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Clinical value screening, prognostic significance and key pathway identification of miR-204-5p in endometrial carcinoma: A study based on the Cancer Genome Atlas (TCGA), and bioinformatics analysis

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ABSTRACT

Background: Endometrial carcinoma is one of the common carcinomas in the female reproductive system. It is reported that miR-204-5p is down-regulated in endometrial carcinoma. However, the mechanism and key pathways of miR-204-5p in endometrial carcinoma have not been clarified.

Material/Methods: We evaluated the expression profiles and prognostic value of miR-204-5p expression in endometrial carcinoma by using bioinformatics analysis of a public dataset from TCGA. Drug of endometrial carcinoma from DrugBank, GO analysis, KEGG analysis, PPI network, mutation, as well as assessment of the prognostic significance were performed to the overlapping target genes of miR-204-5p in endometrial carcinoma. The relative expression levels of miR-204-5p target genes in endometrial carcinoma, including SF3B1, FBXW7, SPOP, and BRD4, were assessed by real-time quantitative polymerase chain reaction (RT-qPCR).

Results: First, through DrugBank website, we obtained target drugs for endometrial carcinoma. MiR-204-5p expression was found to be lower in the endometrial carcinoma tissues than in adjacent normal tissues from TCGA. Next, we identified 143 genes as potential targets of miR-204-5p. Then, through GO enrichment analysis, KEGG signaling pathway and PPI analysis, we revealed the key networks in endometrial carcinoma. Next, mutation and assessment of the prognostic significance of endometrial carcinoma were obtained. At last, in endometrial carcinoma, the relative expression of SF3B1 and BRD4 increased, and the relative expression of FBXW7 decreased.

Conclusions: MiR-204-5p is down-regulated in endometrial carcinoma and affects the prognostic significance of endometrial carcinoma, which might play an important role in the tumorigenesis of endometrial carcinoma.

1. Background

Endometrial carcinoma is the most common carcinoma of the female genital tract [1]. Hormonal and metabolic mechanisms are implicated in the pathogenesis of endometrioid cancer, the numerically predominant subtype [2]. The morbidity of endometrial carcinoma is increasing, and the age of onset is younger than previous years, meanwhile the mortality rate is increasing [3]. The upward trend of endometrial carcinoma is expected to continue [4]. Incidence of endometrial carcinoma is predicted to escalate by 50–100% in 2025 [5]. According to statistics, about 50,000 new cases of endometrial carcinoma are added each year in China, and the number of deaths is 18,000, as well as with the impact of environmental pollution and life stress, the incidence of endometrial carcinoma has increased [6]. The etiology and pathogenesis of endometrial carcinoma have not yet been elucidated. Therefore, insights into the mechanisms of endometrial carcinoma are considered urgent.

MiRNAs are short non-coding RNAs that act as part of the epigenetic machinery. Epigenetic modifications were reported to can be used to explain certain features of complex diseases [7–11]. LncRNA TUG1 sponges miR-204-5p to promote osteoblast differentiation in aortic valve calcification [12]. MiR-204-5p acts as a tumor suppressor in malignant melanoma [13]. MiR-204-5p inhibits invasion and metastasis of laryngeal squamous cell carcinoma [14].

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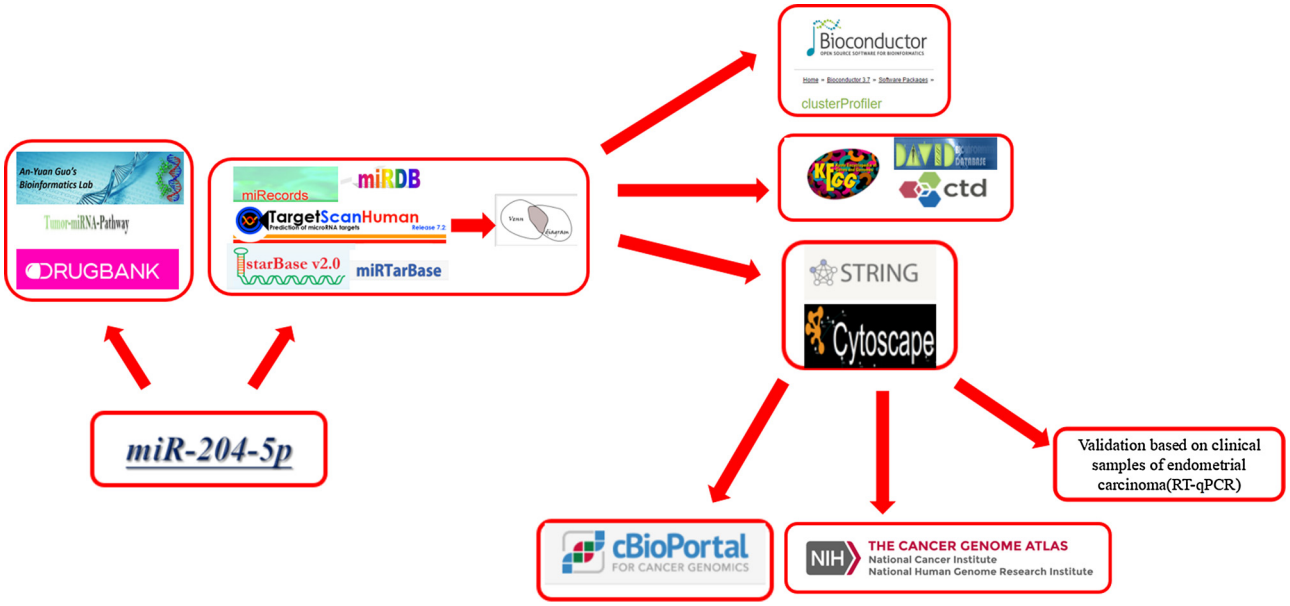

Fig. 1. Flow chart of the multi-parts analysis methodology employed in the endometrial carcinoma study.

