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Original Article

Transcriptomic analysis of stromal cells from patients with endometrial carcinoma

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Abstract: Tumor microenvironment plays a critical role in cancer pathogenesis. In this study, we performed transcriptomic analysis of stromal cells from patients diagnosed with endometrial carcinoma. Endometrial stromal cells from patients and healthy donors were cultured and total RNA was extracted for RNA integrity examination and gene profiling analysis. Gene ontology (GO) and KEGG analysis were also performed. In this study, we found that, in endometrial stromal cells from endometrial cancer patients, a total of 605 genes were changed (fold change ≥2, *p*-value <0.05). From these, 275 were up-regulated and 330 were down-regulated genes. In addition, GO analysis showed that the differentially expressed genes (DEGs) were involved in various biological processes including cell adhesion, biological adhesion, bone development and extracellular matrix organization. Furthermore, KEGG analysis of the DEGs identified four pathways including Wnt signaling pathway, cadherin signaling pathway, ECM-receptor interaction, and focal adhesion. Our study identified 605 DEGs in stromal cells from endometrial carcinoma which mapped to a variety of biological processes. These results may contribute to understanding the molecular mechanisms o of endometrial carcinoma pathogenesis.

Keywords: Endometrial carcinoma, gene expression profiling, stromal cells, microenvironment

Introduction

Endometrial carcinoma is one of the worldwide leading gynecological malignancies in women. Typical clinical symptoms observed include dyspareunia, dysmenorrhea, and infertility [1, 2]. In 2013, 49500 new cases and 8200 deaths caused by endometrial cancer were reported in the United States [3]. To date, surgical resection and hormonal suppressant drugs are the primary therapeutic options for endometrial cancer [4]. To date, little is known about endometrial cancer pathogenesis, therefore identification of altered genes may contribute to understanding the molecular events in endometrial cancer.

It is well established that cancer is a systemic and multiple-step disease, encompassing interaction between tumor cells and host stromal cells [5, 6]. Increasing evidence demonstrates that tumors are composed of tumor parenchyma and stroma, two discrete but closely interactive parts, and that their crosstalk promotes tumor growth [7, 8]. Recently, many studies support the notion that tumor stromal cells play critical roles in malignant behaviors, such as cell growth, invasion, differentiation, and metastasis [9, 10]. In the current study, we examined the gene expression profile of stromal cells isolated from endometrial carcinoma patients.

Materials and methods

Endometrial tissue isolation

Samples of uterine tissue were obtained from endometrial carcinoma patients at the Xuhui Hospital, Zhongshan Hospital, Fudan University. Endometrial stromal cells were isolated and cultured as previously described [11].

Table 1. Preparation of RNAs for microarray analysis

Sample Name	Concentration (μg/μL)	Volume (μL)	Total (μg)	A260/280
JZ-N-1	0.202	100	20.19	1.79
JZ-N-2	0.213	100	21.31	1.76
JZ-N-3	0.186	100	18.57	1.80
JZ-C-1	0.140	100	14.01	1.71
JZ-C-2	0.136	100	13.57	1.75
JZ-C-3	0.121	100	12.06	1.82

JZ-N represents control and JZ-C means cancer. A total of six samples were subjected to microarray analysis with three samples in each group.

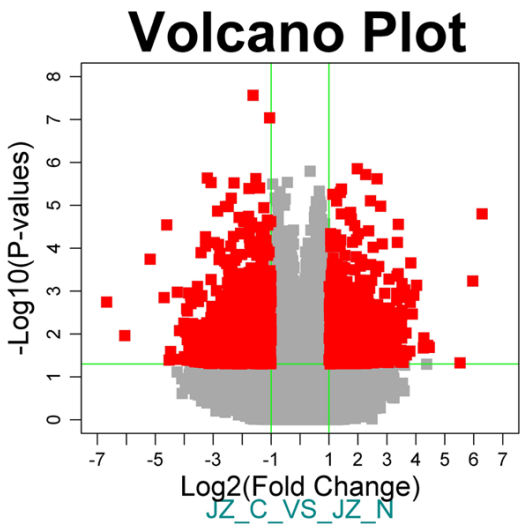

Figure 1. Volcano Plot of differentially expressed genes. X axis represents log2 (fold change) and Y axis is marked with -log10. Differentially expressed genes with fold change ≥2 and P<0.05 are marked in red.

