Genome-wide analysis of the effect of esophageal squamous cell carcinoma on human umbilical vein endothelial cells

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Abstract. A large volume of data indicates that controlling tumor-associated angiogenesis is a promising therapy against cancer. However, angiogenesis is a complex process, little is known about the differential gene expression in the process of normal endothelial cell differentiation toward tumor vascular endothelial cells induced by tumor microenvironment. The aim of this study is to investigate the effect of tumor microenvironment simulated by the supernatant of esophageal squamous cancer cells (KYSE70) on normal endothelial cells (HUVECs) at the whole genome level. The gene expression profile was studied through gene ontology and signal pathway analysis. Compared with the normal HUVECs, a total of 3769 differentially expressed genes in induced HUVECs were detected, including 1609 upregulated genes and 2160 downregulated genes. Moreover, the microarray data analysis showed that 11 significant biological processes and 10 significant signaling pathways changed most, which are associated with angiogenesis and cell differentiation. According to the different expression levels in the microarrays and their functions, four differentially expressed genes involved in tumor angiogenesis and cell differentiation (IL6, VEGFA, S1PR1, TYMP) were selected and analyzed by qRT-PCR. The qRT-PCR results were consistent with the microarray data. Furthermore, we simulated the tumor microenvironment by human esophageal carcinoma tissue homogenate to investigate its effect on HUVECs, the qRT-PCR results indicated that the above genes were highly expressed in HUVECs after induction by esophageal carcinoma tissue homogenate. In conclusion, tumor microenvironment impact on normal endothelial cells differentiated toward tumor vascular endothelial cells, and the selected genes, which are associated with tumor angiogenesis, would be anti-angiogenesis targets against esophageal carcinoma.

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Introduction

Angiogenesis, which is the formation of new capillaries from pre-existing blood vessels, is crucial for normal embryonic development and also responsible for many pathological states including tumor growth and the development of metastases (1,2). When solid tumors grow beyond the diameter of ~1-2 mm, new blood vessels are needed to provide oxygen and nutrients and take metabolic waste away (3). Tumor angiogenesis is a complex process dependent on angiogenic factors and also involve in multiple molecular events in tumor microenvironment (4,5).

The tumor microenvironment is composed of a variety of signaling molecules that influence angiogenesis. Previous studies illustrate that tumor cells create the microenvironment by secreting cytokines and growth factors to activate normal, quiescent cells around them and initiate a cascade of events. For example, tumor cell can release vascular endothelial growth factor (VEGF) to stimulate the sprouting and proliferation of endothelial cells (6,7). Furthermore, it has been proved as a credible method to simulate the tumor microenvironment by the cancer cell supernatant (8,9).

There are many ways for tumor angiogenesis such as sprouting angiogenesis, intussusceptive angiogenesis, vasculogenic mimicry and lymphangiogenesis (10). Moreover, tumor microenvironment secrete numerous cytokines and growth factors to recruit normal endothelial cells differentiate into tumor endothelial cells for tumor angiogenesis, which was considered as an important way for tumor neovascularization (6,7).

Esophageal carcinoma is one of the most common cancers in China. The main type of esophageal cancer is esophageal squamous cell carcinoma which is characterized by poor prognosis as well as strong invasiveness (11). Currently, there are no other good methods to manage it except operation, chemo- or radiotherapy, from which only people who are diagnosed during the early period can benefit. In the 1970s, Folkman hypothesized a link between angiogenesis, tumor growth and

metastasis, thus angiogenesis became a putative target for anticancer therapy (12). Recent studies revealed that blocking tumor vascularization is a crucial way to curtail tumor growth for tumor therapy (13). Moreover, controlling of pathological angiogenesis is known as a potential therapeutic strategy for the prevention of tumor progression and the treatment of vascular diseases (14).

The purpose of the present study is to analyze the effects of tumor microenvironment on gene expression of endothelial cells and clear the link between tumor microenvironment and angiogenesis, which would be clinically important as the potential anti-angiogenesis target for esophageal carcinoma.

Materials and methods

Cell preparation. KYSE70, poorly differentiated esophageal squamous cancer cells, were cultured in RPMI-1640 (Biological Industries, Kibbutz Beit Haemek, Israel) medium with 10% FBS, then replenished with fresh medium after reaching 60-80% confluence, the supernatant was collected and centrifuged after 24 h incubation and stored at -20˚C. Human umbilical vein endothelial cells (HUVECs) were cultured in endothelial cell medium (ECM) (ScienCell, Carlsbad, CA, USA). After reaching 60-80% confluence the HUVECs were induced with condition medium (60% KYSE70 supernatant and 40% ECM) for 48 h as induced HUVECs (I-1, I-2, I-3), while normal HUVECs (N-1, N-2, N-3) were used as control. The preparation of tissue homogenate supernatant of human esophageal carcinoma and peri-carcinoma is according to our previous study (15). Briefly, tumor specimens of ESCC tissue and peri-carcinoma tissue (>5 cm) were collected, to prepare to the tissues homogenate supernatant by grinding and centrifuging. The HUVECs were induced by 40% tissue homogenate supernatant of human esophageal carcinoma or peri-carcinoma for further verified by qRT-PCR.