The dual regulatory role of miR-204-5p in endometrial carcinoma [15]. A TrkB-STAT3-miR-204-5p regulatory circuitry controls endometrial carcinoma cells [16]. Dysregulation of miRNA-204 mediates migration and invasion of endometrial carcinoma [17].

The aim of this study was to explore the role of miR-204-5p in endometrial carcinoma by analyzing and identifying the expression of miR-204-5p and its target genes in endometrial cancer based on TCGA and bioinformatics analysis (Fig. 1).

2. Material/Methods

2.1. Drug of endometrial carcinoma from DrugBank

DrugBank (https://www.drugbank.ca/) [18–21] is a large online database containing biochemical and pharmacological information about all kinds of drugs. In the present study, we sought to identify the drug targeted endometrial carcinoma using the DrugBank online database.

2.2. MiR-204-5p expression in UCEC clinical tissues from TCGA

TCGA is a large repository of high throughput data in various human carcinomas, which contained large cohorts of over 30 human tumors [22]. Through a TCGA data online analysis tool [23] (http://bioinfo.life.hust.edu.cn/), we gained the miR-204-5p expression profile of diverse types of human cancers and adjacent normal tissues, included endometrial carcinoma, and so on.

2.3. MiR-204-5p prediction of target genes and data screening

The target genes of miR-204-5p were predicted through 5 programs, including miRDB [24–26] (http://www.mirdb.org/), miRrecords [27] (http://c1.accurascience.com/miRecords/), miRTarBase [28–30] (http://mirtarbase.mbc.nctu.edu.tw/php/search.php), starBase [31,32] (http://starbase.sysu.edu.cn/), and TargetScan [33,34] (http://www.targetscan.org/). To increase the prediction accuracy of the results, we finally selected the target genes that were overlapped in at least 3 of 5 databases. Venn diagram (http://bioinformatics.psb.ugent.be/webtools/Venn/) made for the integration between predicted target genes from five prediction software.

2.4. Functional analysis of the overlapping target genes in endometrial carcinoma

To further reveal the biofunction of the overlapped target genes of miR-204-5p in endometrial carcinoma, gene ontology (GO) enrichment analysis was conducted for overlapped target genes of miR-204-5p. The overlapping target genes of miR-204-5p were preprocessed using clusterProfiler [35] package in R language ($p < 0.05$).

2.5. Signaling pathway enrichment analysis of the overlapping target genes of miR-204-5p in endometrial carcinoma

The miR-204-5p's overlapping target genes signaling pathway enrichment were conducted using online websites of DAVID [36–38] (https://david.ncifcrf.gov/summary.jsp), KEGG PATHWAY [39–41] (https://www.kegg.jp/), and comparative toxicogenomics database (CTD) [42–44] (http://ctdbase.org/). Pathways related to endometrial carcinoma and PCOS were screened from CTD. We collected pathways which were reported to be involved in endometrial carcinoma and PCOS. Meanwhile, we compared the endometrial carcinoma related pathways with the KEGG pathways of the overlapping target genes of miR-204-5p, $p < 0.05$ as the cut-off criterion.

2.6. Key candidate genes identification in endometrial carcinoma with the overlapping target genes protein–protein interaction network (PPI) analysis

Search Tool for the Retrieval of Interacting Genes(STRING) [45,46] (https://string-db.org/cgi/input.pl) and MCODE plugin in Cytoscape [47,48] (http://www.cytoscape.org/) were used to performed the PPI analysis of the overlapping target genes of miR-204-5p.

2.7. Mutation of the most significant 14 node degree genes of miR-204-5p in endometrial carcinoma

The cBioPortal [49–51] (http://www.cbioportal.org/) online website provides a resource with cancer genomics datasets, which from TCGA database. The mutation of hub genes in endometrial carcinoma from PPI analysis were conducted using cBioPortal.

2.8. Assessment of the prognostic significance of the overlapping target genes of miR-204-5p in endometrial carcinoma from TCGA

The hub genes in endometrial carcinoma from PPI analysis were preprocessed using RTCGAToolbox [52,53] package in R language. Thus, we obtained the assessment of the prognostic significance of the hub gene of miR-205-5p.

2.9. Validation based on clinical samples of endometrial carcinoma

To further verify the data of endometrial carcinoma from TCGA, we conducted real time RT-qPCR to detect the level of mRNA SF3B1, FBXW7, SPOP and BRD4 with 11 paired endometrial carcinoma clinical samples and normal endometrial clinical samples (n = 22) from the Third Affiliated Hospital of Guangzhou Medical University. The control group was from normal endometrial samples of hysterectomy in patients with uterine myoma. The Ethical Committee of Third Affiliated Hospital of Guangzhou Medical University, China approved the present study. All participating patients provided informed consent and agreement for the research use of the clinical endometrial samples. GAPDH was used as internal reference with the primers as follows:Forward-5′-GGAGCGAGATCCCTCCAAAAT-3′, Reverse-5′-GGC TGTTGTCATACTTCTCATGG-3′. The primers were listed as follows: SF3B1, Forward-5′-TTTGCTGGATACGTGACATCAA-3′; Reverse-5′-CCGGTCTGCAATCTTTGGAGG-3′; FBXW7, Forward-5′- CGACGCCGAA TTACATCTGTC-3′; Reverse-5′-CGTTGAAACTGGGGTTCTATCA-3′; SPOP, Forward-5′- GCCCCGTAGCTGAGAGTTG-3′; Reverse-5′-ACTCGC AAACACCATTTCAGT-3′; BRD4, Forward-5′- GAGCTACCCACAGAAGA AACC-3′; Reverse-5′-GAGTCGATGCTTGAGTTGTGTT-3′. *t*-test was performed to compare the difference of relative expression of mRNA between endometrial carcinoma and normal endometrial tissues with SPSS 22.0.