RNA extraction and purification

Total RNA was extracted using TRIZOL Reagent (Life technologies, Carlsbad, CA, USA) following the manufacturer's instructions. RNA quality was determined via Agilent Bioanalyzer 2100 (Agilent technology, Santa Clara, CA, USA). Total RNA was further purified by RNeasy microkit (QIAGEN, GmBH, Germany) and RNase Free DNase Set (QIAGEN, GmBH, Germany).

RNA amplification and labeling

Total RNA was amplified, labeled and purified via GeneChip 3'IVT Express Kit (Affymetrix, Santa Clara, CA, USA) following the manufacturer's instructions to obtain biotin labeled cRNA.

Array hybridization

Array hybridization and wash was performed using GeneChip® Hybridization, wash and stain Kit (Affymetrix, Santa Clara, CA) in Hybridization Oven 645 (Affymetrix, Santa Clara, CA) and Fluidics Station 450 (Affymetrix, Santa Clara, CA) following the manufacturer's instructions.

Data analysis

Slides were scanned by GeneChip® Scanner 3000 (Affymetrix, Santa Clara, CA, US) and Command Console Software 3.1 (Affymetrix, Santa Clara, CA, US) with default settings. The enrichment analysis of GO and KEGG pathway annotations were conducted based on a hypergeometric test.

Results

Transcriptional profiles in stromal cells from endometrial carcinoma patients

As previously described, stromal cells were characterized with cytokeratin (epithelial marker) and vimentin (stromal marker) [11]. Total RNA was extracted f from healthy donors stroma cells and endometrial carcinoma patients, and further subjected to microarray analysis using Affymetrix U133 plus 2.0 array. The concentrations and A260/280 values of RNA prepared for microarray analysis are shown in **Table 1**. We found that stromal cells from endometrial cancer patients exhibited significant variety in gene expression levels when compared to healthy donors. As shown in **Figures 1** and **2**, a total of 605 genes were changed (fold change ≥2, *p*-value <0.05). Changes included 275 up-regulated genes and 330 down-regulated genes.

Functional categories of differentially expressed genes

The differentially expressed genes (DEGs) were further analyzed with GO ontology analysis to determine a correlation between genes and physiology. We found that DEGs were involved in various molecular functions and biological processes (**Figure 3**) including cell adhesion, biological adhesion, bone development and extracellular matrix organization.