Complementary RNA preparation and microarray hybridization. RNA extraction and microarray hybridization were performed at CapitalBio Technology Corp. (Beijing, China). Total RNA isolation was performed using the TRIzol reagent and was further purified using Qiagen RNeasy Mini kit according to the manufacturer's instructions. RNA samples with RIN values >8, 260/280 absorbance ratios >1.8 and 260/230 absorbance ratios >1.5 were considered suitable for microarray analysis. The processes of labeling, hybridization and scanning were performed at a platform of CapitalBio Technology Corp. Aliquots (100 ng) of total RNA from induced HUVECs (I-1, I-2, I-3) and normal HUVECs (N-1, N-2, and N-3) were used for synthesis and amplification of first-strand cDNAs, double stranded cDNAs, and biotin-labeled antisense RNAs, with a Message Amp™ Premier RNA Amplification kit (Ambion) on a PCR apparatus (MJ, PTC-225).

After measuring the concentrations of the labeled RNAs by ultraviolet spectrophotometer, 1 μg of each preparation was fragmented and verified onto 1.2% formaldehyde denatured agarose electrophoresis. The biotinylated cRNAs were hybridized to a commercial gene chip, Affymetrix GeneChip Human Genome U133 (HG-U133) Array. Microarray hybridization was performed at 45˚C for 16 h with constant rotation at the speed of 60 rpm using an Affymetrix GeneChip Hybridization Oven 640. After washing and staining automatically on an Affymetrix fluidics station 450, and using the hybridization, Wash and Stain kit (Affymetrix), the chips were scanned on Affymetrix GeneChip® scanner 3000. Overall, six microarray chips were analyzed in this study.

Data processing and microarray data analysis. The obtained scanned images were first assessed by visual inspection and then analyzed using Affymetrix GeneChip Operating software (GCOS 1.4). An invariant set normalization procedure was performed to normalize the different arrays using DNA-chip analyzer (dChip). In a comparison analysis, we applied a two-class unpaired method of the Significance Analysis of Microarrays software (SAM version 3.02, Stanford University, Stanford, CA, USA) to compare significantly differentially expressed genes (DEGs) in the induced HUVECs and normal HUVECs. The algorithm used to sort the statistically significant DEGs was a modified t-test, and the criteria for DEGs were FDR <0.05 and fold change >2.0 or <0.5. FDR was the corrected p-value by post-hoc test. Each set of DEGs was further subjected to the CapitalBio® Molecule Annotation System V3.0 (MAS3.0) for gene ontology (GO) and KEGG pathway analyses. As for GO and KEGG pathways, we calculated the p-value and Q-value for the significantly affected biological processes and pathways and ranked them by the p-values.

Quantitative RT-PCR. qRT-PCR was conducted on candidate genes that were differentially expressed in the microarrays, and whose functions were found upon a biological function analysis, to be closely related to cell differentiation and angiogenesis. qRT-PCR was performed according to the manufacturer's guideline using Quantifast SYBR Green PCR kit, and qRT-PCR were run using the following program: 95˚C /10 min + 40 x (95˚C/10 sec + 60˚C/30 sec). Each sample was run in triplicate. The qRT-PCR data analysis was performed on 7500 software v2.0.5. The comparative threshold cycle (Ct) method, i.e., $2^{-\Delta\Delta Ct}$ was used to calculate fold amplification.

Statistical analysis. Data were expressed as means ± standard deviation (SD) with at least three separate experiments and analyzed by One-way ANOVA and t-test. Significance was defined as $p<0.05$.

Results

Microarray analysis. We used Affymetrix GeneChip 4.0 Array to analysis differentially expressed genes in induced HUVECs and normal HUVECs. Of the 47000 probe sets on the arrays, 19536 had a present call, except the similar expression between the induced HUVECs and normal HUVECs, there were 3769 genes that had differential expression ($p<0.001$), of which 1609 were upregulated and 2160 were downregulated. Scatter plot for all the detected genes are shown (Fig. 1A). Red spots represent upregulated genes, green spots represent downregulated genes, black spots represent no change genes between the induced HUVECs and normal HUVECs. The distance of red or green spots from the black spots represents markedly up- or down-regulated ($p<0.001$). To compare the functions of differential genes, the