3. Results

3.1. Drug of endometrial carcinoma from DrugBank

Through DrugBank website, we searched for target drugs for endometrial carcinoma. As a result, therapeutic drugs of endometrial carcinoma included medroxyprogesterone acetate, tamoxifen, cisplatin, anastrozole, carboplatin, doxorubicin, megestrol acetate and so on.

3.2. MiR-204-5p expression in UCEC clinical tissues from TCGA

As the chart shows (Fig. 2), On the one hand, the expression profiling of miR-204-5p revealed that it was overexpressed in some human cancers, such as cholangiocarcinoma, liver hepatocellular carcinoma, lung squamous cell carcinoma, skin cutaneous melanoma, and thymoma, of which miR-204-5p expression was found to be higher in the cancerous tissues than in adjacent normal tissues. On the other hand, miR-204-5p have a tumor-suppressing effect on some cancers, including kidney chromophobe, kidney renal clear cell carcinoma, kidney renal papillary cell carcinoma, esophageal carcinoma, cervical squamous cell carcinoma and endocervical adenocarcinoma, breast invasive carcinoma, pancreatic adenocarcinoma, pheochromocytoma and paraganglioma, prostate adenocarcinoma, stomach adenocarcinoma, thyroid carcinoma, and uterine corpus endometrial carcinoma, of which miR-204-5p expression was found to be lower in the cancerous tissues than in adjacent normal tissues. MiR-204-5p expression in UCEC clinical tissues from TCGA were shown in Fig. 2.

3.3. MiR-204-5p prediction of target genes and data screening

599 genes were identified by miRDB prediction software. 19 genes were identified by miRrecords prediction software. 399 genes were identified by miRTarBase prediction software. 1236 genes were identified by starBase prediction software. 524 genes were identified by TargetScan prediction software. A total of 2777 genes were predicted to be targeted by miR-204-5p in five prediction software. By considering the genes were selected as targets that were overlapped in at least 3 of 5 databases (miRDB, miRrecords, miRTarBase, starBase, TargetScan), we identified 143 genes as potential targets of miR-204-5p. Venn diagram for target genes of miR-204-5p were shown in Fig. 3.

3.4. Functional analysis of the overlapping target genes in endometrial carcinoma

We analyzed the functional roles of 143 potential target genes in terms of biological processes in endometrial carcinoma by GO enrichment analysis. In terms of the biological process, the potential targeted genes of endometrial carcinoma were significantly enriched in some cellular processes, such as epithelial cell proliferation, transcription factor activity: RNA polymerase II proximal promoter sequence-specific DNA binding, transcriptional activator activity: RNA polymerase II transcription regulatory region sequence-specific DNA binding, transcriptional activator activity: RNA polymerase II proximal promoter sequence-specific DNA binding, GTPase activity, transcription coactivator activity, protein acetylation, transcription factor activity: RNA polymerase II transcription factor binding, peptidyl–lysine acetylation, RNA polymerase II transcription cofactor activity, regulation of osteoblast differentiation, regulation of substrate adhesion–dependent cell spreading, circadian regulation of gene expression, regulation of protein acetylation, RNA polymerase II transcription coactivator activity, GDP binding, transcriptional activator activity: RNA polymerase II transcription factor binding, core promoter sequence–specific DNA binding, positive regulation of leukocyte apoptotic process, positive regulation of substrate adhesion–dependent cell spreading, response to monoamine, response to catecholamine, positive regulation of response to endoplasmic reticulum stress, positive regulation of endoplasmic reticulum stress–induced intrinsic apoptotic signaling pathway, regulation of hippo signaling. GO enrichment analysis were shown in Fig. 4 and 5.

3.5. Signaling pathway enrichment analysis of the overlapping key genes of miR-204-5p in endometrial carcinoma

The overlapping target genes signaling pathway enrichment were conducted using online websites of DAVID, KEGG PATHWAY, and comparative toxicogenomics database (CTD). The overlapping target genes mainly enriched in AMPK signaling pathway, Aldosterone synthesis and secretion, Oxytocin signaling pathway, cGMP-PKG signaling pathway, Transcriptional misregulation in cancer, Phosphatidylinositol signaling system, Glucagon signaling pathway, Estrogen signaling pathway, Insulin resistance. Transcriptional misregulation in cancer of pathway linked to carcinogenesis. What's more, the screening results of CTD database showed that 7 pathways out of the 9 KEGG pathways were involved in endometrial carcinoma and 8 pathways were correlated with polycystic ovary syndrome. Additionally, 6 pathways were identified to be associated with both endometrial carcinoma and polycystic ovary syndrome, including AMPK signaling pathway, aldosterone synthesis and secretion, transcriptional misregulation in cancer, glucagon signaling pathway, estrogen signaling pathway, and insulin resistance. Signaling pathway enrichment analysis were shown in Table 1.

