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Exploring key genes and pathways underlying metastasis endometrial cancer based on gene expression microarray.

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Abstract

Purpose: To reveal the key genes and pathways involved in the metastasis of endometrial cancer remains.
Methods: Microarray data GSE29436 consisting of 4 progressive endometrial cancer samples and 4 non-progressive controls were downloaded from Gene Expression Omnibus database. Differentially Expressed Genes (DEGs) were screened out using Limma package in R, followed by hierarchical clustering. Gene Ontology and pathway enrichment analysis were performed for DEGs.
Results: Bioinformatic analysis revealed a total of 65 DEGs between progressive and non-progressive samples. Functional annotation showed that those genes were mainly enriched in functions of cell proliferation, MPKA and TGF-beta signaling pathways, which involved with up-regulated genes pleiomorphic adenoma gene 1 (*PLAG1*), Insulin-Like Growth Factor 2 (*IGF2*), and down-regulated genes Fibroblast Growth Factor 20 (*FGF20*) and Thrombospondin 4 (*THBS4*).
Conclusion: Sixty-five identified genes in progressive endometrial cancer samples were mainly associated with tumor metastasis possibly by enhancing cell proliferation and affecting MAPK and TGF-beta signaling pathways. High expression of *PLAG1*, *IGF2* and low expression of *FGF20*, *THBS4*, therefore, appear to play an important role in tumorigenesis and progression of endometrial cancer.

Keywords: Endometrial cancer, Differentially expressed genes, Pathways, Microarray.

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Introduction

Endometrial cancer is the sixth most common cancer affecting women in the western world, with at least 320,000 new cases being diagnosed resulting in 74,000 disease-related deaths in women worldwide [1]. Although endometrial cancer is efficiently diagnosed and successfully treated, the treatment of aggressive and progesterone-resistant cancer is difficult. However, patients with early stage disease have 5 y survival rates over 80% but 15-20% develops metastasis [2].

There are two types of endometrial cancer (types I and II) with different molecular expression profiles and clinical and histopathological behaviors [3,4]. Type I endometrial cancer is estrogen-dependent with endometrioid morphology and accounts for 75% of endometrial cancer [5]. Type II cancer exhibits serious histological alterations and poorly differentiated endometrioid morphology with extra-uterine spread and myometrial invasion. The mechanisms of endometrial cancer metastasis have been partly elucidated, but not fully understood.

In the current study, we aimed to further elucidate the molecular mechanism of endometrial cancer metastasis and provide potential therapeutic target for treating endometrial cancer. Based on the microarray data GSE29436, further analysis was performed by identifying Differentially Expressed Genes (DEGs) in progressive samples compared with non-progressive control, as well as functional annotation of DEGs. Finally, functions and involved biological pathway associated with metastasis of endometrial cancer were detected.

Materials and Methods

Data source

Microarray data GSE29436 were downloaded from Gene Expression Omnibus (GEO, http://www.ncbi.nlm.nih.gov/geo/) database based on the platform of Affymetrix Human Genome U133 Plus 2.0 Array. The dataset included 4 progressive endometrial cancer specimen and 4 non-progressive controls.

Data preprocessing and identification of DEGs

The affy package in R [6] was used to process the CEL files by converting the raw probe intensities to probe set expression values. Raw data were background-corrected and normalized with Robust Multi-Array Average (RMA) procedure [7]. Replicate probes for one gene were summarized by mean value as expression value for one gene. To identify critical genes in endometrial cancer metastasis, DEGs were detected using limma package in R [8] by comparing the expression profile between progressive and non-progressive samples. Batch

effects in microarray expression data were adjusted using empirical Bayes method [9]. Genes were considered to be differentially expressed in progressive samples if |log2FC (fold change)|>1 and adjusted P-value<0.01. Then, hierarchical clustering was carried out for DEGs based on expression value of each sample.

Functional annotation of DEGs in progressive cancer

Gene Ontology (GO) database [10] includes functions from three categories, biological process, cellular component and molecular function. The P value of GO terms was calculated (where n is the total number of genes, m the total number of genes that contain the term, c is the number of the genes of the list and k the genes of the list that contain the term). To study the functions of DEGs in progressive endometrial cancer, GO and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway [11] enrichment analysis were performed with hypergeometric distribution. P value less than 0.05 was set as the cut-off of GO terms and pathways.