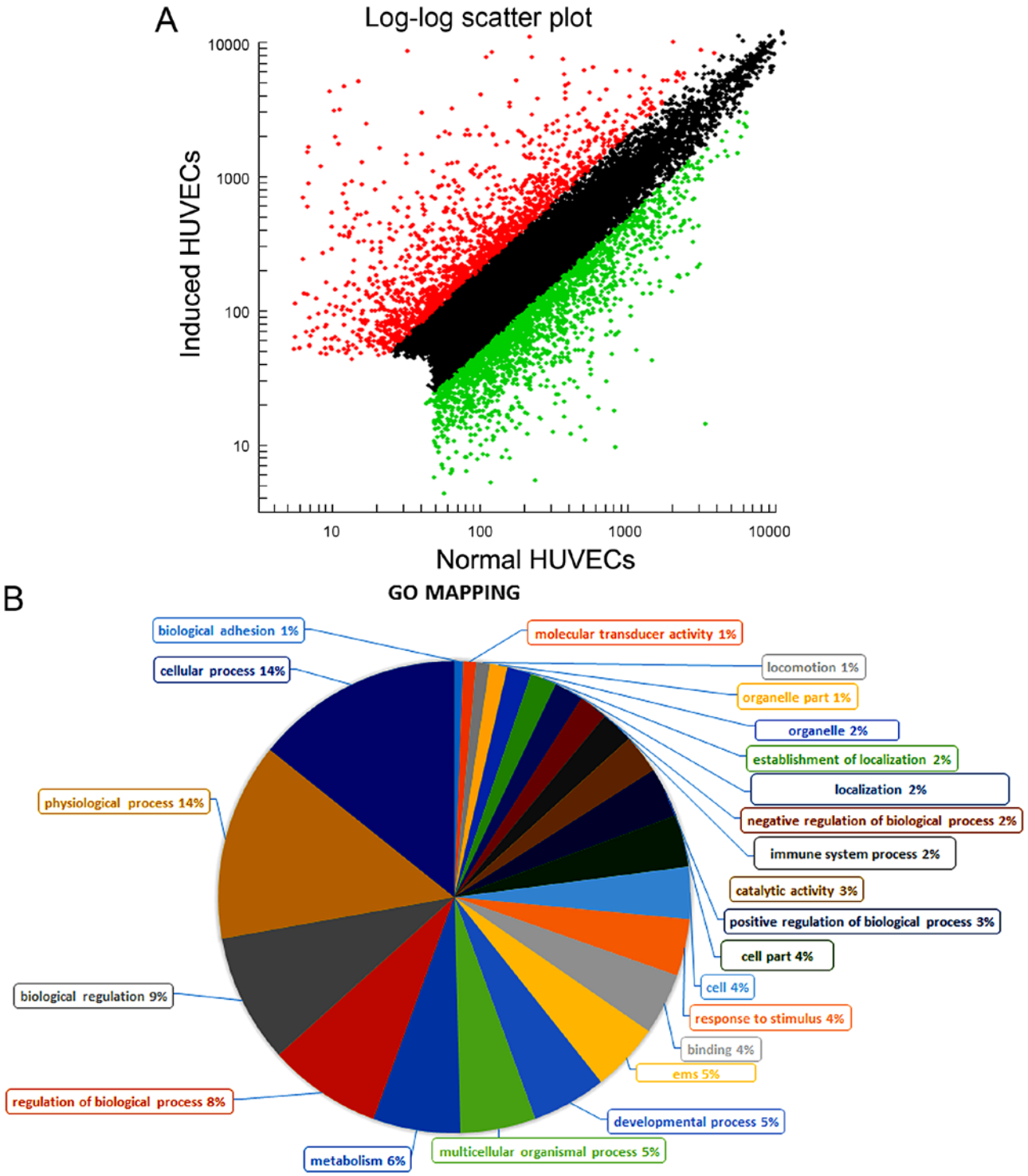

Figure 1. Scatter plots and classification of differentially expressed genes in induced HUVECs and normal HUVECs. (A) The scatter plots of differentially expressed genes in induced HUVECs and normal HUVECs. Black dots, the stably expressed genes in both groups. Red dots, upregulated genes in induced HUVECs compare with normal HUVECs, while green dots, downregulated genes, each group have three duplicate samples. (B) The genes were classified according to their function. The composition of each class of identified genes is given as the percentage of all the individual identified genes.

functional annotations of all identified genes were collected and displayed (Fig. 1B). The following will focus on the genes involved in cell differentiation and angiogenesis, for their potential roles in normal endothelial cells differentiation into tumor vascular endothelial cells. Clustering analysis of the arrays showed the 48 genes differentially expressed in induced HUVECs compared with normal HUVECs, of which 30 were upregulated and 18 were downregulated. Among them, the upregulated genes included IL-6, VEGF-A, S1PR1, and TYMP (Fig. 2).

