et al. Differential expression of ten-eleven translocation genes in endometrial cancers. Tumour Biol. 2017;39(3):1010428317695017.
24. Murakami K, Kurotaki D, Kawase W, Soma S, Fukuchi Y, Kunimoto H, et al. OGT Regulates Hematopoietic Stem Cell Maintenance via PINK1-Dependent Mitophagy. Cell Rep. 2021;34(1):108579.
25. Rashid S, Freitas MO, Cucchi D, Bridge G, Yao Z, Gay L, et al. MLH1 deficiency leads to deregulated mitochondrial metabolism. Cell Death Dis. 2019;10(11):795.
26. Sheng P, Zhu H, Zhang W, Xu Y, Peng W, Sun J, et al. The immunoglobulin superfamily member 3 (IGSF3) promotes hepatocellular carcinoma progression through activation of the NF-kappaB pathway. Ann Transl Med. 2020;8(6):378.
27. Bredholt G, Mannelqvist M, Stefansson IM, Birkeland E, Bo TH, Oyan AM, et al. Tumor necrosis is an important hallmark of aggressive endometrial cancer and associates with hypoxia, angiogenesis and inflammation responses. Oncotarget. 2015;6(37):39676-91.
28. Zhang Q, Lenardo MJ, Baltimore D. 30 Years of NF-kappaB: A Blossoming of Relevance to Human Pathobiology. Cell. 2017;168(1-2):37-57.
29. Durand JK, Baldwin AS. Targeting IKK and NF-kappaB for Therapy. Adv Protein Chem Struct Biol. 2017;107:77-115.
30. Ge QL, Liu SH, Ai ZH, Tao MF, Ma L, Wen SY, et al. RelB/NF-kappaB links cell cycle transition and apoptosis to endometrioid adenocarcinoma tumorigenesis. Cell Death Dis. 2016;7(10):e2402.
31. Liu Z, Li X, Ma J, Li D, Ju H, Liu Y, et al. Integrative Analysis of the IQ Motif-Containing GTPase-Activating Protein Family Indicates That the IQGAP3-PIK3C2B Axis Promotes Invasion in Colon Cancer. Onco Targets Ther. 2020;13:8299-311.
32. Mavrommati I, Cisse O, Falasca M, Maffucci T. Novel roles for class II Phosphoinositide 3-Kinase C2beta in signalling pathways involved in prostate cancer cell invasion. Sci Rep. 2016;6:23277.
33. Yan B, Xiong C, Huang F, Zhang M, Mo Y, Bai H. Big data-based identification of methylated genes associated with drug resistance and prognosis in ovarian cancer. Medicine (Baltimore). 2020;99(27):e20802.
34. Philip CA, Laskov I, Beauchamp MC, Marques M, Amin O, Bitharas J, et al. Inhibition of PI3K-AKT-mTOR pathway sensitizes endometrial cancer cell lines to PARP inhibitors. BMC Cancer. 2017;17(1):638.
35. Chomez P, De Backer O, Bertrand M, De Plaen E, Boon T, Lucas S. An overview of the MAGE gene family with the identification of all human members of the family. Cancer Res. 2001;61(14):5544-51.
36. Hu S, Yuan H, Li Z, Zhang J, Wu J, Chen Y, et al. Transcriptional response profiles of paired tumor-normal samples offer novel perspectives in pan-cancer analysis. Oncotarget. 2017;8(25):41334-47.
37. Liu D, Xing HR, Liu Y, Sun Z, Ye T, Li J, et al. Asymmetric Division Gene Neurl2 Mediates Twist2 Regulation of Self-Renewal of Mouse Lewis Lung Cancer Stem Cells. J Cancer. 2019;10(15):3381-8.

38. Das P, Biswas S, Mukherjee S, Bandyopadhyay SK. Association of Oxidative Stress and Obesity with Insulin Resistance in Type 2 Diabetes Mellitus. Mymensingh Med J. 2016;25(1):148-52.
39. Wu Y, Yakar S, Zhao L, Hennighausen L, LeRoith D. Circulating insulin-like growth factor-I levels regulate colon cancer growth and metastasis. Cancer Res. 2002;62(4):1030-5.

Tables

TABLE 1. Identification of the up-regulated and down-regulated differential genes

DEGs	Genes Name
Up-regulated	GIP TRIM31 ZG16 TRIM15 GNL3L LAMB3 E2F2 TRNP1 YY1 ITPRIPL2 AGO2 MYL12A TRAF3 AKAP7 ZIC1 INF2 CFB AAK1 PIK3C2B PITX1 OTUD7A IRAK2 FAM107B GPR35 LYN PDCD7 KDM2A TGFBRAP1 RAD21 UBXN2A TYMS IGSF3 USP9X KCNQ4 BRD4 DENR PTPN1 EDN1 UBE2H RNF19A FAM170A RRP1 TJP2 EPT1 TPM4 MAPKAPK3 UBE3C ARHGAP5 ZFP91 ERC1 SDCCAG3 FRMD8 ZNF92 SPATA5 VEZT TCF20 GNB1L SPN HES1 CLIC5 MSL3 HRH3 KLF5 GCNT2 FBXW7 CNGB1 UHMK1 BTBD7 SPATA8 GJB3 SLC7A13 SMCR8 CHRNA4 DDX58 ADGRF1 IL4I1 GPR31 SIM2 MYO19 PCDHGA11 CXORF38
Down-regulated	SLC9B2 KCNN3 SLC25A17 FKBP7 FMNL3 MVB12B MOSPD1 ZFP92 NEURL2 P2RY14 RHOBTB1 MAGED1 VPS16 RARA MAP4 ZXDA LOC202181 SCAMP4 COG5 PTGER3 EIF3J-AS1 PFKL SUPT3H LSS LTBP4 SMARCD3 NEK1 ADAM12 PIK3C3 CAB39L MIB1 GPALPP1 VPS13B TCF21 ANKRD50 OGT COL21A1 TMEM178A NPHP4 ZBED8 LINC00938 CAMTA1 ELF2 PTEN LMO4 RYBP GPX8 RERGL SEC63 ZFP90 ZCCHC11 MEG3 UNC45A FAM118A CCDC80 AMPH AZI2 SERPINF1 MYO9A ING3 RUNX1T1 TTC5 MAGED2 TRO ZNF704 PLA2R1 PCDHGA1