3.6. Key candidate genes identification in endometrial carcinoma with the overlapping target genes PPI analysis

To explore the interaction among the overlapped key genes in endometrial carcinoma, STRING and Cytoscape MCODE was performed to construct the PPI network. The most significant 14 node degree genes were SF3B1, KHDRBS1, KHDRBS3, DMTF1, AP1S2, GAPVD1, M6PR,

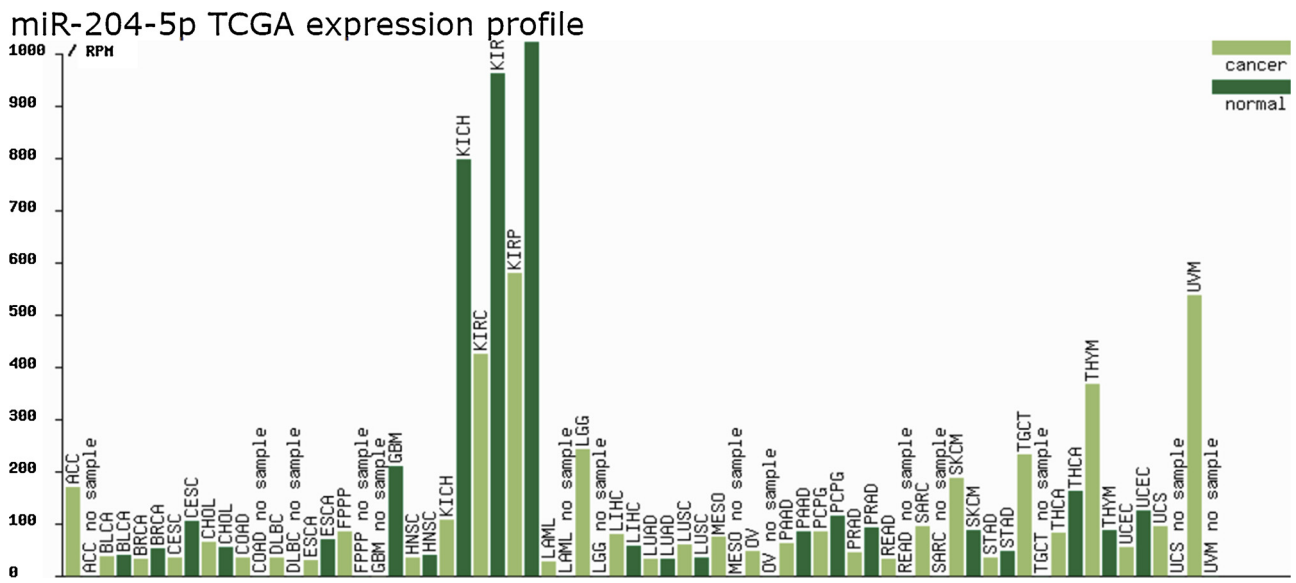

Fig. 2. Expression profile of miR-204-5p from TCGA. MiR-204-5p was expressed lowly in endometrial carcinoma tissues compared with normal tissues.

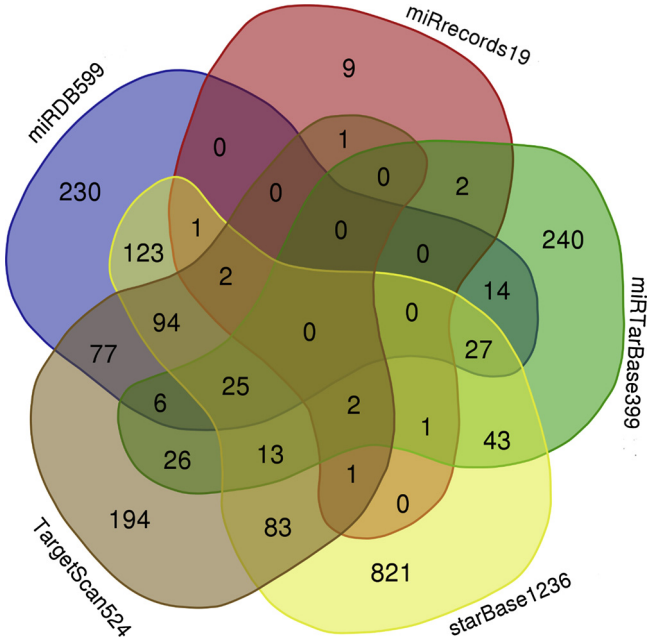

Fig. 3. Venn diagram for the integration between predicted target genes from five prediction software.

DNM2, FBXW7, USP47, SPOP, BRD4, CCNT2, MLLT3. PPI network complex analysis were shown in Fig. 6.

3.7. Mutation of the most significant 14 node degree genes of miR-204-5p in endometrial carcinoma

Given the high frequency of gene amplification in endometrial carcinoma, gene expression in endometrial carcinoma was likely also dysregulated. Therefore, we assessed genetic alterations in endometrial carcinoma data from TCGA, queried with cBioPortal, included SF3B1, KHDRBS1, KHDRBS3, DMTF1, AP1S2, GAPVD1, M6PR, DNM2, FBXW7, USP47, SPOP, BRD4, CCNT2, and MLLT3 [54,55]. cBioPortal results as the chart showed (Fig. 7).

3.8. Assessment of the prognostic significance of the overlapping hub key genes of miR-204-5p in endometrial carcinoma from TCGA

According to the results in endometrial carcinoma from TCGA, difference was found in the overall survival of endometrial carcinoma patients with low expression of the overlapping target genes AP1S2 of miR-204-5p. A favorable prognosis in terms of the overall survival, which was observed in endometrial carcinoma patients with low the overlapping target genes AP1S2 of miR-204-5p expression ($P = 0.027$) (Fig. 8).