All the differential genes were categorized based on their cellular component, molecular function, biological process and pathway. The number of changed genes enriched in the cellular component and molecular function is shown (Fig. 3). Among the differentially genes, 28 were categorized based on

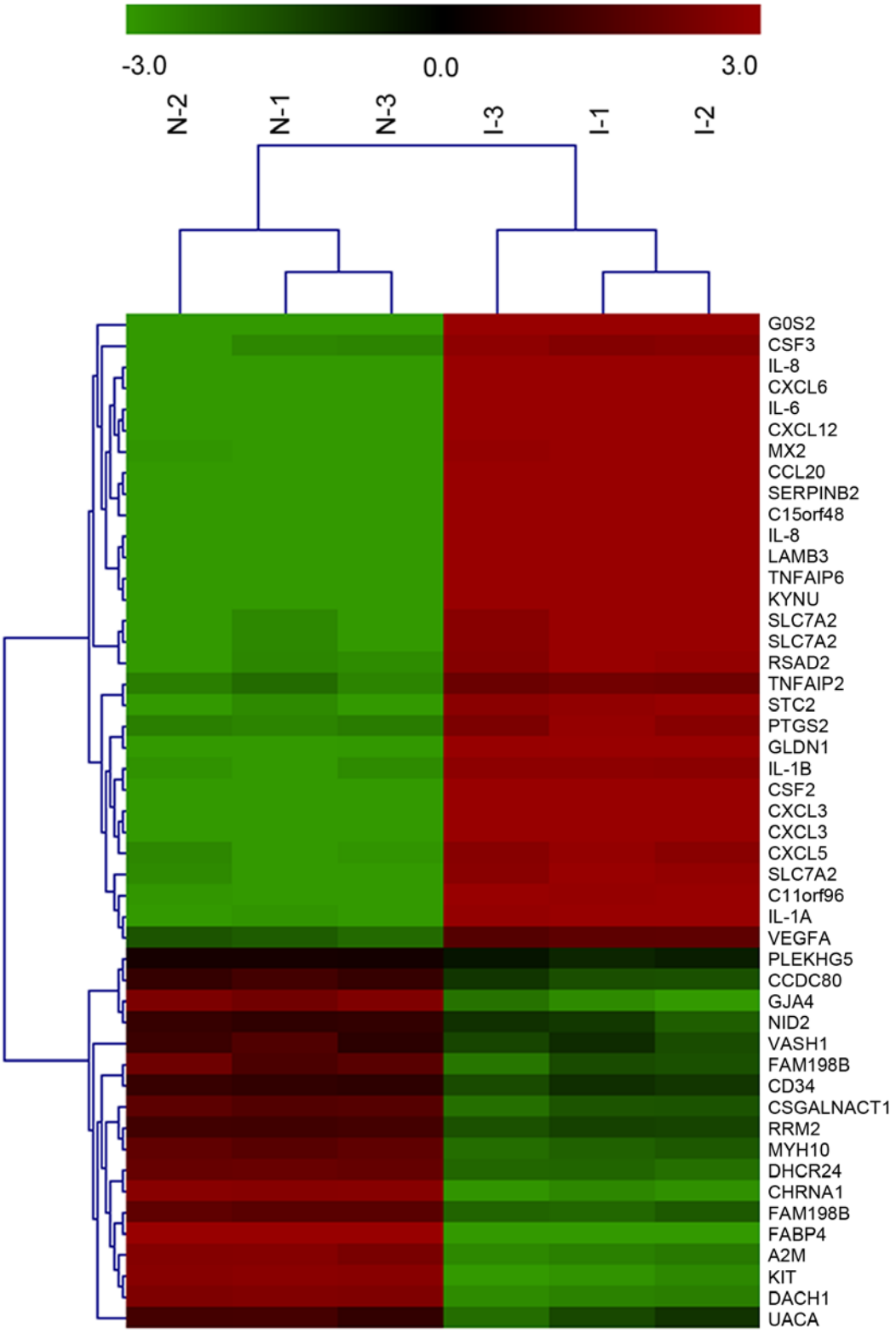

Figure 2. Hierarchical cluster assays of genes expressed with significant difference in induced HUVECs (I-1, I-2, I-3) compare with normal HUVECs (N-1, N-2, N-3). Distinct signatures were observed in induced HUVECs and normal HUVECs. The value of each gene was adjusted by a median-centering algorithm in log scale, and the colors indicate the relative gene expression in the red-green heat map. 0 indicated by pure black represents no change from the median gene expression level in all samples. -3.0 indicated by pure green represents relatively lower expression. +3.0 indicated by pure red represents relatively higher expression.

their involvement in one or more of the biological processes. Of these processes, angiogenesis, cell differentiation, positive regulation of angiogenesis, positive regulation of cell migration, endothelial cell differentiation, signal transduction, cell adhesion, positive regulation of cell proliferation, cell-cell signaling, were significantly represented (Table I). Differential genes (3769) were submitted to online DAVID for KEGG analysis. Compared with the normal HUVECs, the

Table I. Genes differentially expressed in induced HUVECs vs. normal HUVECs categorized based on biological processes.