TABLE 2. Gene ontology analysis of biological processes,cellular components and molecular functional components of different expressed genes

GO	Expression	Category	Term	Count %	*P*-Value
GO:0045944	BP	positive regulation of transcription from RNA polymerase II promoter	18	12.2	0.0023
GO:0045893	BP	regulation of transcription, DNA-templated	11	7.5	0.0087
GO:0051091	BP	positive regulation of sequence-specific DNA binding transcription factor activity	5	3.4	0.0102
GO:0071300	BP	phosphatidylinositol-mediated signaling	5	3.4	0.0105
GO:0034696	BP	response to prostaglandin F	2	1.4	0.0160
GO:0005515	MF	protein binding	80	54.4	0.022
GO:0008270	MF	zinc ion binding	17	11.6	0.017
GO:0000987	MF	transcription factor activity, sequence-specific DNA binding	14	9.5	0.032
GO:0003713	MF	transcription coactivator activity	7	4.8	0.0037
GO:0008134	MF	transcription factor binding	7	4.8	0.022
GO:0005737	CC	cytoplasm	55	37.4	0.005
GO:0005654	CC	nucleoplasm	31	21.1	0.022
GO:0009898	CC	cytoplasmic side of plasma membrane	3	2	0.04
GO:0016459	CC	myosin complex	3	2	0.05
GO:0005829	CC	cytosol	33	22.45	0.081

BP=Biological Processes,MF=MolecularFunctional,CC= Cellular Components

TABLE 3. KEGG pathway analysis of differentially expressed genes

Pathway	Gene	%	*P*-Value	Genes ID
Small Cell Lung Cancer	4	2.721088	0.028738	LAMB3, TRAF3, PTEN, E2F2
NF-kappa B signaling pathway	4	2.721088	0.030506	LYN, DDX58, TRAF3, ERC1
Epstein-Barr virus infection	4	2.721088	0.070305	LYN, SPN, DDX58, TRAF3
Pathways in cancer	7	4.761904	0.085880	LAMB3, TRAF3, PTGER3, PTEN, RARA, E2F2, RUNX1T1
Ubiquitin mediated proteolysis	4	2.721088	0.092106	UBE2H, UBE3C, FBXW7, RHOBTB1

TABLE 4. The relationship between hub gene with EC or IR

Hube Gene	*p- Value*		*Degree*	*EcCentricity*	*Closeness*	*Radiality*	*MNC*
	EC	*IR*					
PIK3C2B	*0.0173*	*0.0100839*	*2*	*0.095238095*	*17.05*	*4.571428571*	*2*
OGT	*0.013*	*0.0456398*	*3*	*0.095238095*	*17.58333333*	*4.595744681*	*2*
PTEN	*0.0415*	*0.0275705*	*10*	*0.114285714*	*24.85*	*5.05775076*	*5*
YY1	*0.000234*	*0.0003979*	*10*	*0.095238095*	*23.98333333*	*4.984802432*	*6*
KLF5	*0.0000535*	*0.0499418*	*4*	*0.095238095*	*17.78333333*	*4.547112462*	*1*
RARA	*0.0252*	*0.0459215*	*4*	*0.095238095*	*18.8*	*4.680851064*	*1*
ING3	*0.0445*	*0.0412682*	*2*	*0.081632653*	*16.52619048*	*4.498480243*	*1*
HES1	*0.0308*	*0.0115331*	*3*	*0.095238095*	*18.33333333*	*4.680851064*	*2*
BRD4	*0.00511*	*0.0310984*	*2*	*0.095238095*	*17.66666667*	*4.656534954*	*2*
TRO	*0.0237*	*0.0172506*	*2*	*0.071428571*	*11.6047619*	*3.635258359*	*1*
E2F2	*0.0315*	*0.0407442*	*5*	*0.095238095*	*19.5*	*4.717325228*	*3*
IGSF3	*0.0195*	*0.0224513*	*1*	*0.081632653*	*13.57619048*	*4.158054711*	*1*
FBXW7	*0.0152*	*0.0364503*	*7*	*0.095238095*	*21.35*	*4.838905775*	*3*
LYN	*0.00235*	*0.0457824*	*2*	*0.095238095*	*16.58333333*	*4.522796353*	*2*
PIK3C3	*0.018*	*0.0230926*	*5*	*0.095238095*	*19.13333333*	*4.656534954*	*2*
TRAF3	*0.000529*	*0.0385245*	*3*	*0.081632653*	*13.77857143*	*4.012158055*	*2*
NEURL2	*0.00402*	*0.0421525*	*2*	*0.081632653*	*15.85952381*	*4.41337386*	*1*
RAD21	*0.00304*	*0.0363896*	*2*	*0.081632653*	*16.22619048*	*4.462006079*	*2*
PTPN1	*0.0439*	*0.049296*	*3*	*0.095238095*	*17.08333333*	*4.534954407*	*2*
DDX58	*0.0336*	*0.0324484*	*3*	*0.095238095*	*16*	*4.41337386*	*2*
GJB3	*0.0365*	*0.0175177*	*2*	*0.035714286*	*4.5*	*0.267857143*	*1*
MYL12A	*0.000214*	*0.0435537*	*2*	*0.063492063*	*11.54087302*	*3.489361702*	*1*
IRAK2	*0.0462*	*0.0009649*	*2*	*0.081632653*	*12.81190476*	*3.939209726*	*2*
TGFBRAP1	*0.00224*	*0.0016201*	*2*	*0.081632653*	*14.11190476*	*4.133738602*	*2*
RYBP	*0.0273*	*0.0329682*	*2*	*0.081632653*	*16.30952381*	*4.462006079*	*2*