3.9. Validation based on clinical samples of endometrial carcinoma

To elucidate biological mechanisms underlying the role of miR-204-5p in promoting endometrial carcinoma cell proliferation, we investigated potential key targets of miR-204-5p, including SF3B1, FBXW7, SPOP, BRD4. We performed real time RT-qPCR to confirm the relative expression of SF3B1, FBXW7, SPOP and BRD4 in the 11 paired endometrial carcinoma clinical samples and normal endometrial clinical samples. The control group was from normal endometrial samples of hysterectomy in patients with uterine myoma. In these patients, the mean relative expression level of SF3B1 was notably higher in endometrial carcinoma tissues (2.5637 ± 0.3947) than that of normal endometrial tissues (1.0284 ± 0.2731). The mean relative expression level of FBXW7 was notably lower in endometrial carcinoma tissues (0.3712 ± 0.1169) than that of normal endometrial tissues (1.0060 ± 0.2130). The mean relative expression level of SPOP was approximately equal to in endometrial carcinoma tissues (0.9877 ± 0.1746) than that of normal endometrial tissues (1.0105 ± 0.1283). Meanwhile, the mean relative expression level of BRD4 was notably higher in endometrial carcinoma tissues (2.2347 ± 0.0990) than that of normal endometrial tissues (1.0485 ± 0.0658). (***$P < 0.001$) (Fig. 9).

4. Discussion

In this study, we identified the aberrantly expressed miR-204-5p involved in endometrial carcinoma through the comparison of miRNA expression profiles in the cancerous tissues than in normal tissues based on validation from TCGA datasets. In addition, we discovered the therapeutic drugs, mutation site and prognostic significance of endometrial carcinoma, as well as novel markers and potential targets for

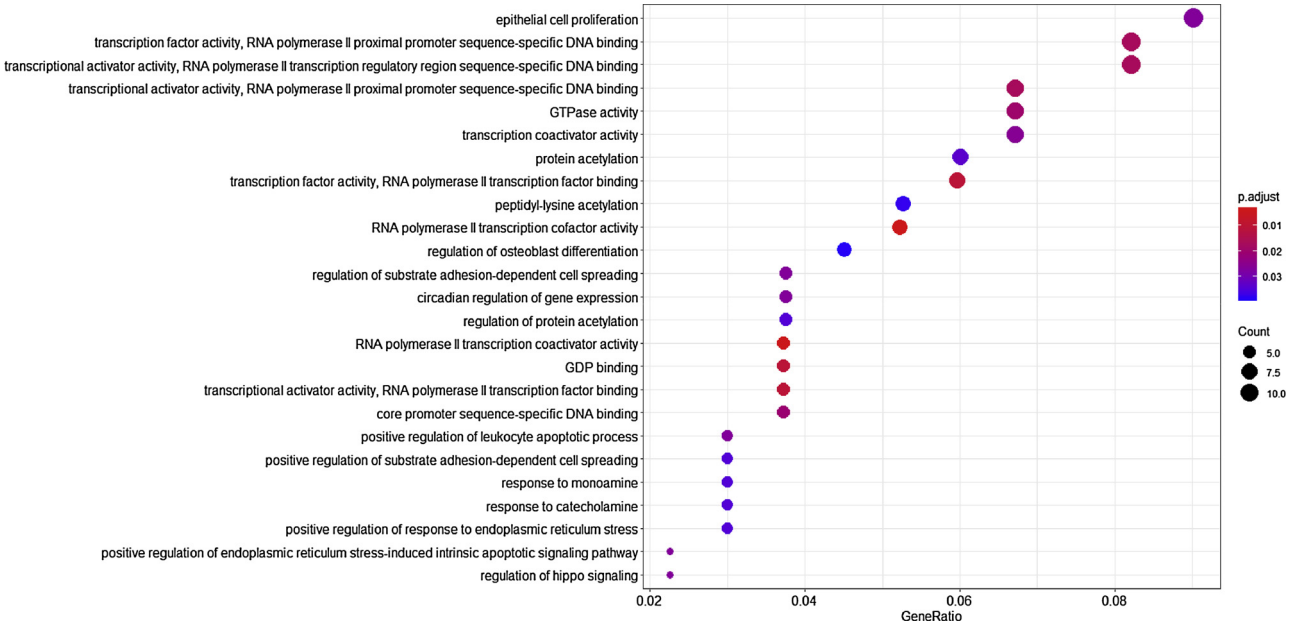

Fig. 4. GO enrichment analysis of the overlapping target genes in endometrial carcinoma (A).

miR-204-5p. At the same time, the target genes of miR-204-5p were involved in the regulation of biological processes in endometrial carcinoma by GO analysis and KEGG pathway annotation.

There have been some studies concerning the characteristics of miR-204-5p in endometrial carcinoma. MiR-204-5p appeared to be significantly lower in endometrial carcinoma patients compared with control subjects, which exhibited the best diagnostic performance for discriminating endometrial carcinoma patients from control subjects [56]. Further investigation is required to elucidate the role of miR-204-5p in endometrial carcinoma.

In this study, we identified that novel candidate target genes for miR-204-5p were involved in the regulation of crucial biological processes in endometrial carcinoma using a bioinformatics analysis. The target gene SF3B1 of miR-204-5p is oncogenes in endometrial carcinoma [57]. FBXW7 activity results in specific and adjustable regulation of the abundance and activity of its stromas in endometrial carcinoma [58]. Mutations in FBXW7 significantly correlate with tumor grade, endometrial carcinoma type and lymph node status [59]. Targetable FBXW7 mutations (6%) in endometrial carcinoma were frequently found [60]. Endometrial carcinoma frequently mutated genes (≥ 10% tumors) included FBXW7 (10.5%) and so on [61]. In vitro effects of FBXW7 mutation in serous endometrial cancer has increased levels of potentially druggable proteins as well as sensitivity to SI-2 and dinaciclib [62]. In POLE wild-type endometrial clear cell carcinomas, FBXW7 and SPOP were the genes most commonly affected by mutations [63]. It was reported that frequent somatic mutations in SPOP of clear cell endometrial cance identified [64]. BRD4 proteins as substrates that are degraded by endometrial cancer-associated SPOP mutants [65].