Gene ID	Gene description	Probe set	Fold
Positive regulation of cell migration (n=5)[a]			
S1PR1	Sphingosine 1-phosphate receptor 1	11743816_s_at	3.16
RRAS2	Ras-related protein R-Ras2	11747262_s_at	3.2541
EGFR	Epidermal growth factor receptor	11725104_a_at	2.8712
CDH13	Cadherin 13, H-cadherin	11744837_a_at	2.7747
PDGFA	Platelet derived growth factor A/B	11729062_a_at	2.1934
Positive regulation of angiogenesis (n=2)[a]			
IL1A	Interleukin 1, α	11725198_at	72.8526
IL1B	Interleukin 1, β	11719916_at	54.0777
Development (n=6)[a]			
CSF3	Granulocyte colony-stimulating factor 3	11729514_a_at	45.4721
TNFAIP2	Tumor necrosis factor α-induced protein 2	11717823_s_at	23.9717
VEGFA	Vascular endothelial growth factor A	11725372_x_at	12.5152
TYMP	Thymidine phosphorylase	11722449_x_at	9.2008
TNF	Tumor necrosis factor superfamily, member 2	11721577_at	3.8156
VEGFC	vascular endothelial growth factor C	11720163_at	2.4673
Positive regulation vascular endothelial growth factor production (n=2)[a]			
IL1A	Interleukin 1, α	11725198_at	72.8526
IL1B	Interleukin 1, β	11719916_at	54.0777
Cell adhesion (n=3)[a]			
TNFAIP6	Tumor necrosis factor, α-induced protein 6	11743636_at	186.9013
LAMB3	Laminin, β3	11719487_a_at	126.4414
CLDN1	Claudin 1	11728234_a_at	32.8574
Signal transduction (n=4)[a]			
CCL20	Chemokine(C-C motif) ligand 20	11724828_at	334.6801
TNFAIP6	Tumor necrosis factor, α-induced protein 6	11743636_at	186.9013
CXCL5	Chemokine (C-X-C motif) ligand 5	11728716_x_at	51.4412
VEGFC	Vascular endothelial growth factor C	11720163_at	2.4673
Endothelial cell differentiation (n=2)[a]			
JAG1	Jagged	11720825_s_at	4.3523
S1PR1	Sphingosine 1-phosphate receptor 1	11743816_s_at	3.16
Positive regulation of cell proliferation (n=1)[a]			
CXCL5	Chemokine (C-X-C motif) ligand 5	11728716_x_at	51.4412
Cell-cell signaling (n=6)[a]			
IL8	Interleukin 8	11763226_x_at	385.6941
CCL20	Chemokine (C-C motif) ligand 20	11724828_at	334.6801
STC2	Stanniocalcin 2	11721436_a_at	63.1026
CXCL6	Chemokine (C-X-C motif) ligand 6	11730801_at	158.1146
IL1B	Interleukin 1, β	11719916_at	54.0777
CXCL5	Chemokine (C-X-C motif) ligand 5	11728716_x_at	51.4412
Angiogenesis (n=6)[a]			
IL8	Interleukin 8	11763226_x_at	385.6941
TNFAIP2	Tumor necrosis factor α-induced protein 2	11717823_s_at	23.9717
VEGFA	Vascular endothelial growth factor A	11725372_x_at	12.5152
TYMP	Thymidine phosphorylase	11722449_x_at	9.2008
S1PR1	Sphingosine 1-phosphate receptor 1	11743816_s_at	3.16
VEGFC	Vascular endothelial growth factor C	11720163_at	2.4673
Cell differentiation (n=5)[a]			
TNFAIP2	Tumor necrosis factor α-induced protein 2	11717823_s_at	23.9717
TYMP	Thymidine phosphorylase	11722449_x_at	9.2008
VEGFC	Vascular endothelial growth factor C	11720163_at	2.4673
NOTCH2	Notch	11732189_at	2.459
MMP19	Matrix metalloproteinase-19	11719531_a_at	2.3774

[a]$p<0.001$

Table II. Genes differentially expressed in the induced HUVECs vs. normal HUVECs based on the significantly changed pathway.