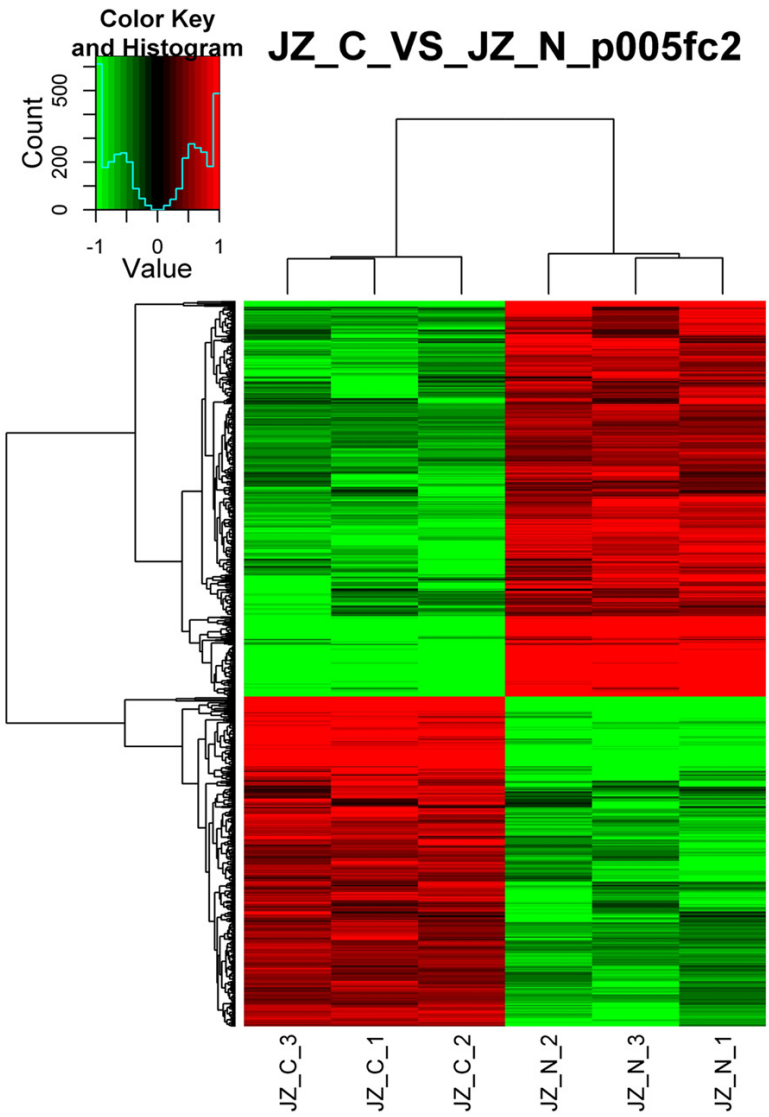

Figure 2. Cluster analysis of transcriptional profiles in stromal cells. Transcriptional profiles were measured in stromal cells from endometrial carcinoma (JZ-C-1,2,3) and normal controls (JZ-N-1,2,3). Fold change ≥2, P<0.05.

Differential gene expression profile with KEGG analysis

To further characterize the potential functions of the stromal genes, the DEGs were subjected to KEGG pathway analysis. As a result, 4 pathways were assigned including Wnt signaling pathway, cadherin signaling pathway, ECM-receptor interaction, and focal adhesion. Analysis of the pathways relative to Wnt signaling, cadherin signaling, ECM-receptor interaction and focal adhesion identified 28, 18, 10, and 11 differentially expressed genes, respectively (**Table 2**).

Discussion

Endometrial carcinoma is a multiple-step process involved in initiation, promotion and progression [5]. Accumulating evidence supports the notion that cancer development is intimately related to the complex tumor microenvironment [7, 12]. The importance of the local tumor microenvironment in tumor progression has been recognized for many years. In the current study, we examined gene transcription expression levels in stromal cells derived from endometrial carcinoma patients.

The tumor microenvironment, is primarily composed of immune cells, stromal fibroblasts, extracellular matrix proteins, and endothelial cells derived from various cell types, which can directly regulate the cell phenotypes, representing a unique approach for cancer diagnosis and treatment [13]. The primary cell types in the tumor microenvironment that are required for cancer progression consist of cancer-associated fibroblasts, endothelial cells, and cancer-associated adipocytes [14]. A recent study has reported that transforming growth factor-β (TGF-β) synthesized by stromal cells participates in bone resorption induced by oral squamous cell carcinoma [15]. Important biomolecules in endometrial carcinoma stromal cells include genes that are intricately related to biological functions such as cell growth, apoptosis, and metastasis. Our previous study demonstrated that stromal cells of endometrial carcinoma promoted epithelial cell growth via regulating HGF/c-Met/Akt signaling pathway [11]. However, the transcriptional profile of stromal cells derived from endometrial cancer has not been previously reported.