Figures

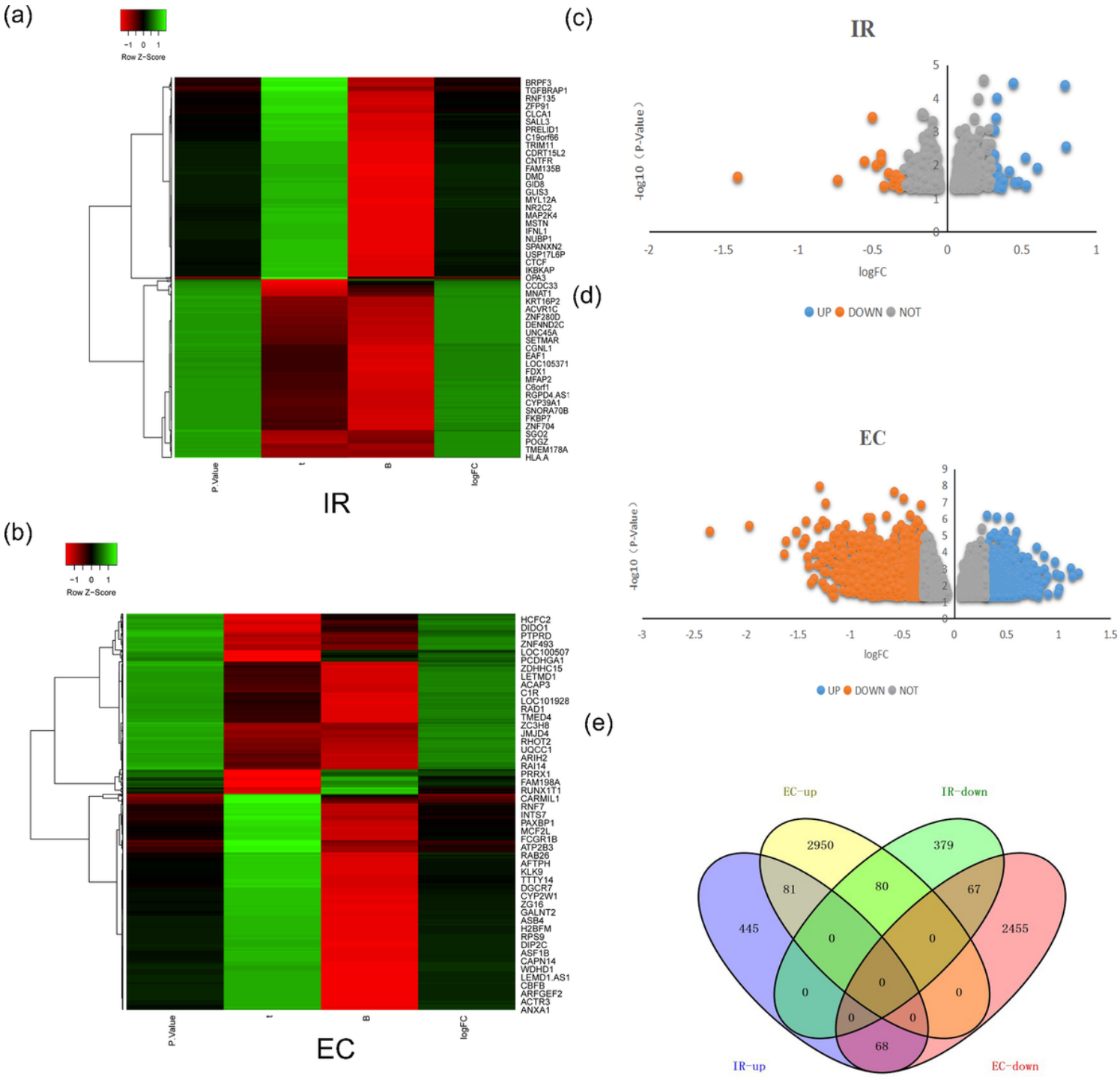


Figure 1

Differentially expressed genes between EC and IR. (a).The expression of the significant genes in IR was shown in the heatmap.The data are from HeatMapper (http://www.heatmapper.ca/expression/). (b). The expression of the significant genes in EC was shown in the heatmap.The data are also from HeatMapper. (c)The volcano plot was showed the expression of the significant genes in IR. (d).The volcano plot was showed the expression of the significant genes in EC. (e). Differentially expressed genes among IR and EC. The data are from VENNY (https://bioinfogp.cnb.csic.es/tools/venny/index.html),there were including 81 communal up-regulated genes and 67 communal down-regulated genes between IR and EC.