Results

Results of preprocessing

After normalization of gene expression profile, intensity of genes in 8 samples were obtained, which was shown with box plot in Figure 1.

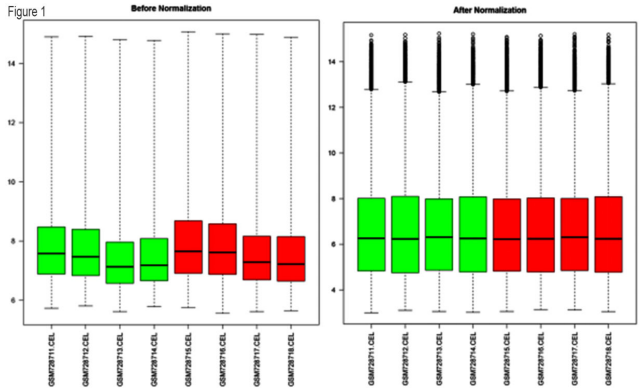

Figure 1. *Box plots of microarray data before and after normalization. Green box represents non-progressive endometrial cancer sample and red box represents progressive sample.*

Identification of DEGs in progressive cancer

By applying statistical analysis, 65 DEGs were screened out between progressive and non-progressive samples, including 35 up- and 30 down-regulated genes. The volcano plot for DEGs, an effective and easy-to-interpret graph that summarizes both P value and fold-change, was shown in Figure 2. Results of hierarchical clustering were shown with heatmap, which indicated that progressive and non-progressive samples were clustered into two classes. The heatmap was shown in Figure 3. The top 10 up- and down-regulated DEGs were shown in Figure 4.

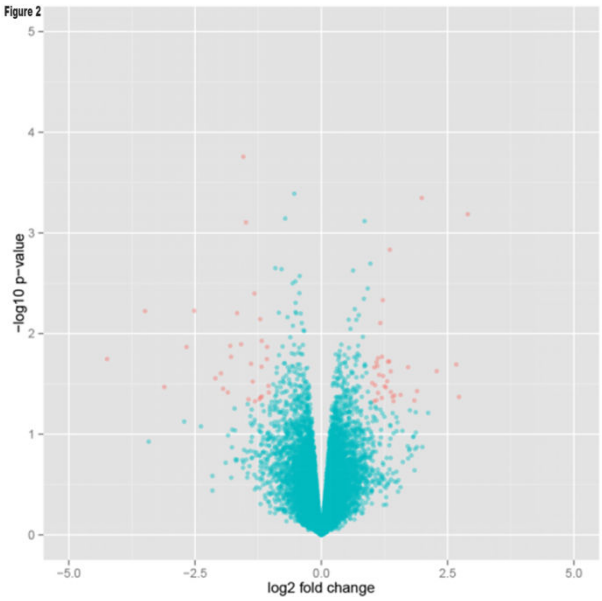

Figure 2. *The volcano plot of differentially expressed genes. The vertical axis represents log2FC (fold change) and the horizontal axis represents-log10 (P value). The red pots represent differentially expressed genes and blue pots represent non-differentially expressed genes.*

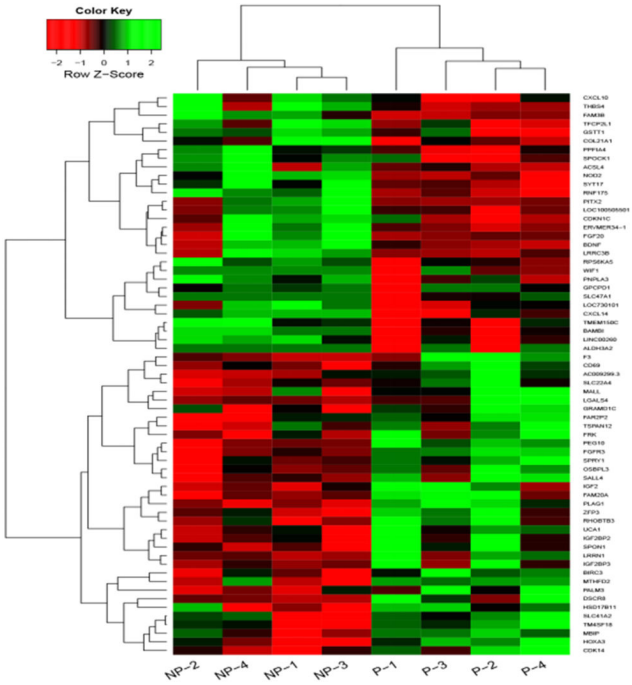

Figure 3. *Heatmap of differentially expressed genes. The vertical axis represents sample. The horizontal axis represents differentially expressed genes.*