Gene ID	Gene description	Probe set	Fold
Cytokine-cytokine receptor interaction (n=11)[a]			
IL8	Interleukin 8	11763226_x_at	385.6941
CCL20	Chemokine(C-C motif) ligand 20	11724828_at	334.6801
CXCL3	Chemokine (C-X-C motif) ligand 3	11728476_a_at	298.7616
IL6	Interleukin 6	11760425_a_at	4.3931
CSF2	Colony stimulatingfactor 2	11728876_at	143.5679
IL1A	Interleukin 1, α	11725198_at	72.8526
CXCL2	Chemokine (C-X-C motif) ligand 2	11744127_at	70.206
IL1B	interleukin 1, β	11719916_at	54.0777
CXCL5	Chemokine (C-X-C motif) ligand 5	11728716_x_at	51.4412
CSF3	Colony stimulating factor 3	11729514_a_at	45.4721
Toll-like receptor signaling pathway (n=3)[a]			
IL8	Interleukin 8	11763226_x_at	385.6941
IL6	Interleukin 6	11760425_a_at	4.3931
IL1B	Interleukin 1, β	11719916_at	54.0777
JAK-STAT signaling pathway (n=3)[a]			
IL6	Interleukin 6	11760425_a_at	4.3931
CSF2	Colony stimulating factor 2	11728876_at	143.5679
CSF3	Colony stimulating factor 3	11729514_a_at	45.4721
VEGF signaling pathway (n=3)[a]			
PTGS2	Prostaglandin-endoperoxide synthase 2	11724038_a_at	5.9638
VEGFA	Vascular endothelial growth factor A	11725372_x_at	12.5152
PRKCA	Classical protein kinase C	11754557_s_at	2.2595
Wnt signaling pathway (n=2)[a]			
MYC	Myc proto-oncogene protein	11745021_a_at	4.5211
JUN	Transcription factor AP-1	11718397_s_at	3.6646
TGF-β signaling pathway (n=1)[a]			
MYC	Myc proto-oncogene protein	11745021_a_at	4.5211
mTOR signaling pathway (n=2)[a]			
VEGFA	Vascular endothelial growth factor A	11725372_x_at	12.5152
VEGFC	Vascular endothelial growth factor C	11720163_at	2.4673
Cell adhesion molecules (CAMs) (n=1)[a]			
CLDN1	Claudin 1	11728234_a_at	32.8574
MAPK signaling pathway (n=2)[a]			
IL1A	Interleukin 1, α	11725198_at	72.8526
IL1B	Interleukin 1, β	11719916_at	54.0777
ECM-receptor interaction(n=1)[a]			
LAMB3	Laminin, β3	11719487_a_at	126.4414

[a]p<0.001

induced HUVECs had 23 differential gene-involved significant pathways (p<0.001, Table II). These pathways included Cytokine-cytokine receptor interaction, JAK-STAT signaling pathway, MAPK signaling pathway, TGF-β signaling pathway, Wnt signaling pathway, VEGF signaling pathway, mTOR signaling pathway, and Cell adhesion molecules (CAMs). Most of these pathways are associated with angiogenesis and cell differentiation.

Confirmation the changes in gene expression by qRT-PCR. According to the relevant functional annotations and their high fold-changed values, IL6 which is involved in the JAK-STAT signaling pathway and Toll-like receptor signaling pathway, VEGFA which is involved in VEGF signaling pathway and mTOR signaling pathway, S1PR1 and TYMP which are involved in the biological process of angiogenesis and cell differentiation were chosen for qRT-PCR validation. The