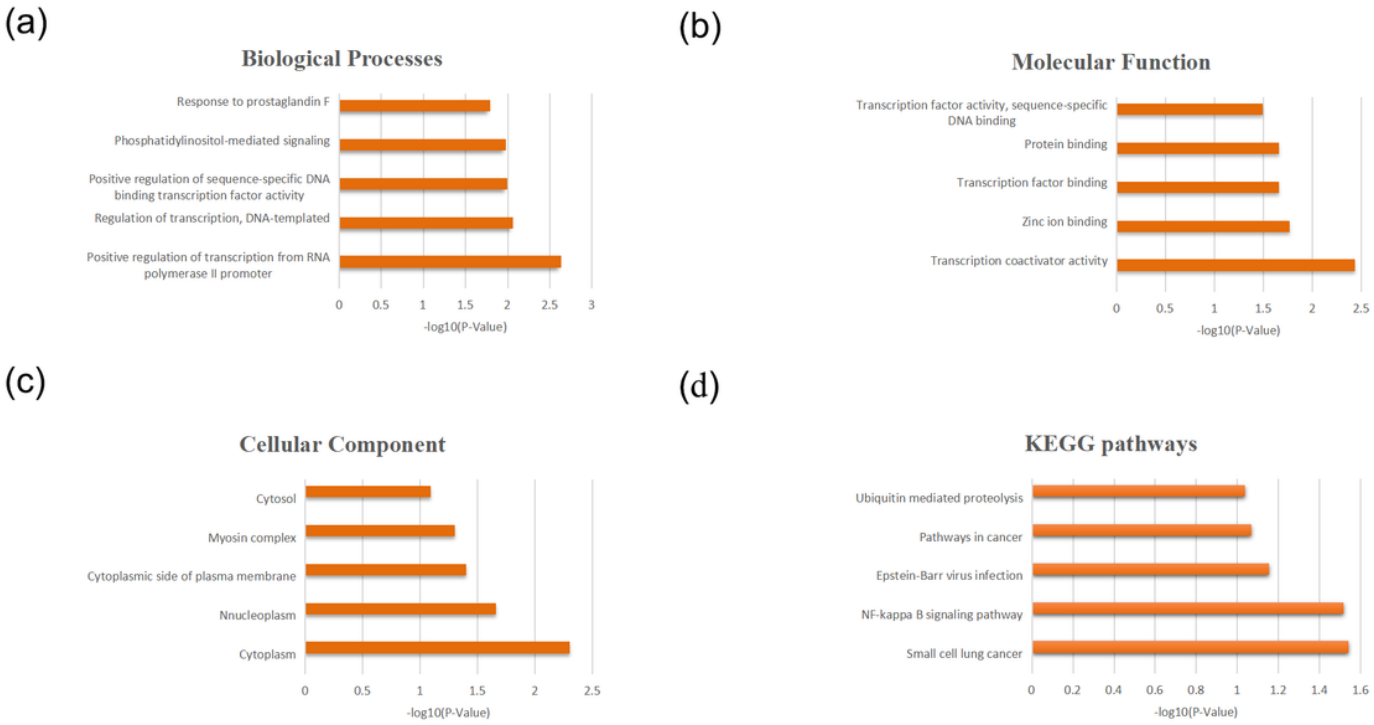
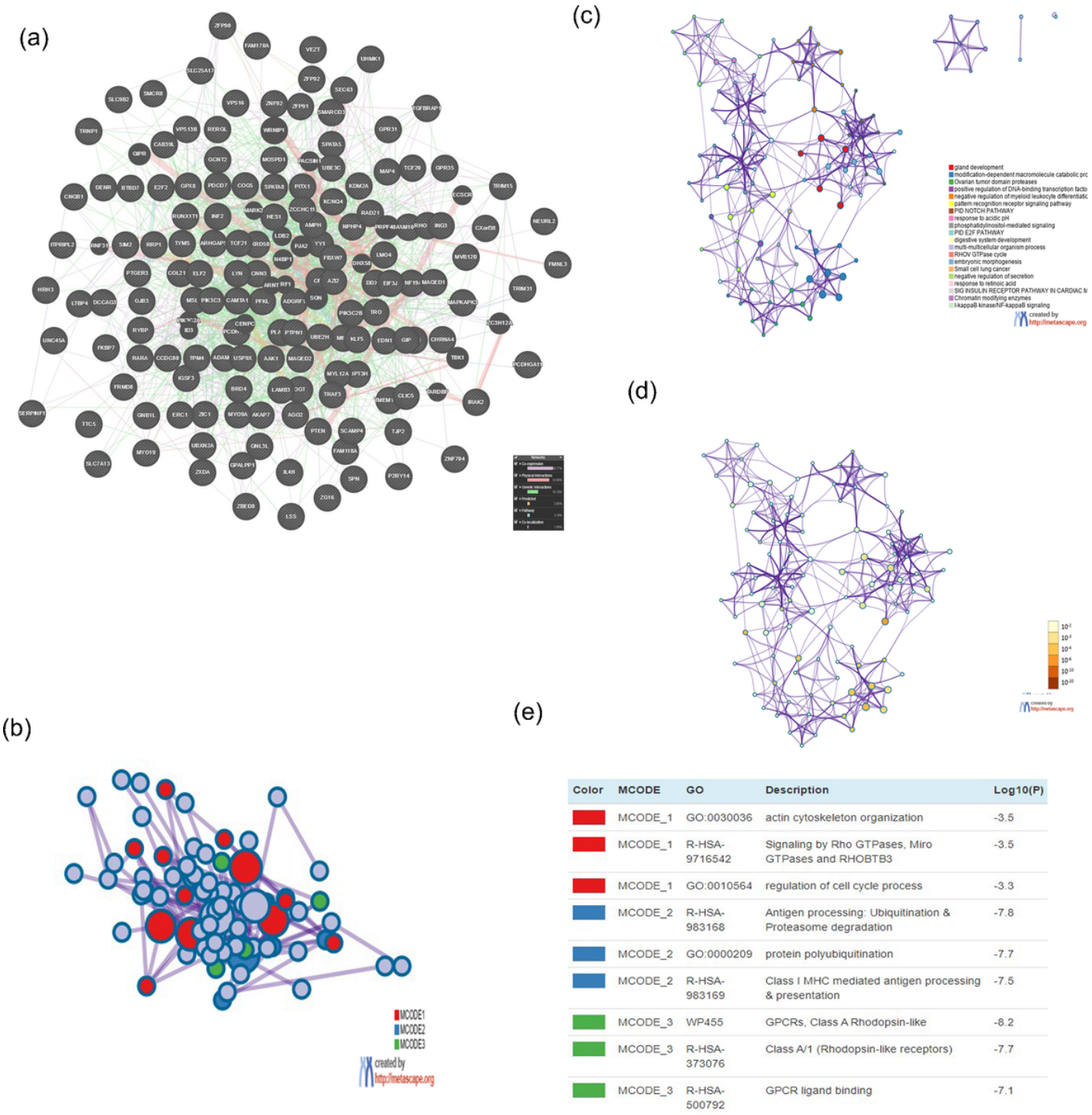


Figure 2

GO enrichment analysis of intersected differential genes. (a) Biological processes analysis of DEGs.(b)Molecular function analysis of DEGs. (c)Cellular component analysis of DEGs. (d).KEGG pathways analysis of DEGs.