GO enrichment analysis of DEGs

To study the functions of DEGs, GO enrichment analysis was performed and DEGs were mainly enriched in biological processes associated with cell proliferation which was involved up-regulated genes pleiomorphic adenoma gene 1 (*PLAG1*), insulin-like growth factor 2 (*IGF2*), coagulation factor III (*F3*),

down-regulated genes fibroblast growth factor 20 (*FGF20*) and thrombospondin 4 (*THBS4*), categories of cell components including cytoplasmic cyclin-dependent protein kinase holoenzyme complex and categories of molecular functions such as mRNA 5'-UTR binding, protein kinase regulator activity (Table 1). Additionally, according to the criteria of P value less than 0.05, DEGs were significantly enriched in bladder cancer, fatty acid metabolism, MAPK signaling pathway involved with *FGF20*, glycerolipid metabolism, NOD-like receptor signaling pathway and TGF-beta signaling pathways involved with *THBS4* (Table 2). Especially, MAPK signaling pathway, which was associated with most genes, was shown in Figure 5.

Table 1. *Results of Gene Ontology (GO) enrichment analysis of differentially expressed genes.*

	GOBPID	Term	P value	Count	Symbols
BP	GO: 0042127	Regulation of cell proliferation	1.50E-05	15	*BDNF; CDKN1C; F3; FGFR3; FRK; HOXA3; IGF2; CXCL10; PITX2; PLAG1; THBS4; SPRY1; BAMBI; FGF20; NOD2*
BP	GO: 0008284	Positive regulation of cell proliferation	2.63E-05	11	*F3; FGFR3; HOXA3; IGF2; CXCL10; PITX2; PLAG1; THBS4; BAMBI; FGF20; NOD2*
BP	GO: 0008283	Cell proliferation	9.11E-05	16	*BDNF; CDKN1C; F3; FGFR3; FRK; HOXA3; IGF2; CXCL10; PITX2; PLAG1; THBS4; SPRY1; BAMBI; FGF20; SALL4; NOD2*
BP	GO: 0007584	Response to nutrient	0.000197	5	*BDNF; ACSL4; CXCL10; PITX2; NOD2*
BP	GO: 0051239	Regulation of multicellular organismal process	0.000554	16	*BIRC3; BDNF; F3; FGFR3; CXCL10; PLAG1; SPOCK1; THBS4; RPS6KA5; SPRY1; IGF2BP3; IGF2BP2; TSPAN12; BAMBI; FGF20; NOD2*
BP	GO: 0009653	Anatomical structure morphogenesis	0.0006	17	*BDNF; F3; FGFR3; HOXA3; CXCL10; PITX2; PLAG1; THBS4; RPS6KA5; SPRY1; IGF2BP3; IGF2BP2; TSPAN12; BAMBI; TFCP2L1; FAM20A; SALL4*
BP	GO: 0050920	Regulation of chemotaxis	0.000657	4	*F3; CXCL10; THBS4; NOD2*
BP	GO: 0042490	Mechanoreceptor differentiation	0.000723	3	*BDNF; FGFR3; FGF20*
BP	GO: 0042325	Regulation of phosphorylation	0.000789	11	*BIRC3; CDKN1C; FGFR3; IGF2; CXCL10; THBS4; RPS6KA5; SPRY1; FGF20; MBIP; NOD2*
BP	GO: 0015695	Organic cation transport	0.000859	2	*SLC22A4; SLC47A1*
CC	GO: 0000308	Cytoplasmic cyclin-dependent protein kinase holoenzyme complex	0.006847	1	*CDK14*
CC	GO: 0009986	Cell surface	0.009575	6	*CD69; F3; FGFR3; CXCL10; PPFIA4; NOD2*
MF	GO: 0048027	Mrna 5'-UTR binding	0.00015	2	*IGF2BP3; IGF2BP2*
MF	GO: 0019887	Protein kinase regulator activity	0.000437	4	*CDKN1C; IGF2; CXCL10; MBIP*
MF	GO: 0019207	Kinase regulator activity	0.000793	4	*CDKN1C; IGF2; CXCL10; MBIP*
MF	GO: 0008083	Growth factor activity	0.001791	4	*BDNF; IGF2; THBS4; FGF20*
MF	GO: 0015491	Cation: cation antiporter activity	0.00224	2	*SLC22A4; SLC47A1*
MF	GO: 0005169	Neurotrophin TRKB receptor binding	0.003211	1	*BDNF*
MF	GO: 0032500	Muramyl dipeptide binding	0.003211	1	*NOD2*
MF	GO: 0046577	Long-chain-alcohol oxidase activity	0.003211	1	*ALDH3A2*
MF	GO: 0047389	Glycerophosphocholine phosphodiesterase activity	0.003211	1	*GPCPD1*
MF	GO: 0047676	Arachidonate-coa ligase activity	0.003211	1	*ACSL4*