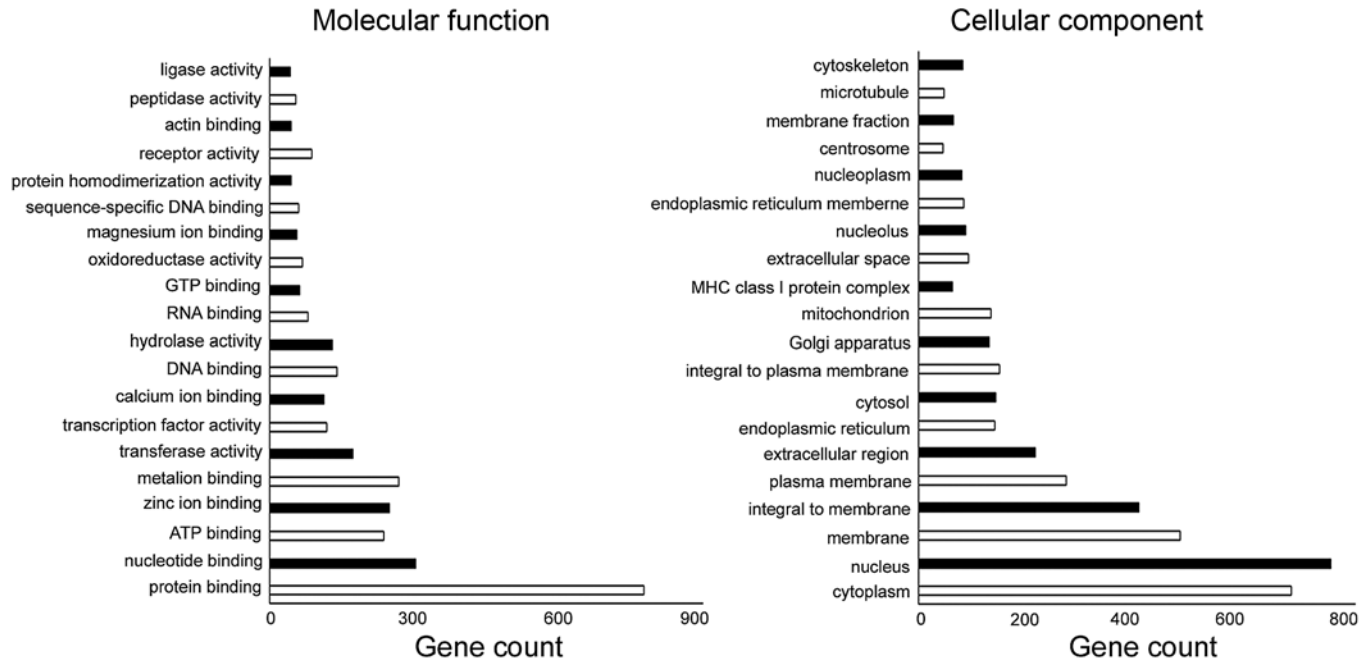

Figure 3. Analysis of the top 20 significant Go terms and their gene numbers in HUVECs genome after induction by KYSE70 supernatant. (A) Cell component analysis showed that the proteins encoded by differently expressed genes were mainly distributed in the extracellular parts and the cell membrane. (B) Molecular function analysis showed that besides some biological macromolecule biding functions, the proteins encoded by differently expressed genes were closely related to the cytokine network in that they influenced ligase activity, peptidase activity, receptor activity, protein homodimerization activity and transferase activity.