Color	MCODE	GO	Description	Log10(P)
	MCODE_1	GO:0030036	actin cytoskeleton organization	-3.5
	MCODE_1	R-HSA-9716542	Signaling by Rho GTPases, Miro GTPases and RHOBTB3	-3.5
	MCODE_1	GO:0010564	regulation of cell cycle process	-3.3
	MCODE_2	R-HSA-983168	Antigen processing: Ubiquitination & Proteasome degradation	-7.8
	MCODE_2	GO:0000209	protein polyubiquitination	-7.7
	MCODE_2	R-HSA-983169	Class I MHC mediated antigen processing & presentation	-7.5
	MCODE_3	WP455	GPCRs, Class A Rhodopsin-like	-8.2
	MCODE_3	R-HSA-373076	Class A/1 (Rhodopsin-like receptors)	-7.7
	MCODE_3	R-HSA-500792	GPCR ligand binding	-7.1

Figure 3

Comprehensive analysis of DEGs. (a)DEGs Protein-Protein Interaction (PPI) Network Complex used by GeneMANIA. (b)Network of GO enriched terms, and the network colored by the color on cluster ID, and nodes with the same color are close to each other. (c)Network of GO enriched terms colored by P-value. (d)-(e).Protein–protein interaction (PPI) network and three most significant MCODE components form the PPI network,functional enrichment analysis of MCODE components.

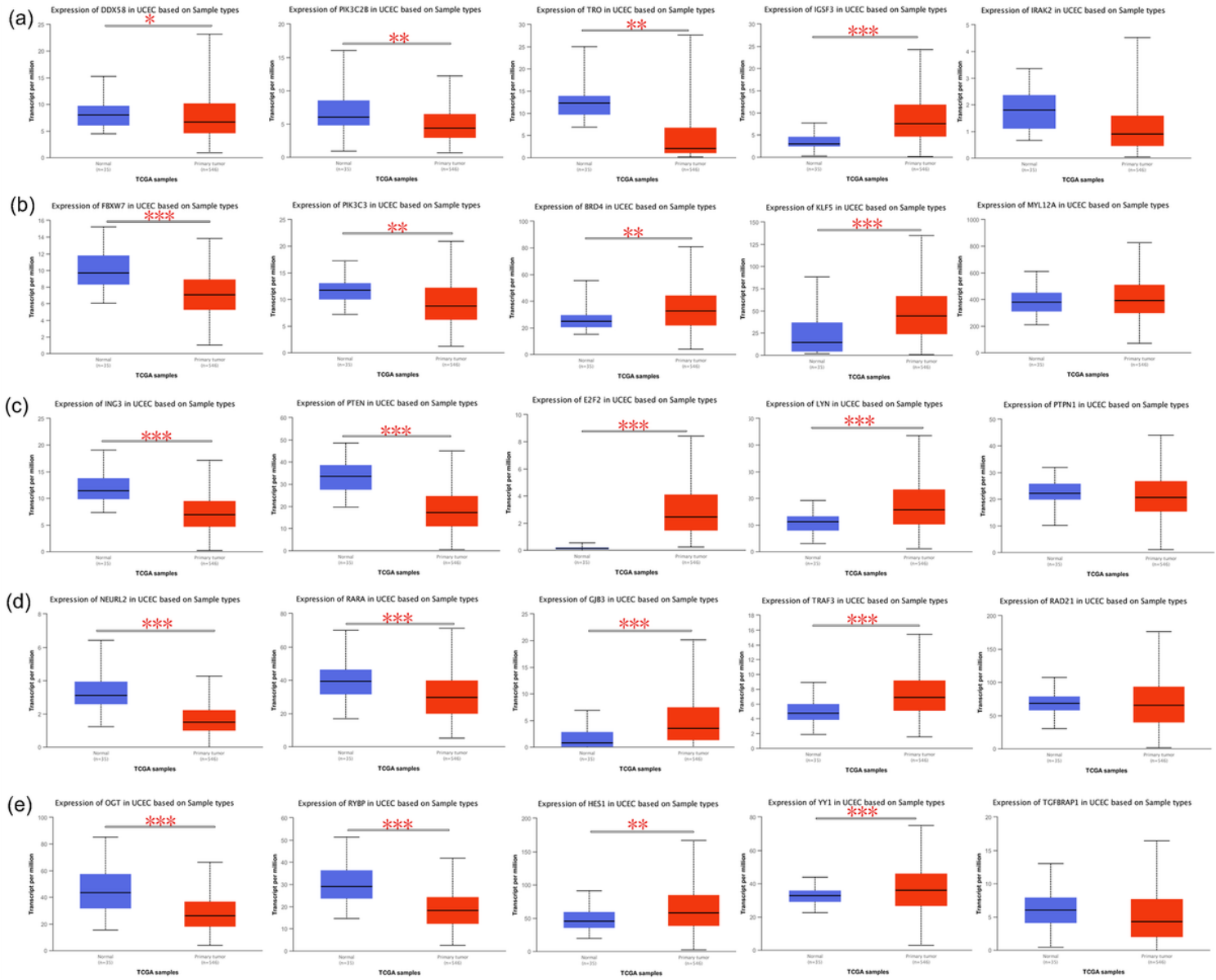


Figure 4

The transcript levels of hub genes in EC patients (UALCAN), the P-value was set at 0.05.*Indicate that the results are statistically significant. *P < 0.05, **P<0.01,***P < 0.001. (a)The expression of DDX58,PIK3C2B,TRO,IGSF3 and IRAK2. (b)The expression of FBXW7,PIK3C3,BRD4,KLF5 and MYL12A. (c)The expression of ING3,PTEN,LYN,E2F2 and PTPN1. (d)The expression of NEURL2,RARA,GJB3, TRAF3 and RAD21.(e)The expression of OGT,RYBP,HES1,YY1 and TGFBRAP1.