In this study, we clearly identified that GO functional annotation and KEGG PATHWAY for miR-204-5p were involved in the regulation of crucial biological processes in endometrial carcinoma using a bioinformatics analysis. Endometrial cancer interstitial cells stimulate

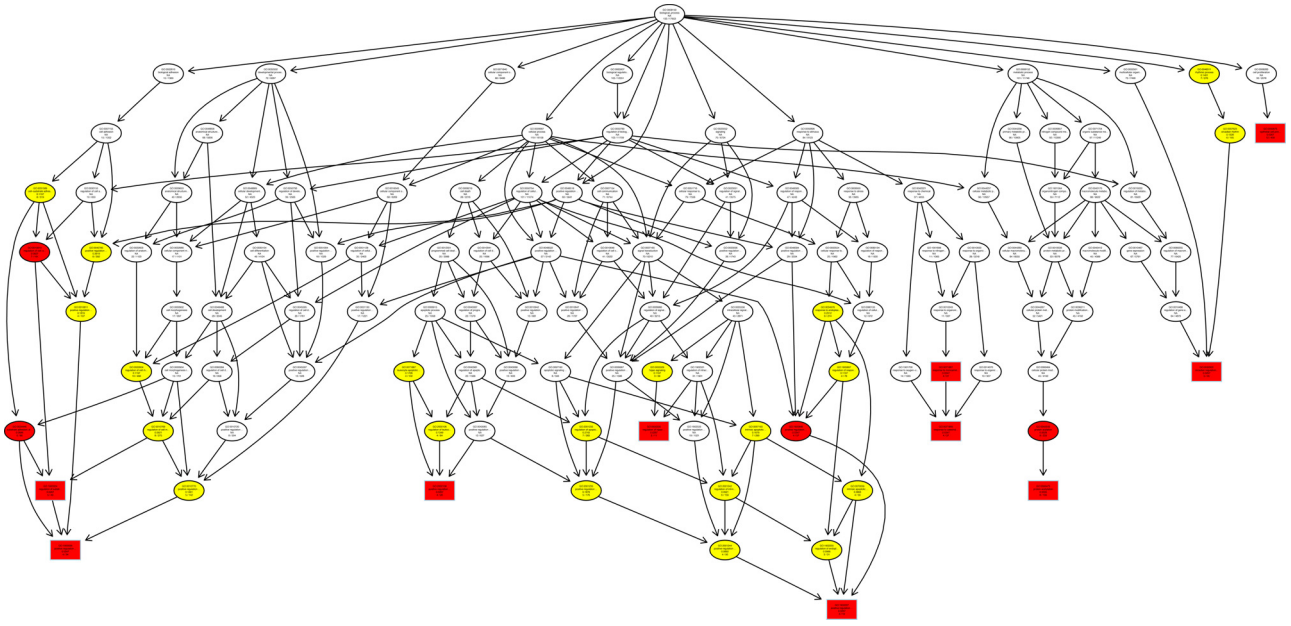

Fig. 5. BP GO enrichment analysis of the overlapping target genes in endometrial carcinoma (B).

Table 1
Signaling pathway enrichment analysis of the overlapping target genes in endometrial carcinoma.

Pathway	Name	Gene Count	p-Value	Genes
hsa04152	AMPK signaling pathway	6	$2.12*10^{-3}$	PIK3CB, RAB14, CREB5, RAB10, SIRT1, PPARGC1A
hsa04925	Aldosterone synthesis and secretion	4	$2.27*10^{-2}$	ADCY6, CAMK1, CREB5, ITPR1
hsa04921	Oxytocin signaling pathway	5	$3.05*10^{-2}$	PIK3CB, ADCY6, CAMK1, NFATC3, ITPR1
hsa04022	cGMP-PKG signaling pathway	5	$3.56*10^{-2}$	PIK3CB, ADCY6, CREB5, NFATC3, ITPR1
hsa05202	Transcriptional misregulation in cancer	5	$3.70*10^{-2}$	CCNT2, SIN3A, RUNX2, MEIS1, MLLT3
hsa04070	Phosphatidylinositol signaling system	4	$3.71*10^{-2}$	PIK3CB, INPP4A, ITPR1, PIP4K2B
hsa04922	Glucagon signaling pathway	4	$3.80*10^{-2}$	CREB5, SIRT1, PPARGC1A, ITPR1
hsa04915	Estrogen signaling pathway	4	$3.80*10^{-2}$	PIK3CB, ADCY6, CREB5, ITPR1
hsa04931	Insulin resistance	4	$4.72*10^{-2}$	PIK3CB, CREB5, OGT, PPARGC1A