BP: Biological Process; CC: Cellular Component; MF: Molecular Function.

Table 2. *Results of Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis of differentially expressed genes.*

KEGGID	Term	P value	Count	Symbols
hsa05219	Bladder cancer	0.007913	2	*FGFR3; RPS6KA5*

hsa00071	Fatty acid metabolism	0.008283	2	*ALDH3A2; ACSL4*
hsa04010	MAPK signaling pathway	0.009565	4	*BDNF; FGFR3; RPS6KA5; FGF20*
hsa00561	Glycerolipid metabolism	0.011086	2	*ALDH3A2; PNPLA3*
hsa04621	NOD-like receptor signaling pathway	0.014731	2	*BIRC3; NOD2*
hsa04350	TGF-beta signaling pathway	0.029556	2	*PITX2; THBS4*

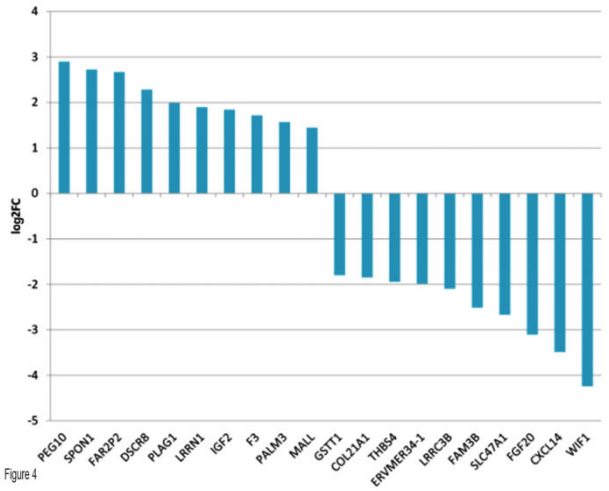

Figure 4. *Top 10 differentially expressed genes.*

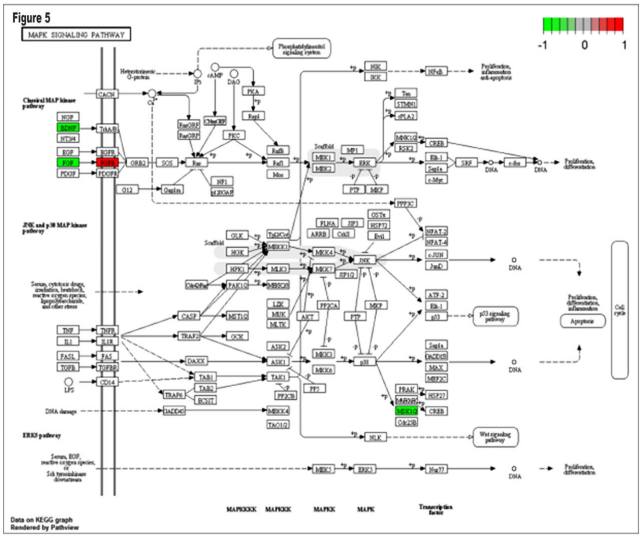

Figure 5. *MAPK signaling pathway. Red represents up-regulated gene and green represents down-regulated gene.*

Discussion

Endometrial cancer spreads by exfoliation of cells that are shed through the fallopian tubes, by lymphatic and or hematogenous dissemination or by direct extension through the myometrium [12]. The most common route of endometrial cancer metastasis is the direct extension of the tumor cells to the myometrium, which results from several interdependent processes including breaking the basement membrane and extracellular matrix by proteolytic enzymes, thereby promoting cell invasion and distant metastasis [13,14].