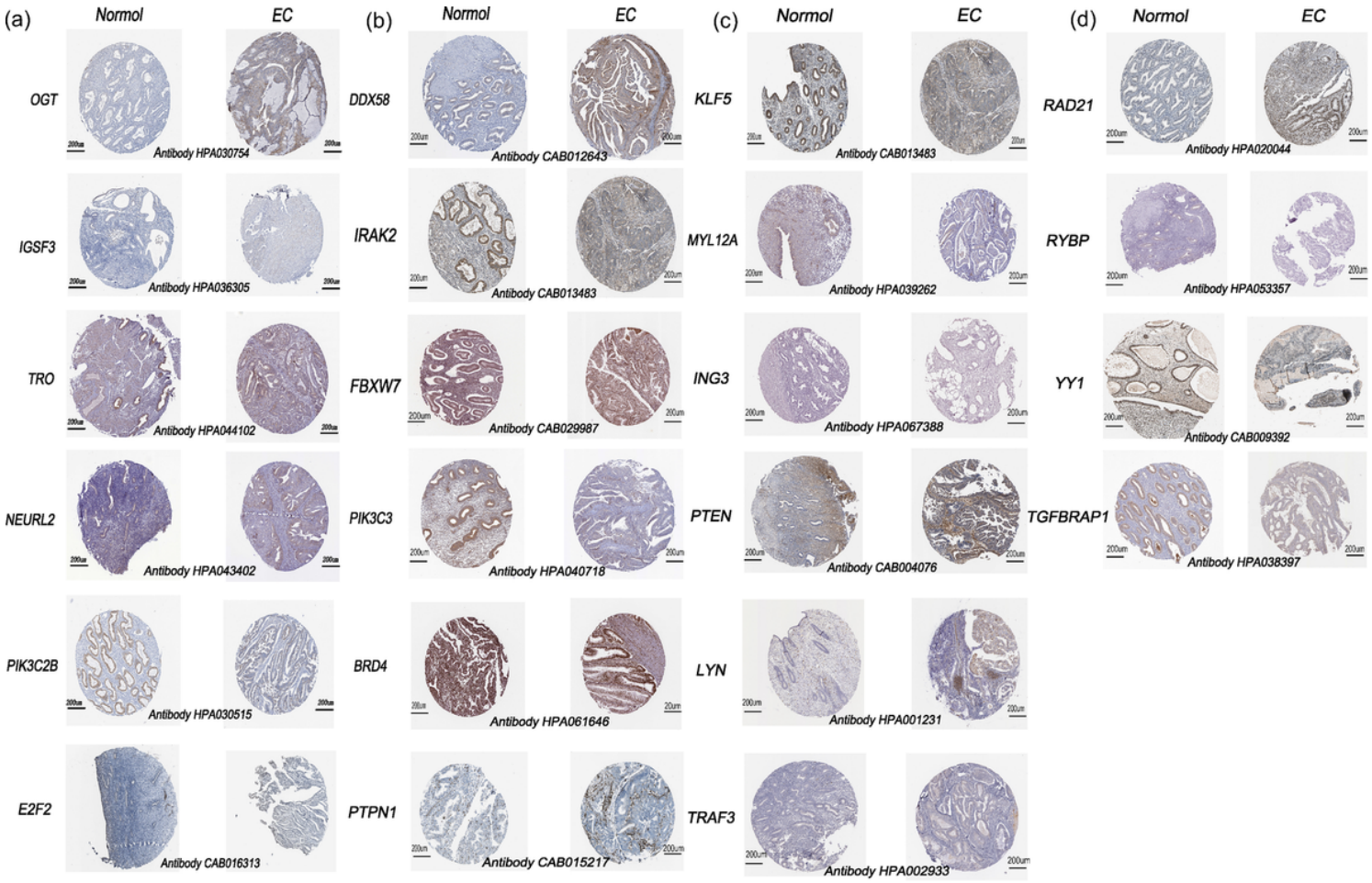


Figure 5

The immunohistochemistry analysis of hub genes in EC patients (HPA). (a)The expression of OGT,IGSF3,TRO,NEURL2,PIK3C2B and E2F2. (b)The expression of DDX58,IRAK2,FBXW7, PIK3C3,BRD4 and PTPN1. (c)The expression of KLF5,MYL12A,ING3,PTEN,LYN and TRAF3. (d)The expression of RAD21,RYBP,YY1 and TGFBRAP1.