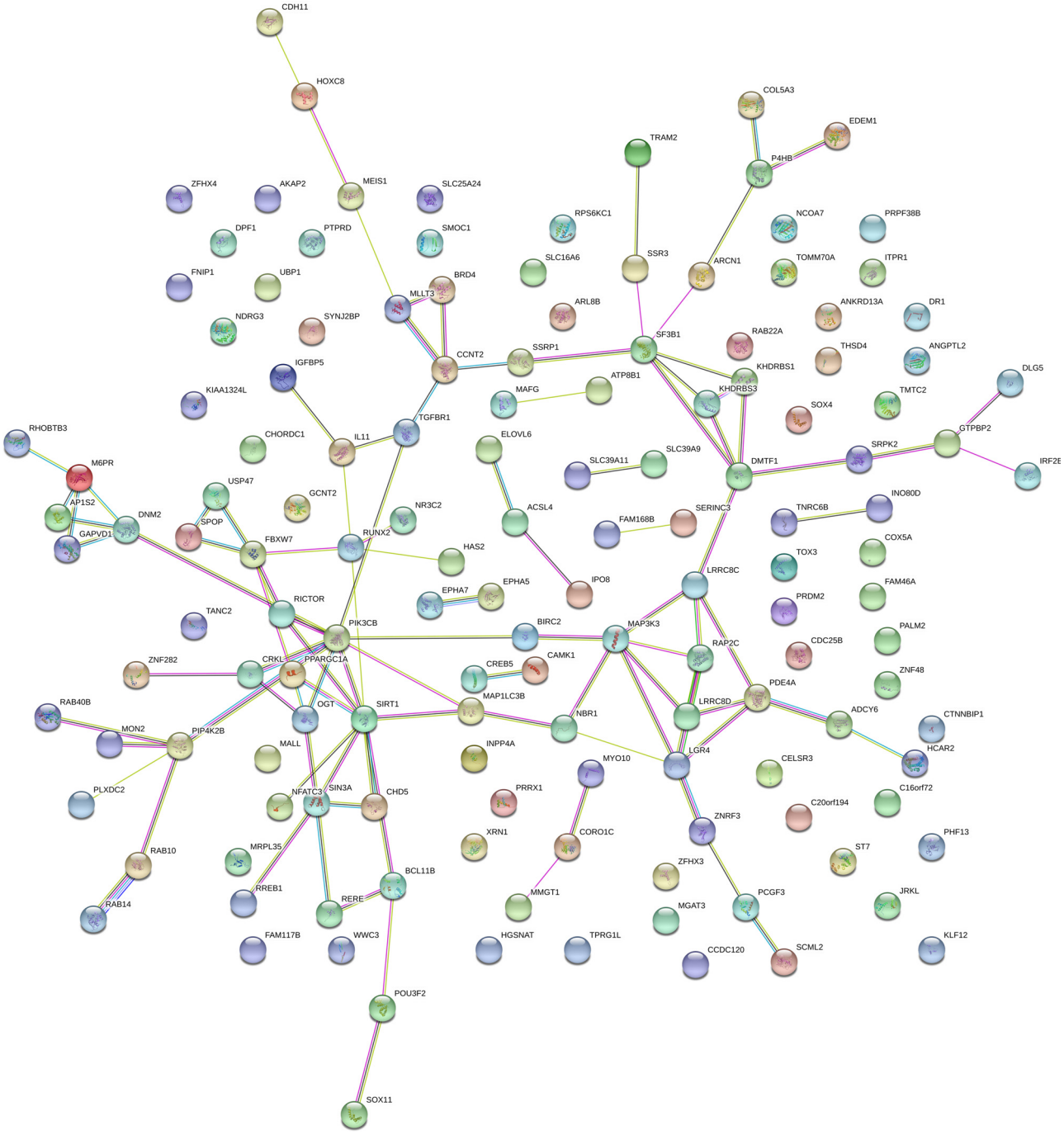

Fig. 6. The overlapping target genes protein–protein interaction (PPI) network complex analysis.

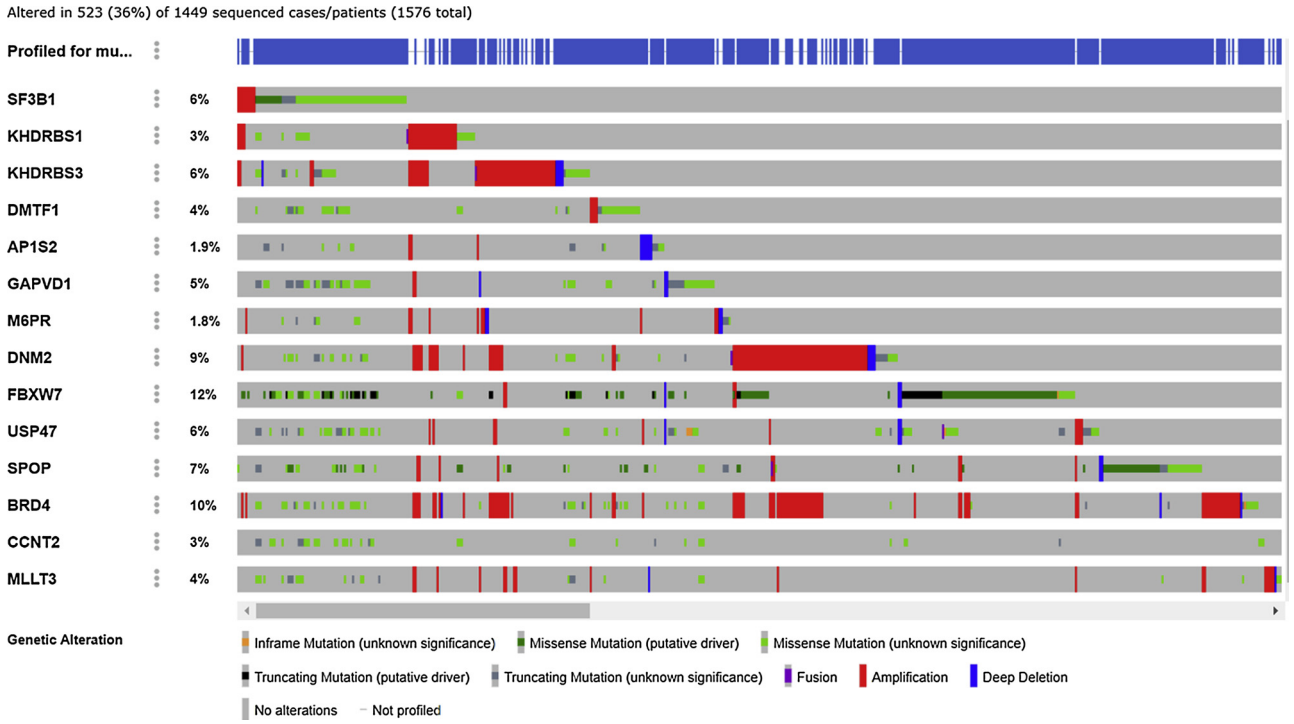

Fig. 7. Mutation of the most significant 14 node degree genes of miR-204-5p in endometrial carcinoma.