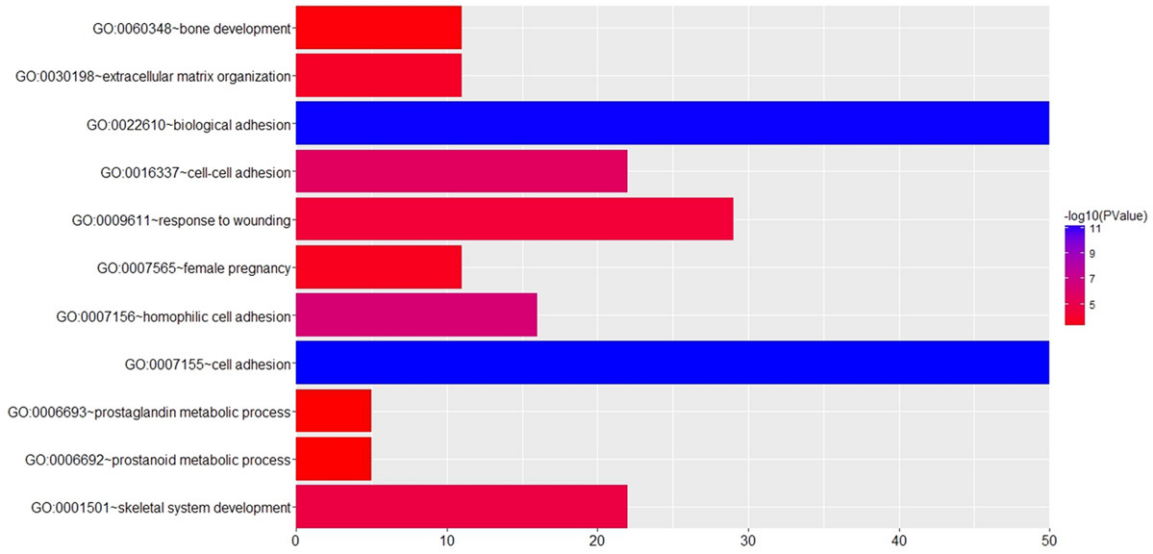

Figure 3. GO analysis of differentially expressed genes. The Y-axis shows significantly enriched gene ontology (GO) terms relative to the genome, and the X-axis shows the enrichment scores of these terms.

Table 2. KEGG analysis of DEGs

Term	Count	*P* Value	Genes
Wnt signaling pathway	28	<0.05	PCDHA6, PCDHA7, PCDHA8, PCDHA9, PCDHA2, CORIN, PCDHA3, PCDHA4, PCDHA5, PCDHA1, PCDHAC2, PCDHAC1, HOXC6, PLCB4, HOXC4, PCDHA10, PCDHA11, PCDHA12, PCDHA13, AXIN2, FZD8, TCF7, MYH3, PCDH10, PCDHB2, PCDH9, PCDH7, TLE2, EP300, HOXB7, SFRP1, KREMEN1, CDH10
Cadherin signaling pathway	18	<0.05	PCDHA6, FZD8, PCDHA7, PCDHA8, TCF7, PCDHA9, CORIN, PCDHA2, PCDHA3, PCDHA4, PCDH10, PCDHA5, PCDHB2, PCDH9, PCDHA1, PCDH7, PCDHAC2, PCDHAC1, PCDHA10, PCDHA11, PCDHA12, PCDHA13, CDH10
ECM-receptor interaction	10	<0.05	COL4A4, LAMA1, TNXB, TNXA, ITGA1, COL6A1, LAMC2, COL1A1, COL11A1, HMMR, SPP1
Focal adhesion	11	<0.05	COL4A4, LAMA1, TNXB, TNXA, ITGA1, COL6A1, LAMC2, HGF, COL1A1, PDGFD, COL11A1, SPP1

In the current study, we compared the gene expression levels in endometrial carcinoma-derived stromal cells to those in the healthy controls. Our results indicated that a total of 605 genes were changed (fold change ≥2, *p*-value <0.05). Among these, 275 were up-regulated and 330 were down-regulated genes. In addition, GO ontology analysis showed that the DEGs were involved in various molecular functions and biological processes.

Furthermore, KEGG analysis revealed that DEGs were involved in Wnt signaling pathway, cadherin signaling pathway, ECM-receptor interaction, and focal adhesion. More specifically, 28 genes belonged to Wnt signaling pathway. The Wnt signaling is a signal transduction pathway made of proteins that passes signals into a cell through cell surface receptors. In mammals, Wnt signaling is important in body axis patterning, cell growth, and cell migration [16]. Constitutive activation of this pathway is closely associated with human cancers [17]. Our results indicate that, many genes mapped to the protocadherins (PCDHs) family including PCDHA1-13. PCDHs are a large family of cadherin-like cell adhesion proteins that are involved in the establishment and maintenance of cellular connections. Adhesive properties of these proteins are critical for cell rearrangement, migration, and cell sorting [18, 19]. In addition, a recent study showed that PCDHs mediate cell growth, cell cycle arrest, apoptosis, and migration of endometrial cancer, underlining their critical roles in endometrial carcinogenesis [20].

In conclusion, our study identified 605 differentially expressed genes, mapping to a variety of biological processes including Wnt signaling pathway, cadherin signaling, ECM-receptor interaction and focal adhesion. These results may contribute to understanding the molecular mechanisms of endometrial carcinoma.

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Disclosure of conflict of interest

None.

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