In a previous study, a novel cytokine system which is composed of the receptor activator of nuclear factor- κB (RANK) and its ligand RANKL, has been indicated to be overexpressed in human endometrial cancer tissues [15]. Briefly, RANK/RANKL could promote cancer cell proliferation, migration and invasion. However, medroxyprogesterone acetate-mediated progesterone receptor B plays critical roles in inhibiting migration and invasion of endometrial cancer cells [15]. More recently, another gene alpha-enolase (*ENO1*), has been found to be up-regulated in several tumors [16]. Meanwhile, *ENO1* silencing has obviously reduced cell proliferation, migration and invasion in endometrial cancer cells, as well as metastasis *in vivo*, as a result, it has been considered as a therapeutic target in treatment of endometrial carcinoma [17]. Additionally, a lysosomal cysteine protease, cathepsion B has also been overexpressed in endometrial cancer tissues and its silencing can inhibit cell migration and invasion [18]. Nevertheless, the molecular mechanism involved in the metastasis of endometrial cancer remains further elucidated.

In the present study, microarray analysis was performed to elucidate the molecular basis of progressive endometrial cancer and showed that 65 DEGs between progressive and non-progressive cancer samples. From the results of functional annotation, we found that those genes were significantly enriched in GO terms of cell proliferation, including *PLAG1*, *IGF2*, *F3*, *FGF20* and *THBS4*. Additionally, most of genes were involved in MAPK and TGF-beta signaling pathways.

PLAG1 is a proto-oncogene whose ectopic expression presumably results in the deregulation of target genes and leads to uncontrolled cell proliferation in tumorigenesis [19]. Studies have indicated that *PLAG1* is involved in various human tumors including pleomorphic adenomas of the salivary glands [20], lipoblastomas [21], hepatoblastoma [22] and leukaemia [23]. Collectively, the *PLAG1* proto-oncogene seems to exert its oncogenic potential at least partially *via IGF2* mitogenic signaling pathway which leads to downstream signaling responsible for cell proliferation [24]. In our results, *PLAG1* was up-regulated in progressive endometrial cancer samples and exerted important role in tumor cell proliferation, which was consistent with previous results indicated by other study. Furthermore, considering the critical function of *IGF2* mitogenic signaling pathway, we also found that *IGF2* was up-regulated in progressive cancer samples and mainly involved in cell proliferation-associated functions. These findings were in

line with results that increased plasma levels of *IGF2* were associated with endometrial cancer risk [25].

It was also found that down-regulated genes *FGF20* and *THBS4* were not only associated with functions of cell proliferation, but also involved in MAPK and TGF-beta signaling pathways. *FGF20* belongs to a large class of FGFs which encoding secretory proteins with potent mitogenic and angiogenic roles in tumor development [26]. Previous study has shown that *FGF20* was expressed at markedly elevated levels in part of human ovarian endometrioid adenocarcinomas harboring β-catenin defects [27]. However, *FGF20* was down-regulated in progressive cancer sample, and one possible explanation is that the gene was under the control of more than one pathway. Notably, *FGF20* has been proved to activate the MAPK signaling pathway which is the major intracellular signaling pathway of FGFs and plays a crucial role in the neurotrophic activity of *FGF20* [28]. Consistently, our bioinformatics results indicated that *FGF20* exerted crucial role in MAPK signaling pathway. *THBS4*, a member of the extracellular calcium-binding protein family, is involved in cell adhesion and migration, and considered as a putative tumor suppressor gene in tumorigenesis [29,30]. In our study, expression levels of *THBS4* were significantly decreased in progressive samples compared with non-progressive control. Moreover, *THBS4* was involved in TGF-beta signaling pathway, which acted as a suppressor of epithelial cell tumorigenesis at early stages, but promoted cancer progression by contributing to tumor cell migration and invasion during the later stages. Taken together, these results suggest these genes have metastatic potential in endometrial cancer.

In conclusion, our results showed that 65 genes were differentially expressed in progressive endometrial cancer samples compared with non-progressive control, which were mainly associated with tumor metastasis possibly by enhancing cell proliferation and affecting MAPK and TGF-beta signaling pathways. High expression of *PLAG1*, *IGF2* and low expression of *FGF20*, *THBS4*, therefore, appear to play an important role in tumorigenesis and progression of endometrial cancer. However, these findings warrant further prospective studies to be validated and determine if these genes could serve as new molecular targets for endometrial cancer therapy.

Acknowledgment

None

Conflict of Interest

We certify that regarding this paper, no actual or potential conflicts of interests exist.

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