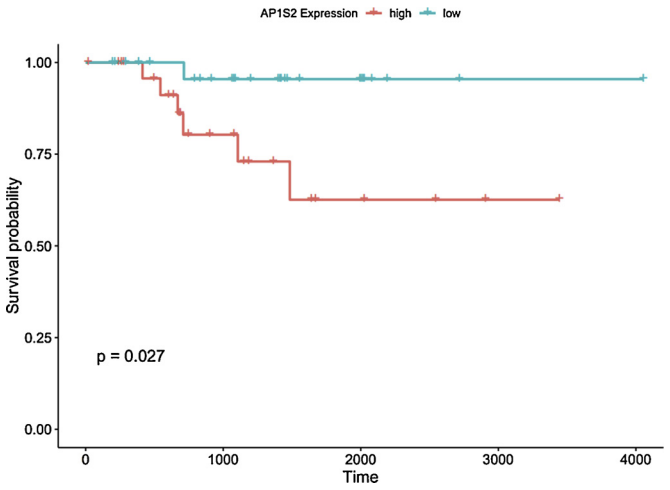

Fig. 8. Prognostic significance of the overlapping target genes AP1S2 of miR-204-5p in endometrial carcinoma from TCGA.

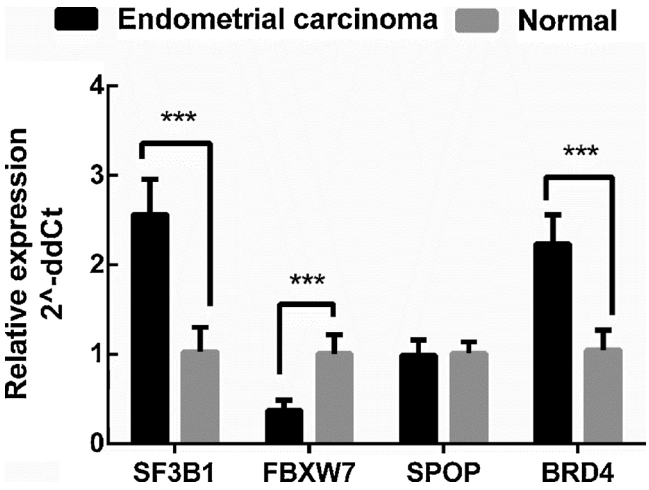

Fig. 9. Validation of SF3B1, FBXW7, SPOP, and BRD4 based on 11 paired endometrial carcinoma clinical samples and normal endometrial clinical samples (n = 22). The level of relative expression of SF3B1, FBXW7, SPOP and BRD4 between endometrial carcinoma tissues and normal endometrial tissues (RT-qPCR). Biological replicates used in this study were from 22 separate patients. GAPDH was used as internal reference, columns represent average, and error bars represent the standard deviation. (***P < 0.001 compared to control group).

normal epithelial cell proliferation [66]. Adiponectin maybe inhibit proliferation and migration of endometrial carcinoma cells through AMPK signal pathway and regulate insensitizes insulin signaling [67]. High glucose increased glucose uptake as well as glycolytic activity through modulating the AMPK pathways in endometrial carcinoma [68]. Metformin decreased expression of phosphorylated (p)-AMPK in endometrial carcinoma [69]. In vitro, oxytocin inhibits proliferation of neoplastic cells of endometrial carcinoma epithelial [70]. Hyperinsulinemia has been identified as a risk factor for endometrial carcinoma [71]. The combined treatment of a PI3K inhibitor and a MEK inhibitor induced a combined antitumor effect in endometrial carcinoma [72]. Visfatin promotes the malignant progression of endometrial cancer through activation of PI3K/Akt signalling [73]. Estrogen signaling and miRNAs to the regulatory mechanisms that drive epithelial-mesenchymal transition in endometrial carcinoma [74]. Estrogen-stimulated Ca(2+) influx required Ca2+ channel and contributes to the development of endometrial carcinoma [75]. Significantly higher risk of endometrial carcinoma in women with high fasting insulin, HOMA-IR values [76]. After the validation of miR-204-5p expression in endometrial carcinoma, as well as we identified the associated assessment of the prognostic significance of target genes of miR-204-5p. Our speculation that lower-AP1S2 target gene of miR-204-5p expression would indicate higher survival rates of endometrial carcinoma patients was confirmed in the prognostic analysis of TCGA.

5. Conclusions

Our bioinformatics analysis showed that miR-204-5p plays an important role in the pathogenesis of endometrial carcinoma. Through bioinformatics analysis, we have increased our understanding of the etiology, molecular and potentially pathways of endometrial carcinoma, which can be used as a therapeutic target. In conclusion, miR-204-5p is down-regulated in endometrial carcinoma and affects the prognostic significance of endometrial carcinoma.

Conflicts of interest

There is no conflict of interest in the study.

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