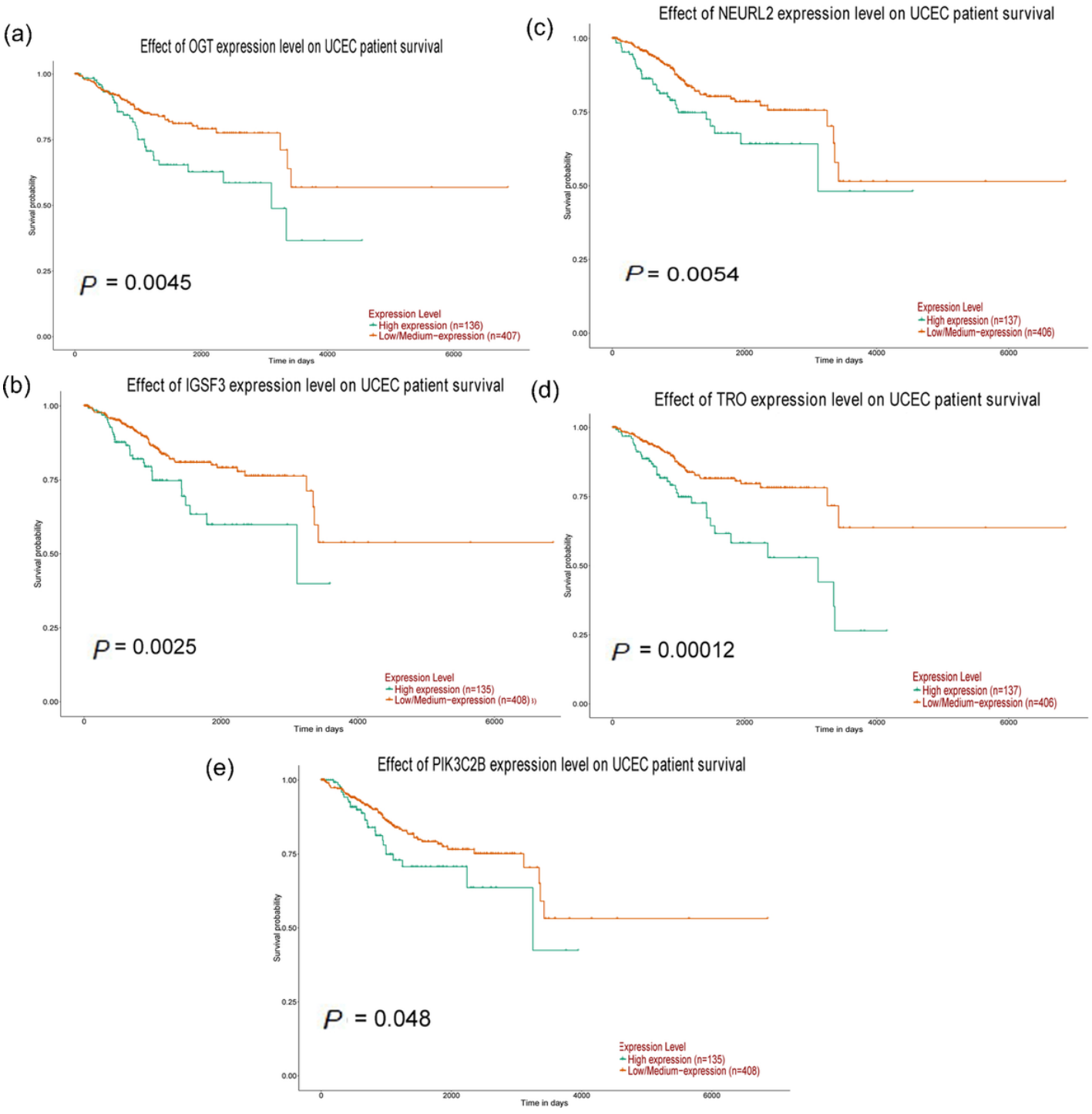


Figure 6

Survival analysis of the hub genes in EC.Red line represents low or medium expression level of a hub gene, and green line represents high expression level.These genes were statistically significant.The X axis indicates overall survival time (day), and the Y axis indicates the survival probability.

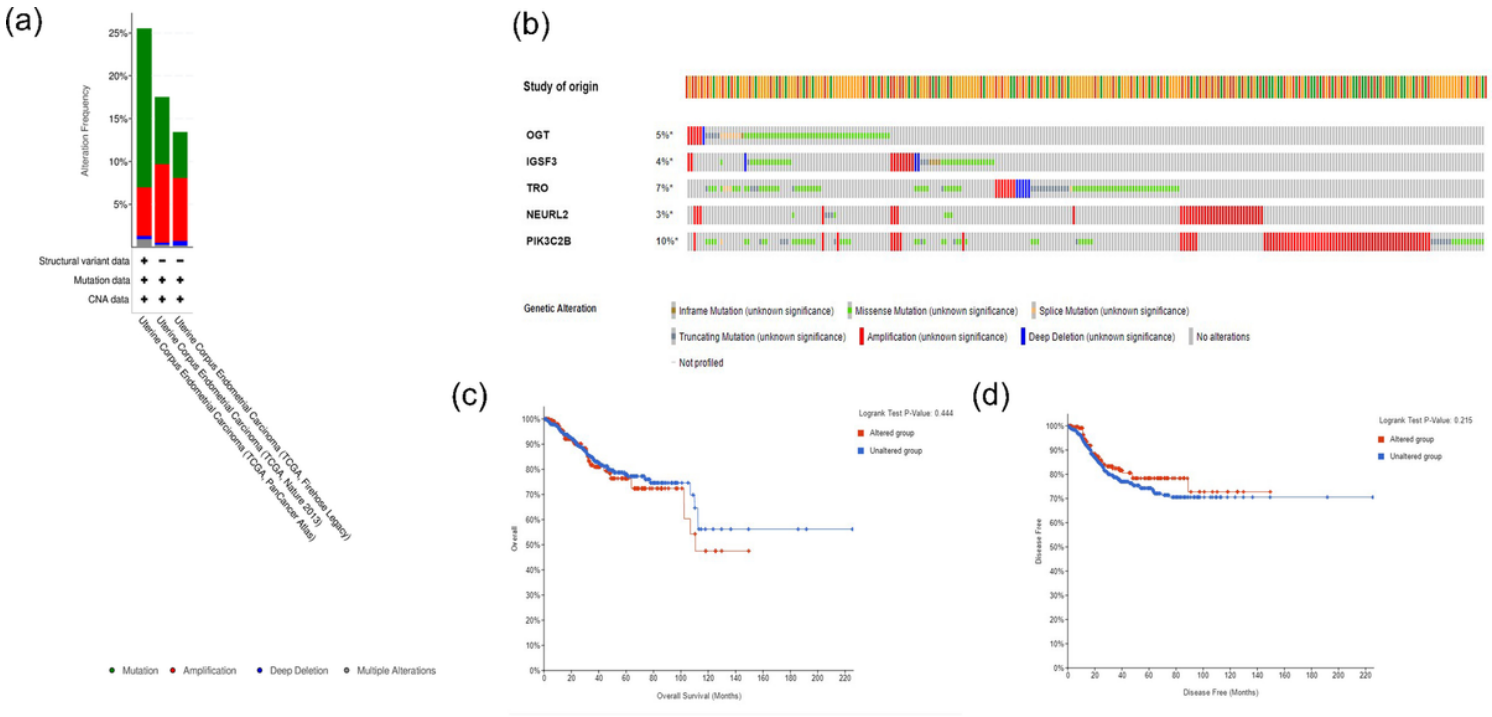


Figure 7

The example shows a visual analysis of the genetic alteration of five hub genes. (a)The summary of mutation of five hub genes. (b)OncoPrint analysis of mutation of five hub genes. (c)Kaplan–Meier plots comparing OS in cases with or without five hub genes mutations.(d)Kaplan–Meier plots comparing DFS in cases with or without five hub genes mutations.

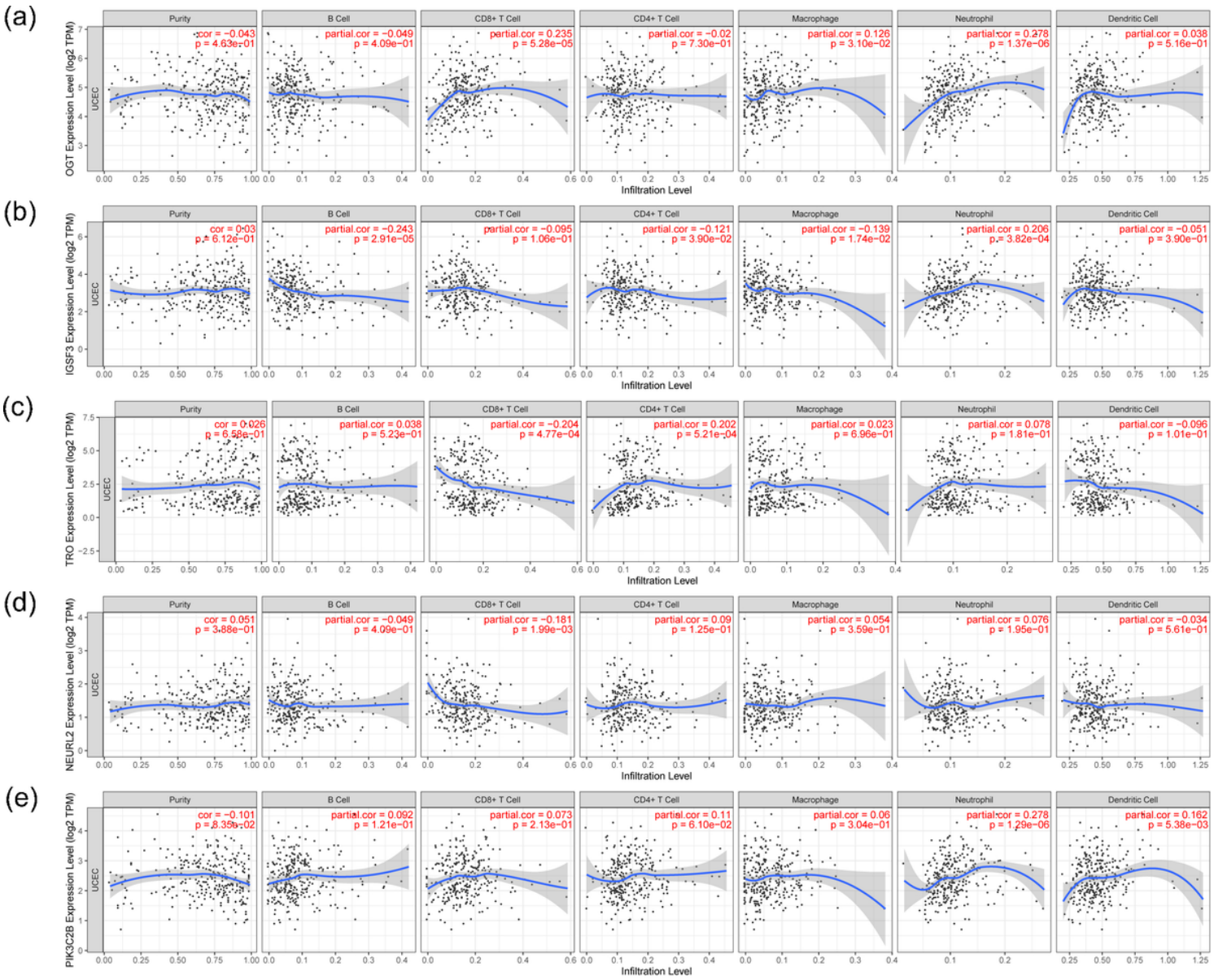


Figure 8

Immune infiltration levels of five hub genes in EC. (a)The transcription levels of OGT in infiltrating immune in EC. (b)The transcription levels of IGSF3 in infiltrating immune in EC. (c)The transcription levels of TRO in infiltrating immune in EC.(d)The transcription levels of NEURL2 in infiltrating immune in EC. (e)The transcription levels of PIK3C2B in infiltrating immune in EC.