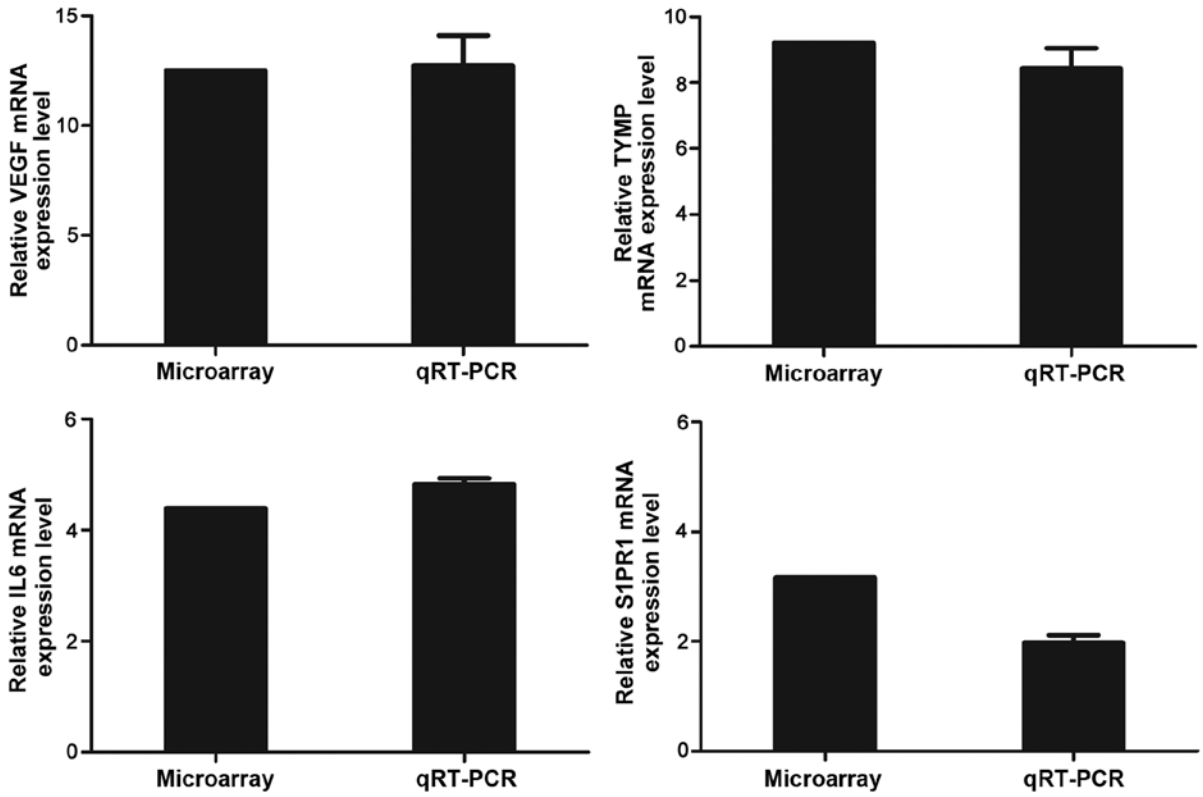

Figure 4. Differentially expressed genes (VEGFA, IL6, S1PR1, and TYMP) studied through microarray experiments and validated by qRT-PCR. The qRT-PCR results were in line with the microarray data set. The HUVECs used for qRT-PCR and microarray analysis were induced in the same conditions. The y-axis represents the selected genes fold change of induced HUVECs compare with normal HUVECs.

HUVECs used for qRT-PCR and microarray analysis were induced by KYSE70 supernatant at the same condition. For all the four genes, the qRT-PCR results were in accordance with the microarray date (Fig. 4).

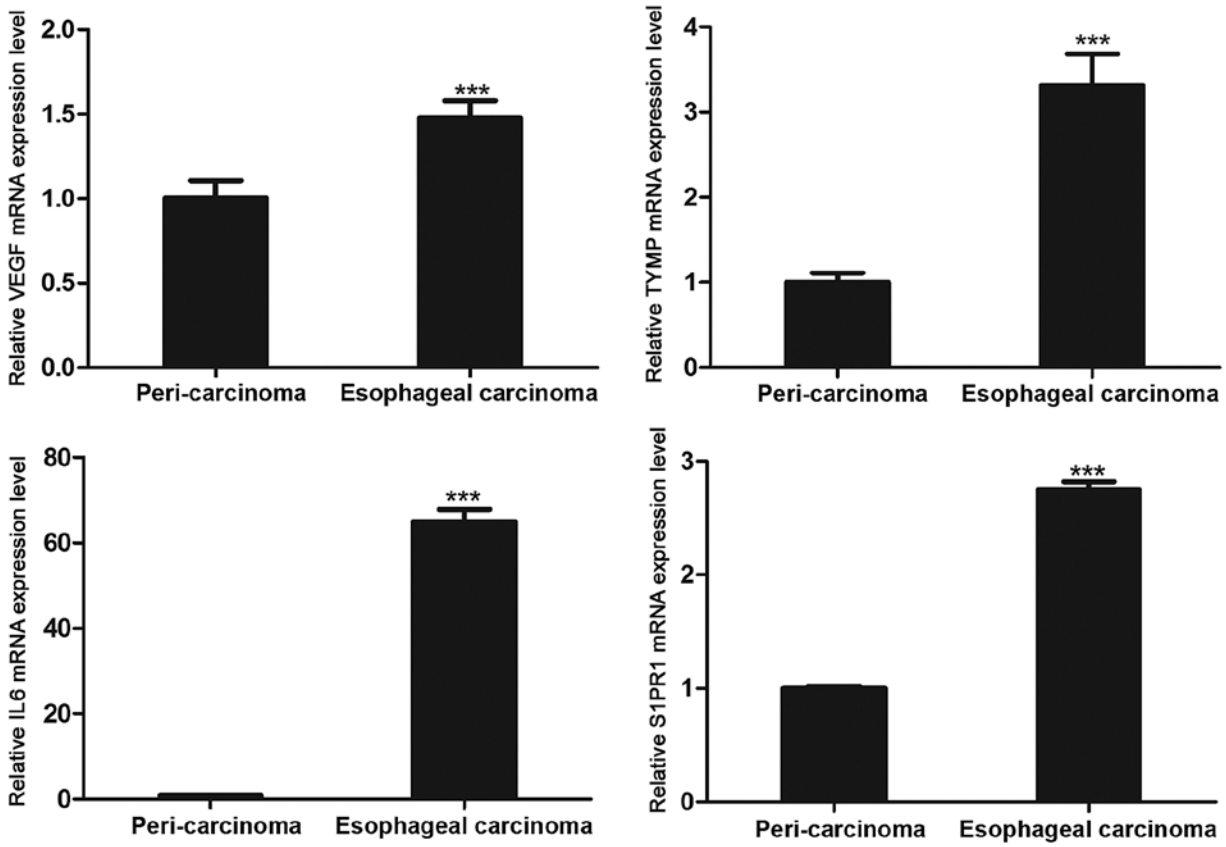

Figure 5. mRNA expression of candidate genes (VEGFA, IL6, S1PR1, and TYMP) in HUVECs were significantly increased after induction by esophageal carcinoma tissue homogenate supernatant.

Differences in the mRNA levels of the target genes analyzed in HUVECs induced by human esophageal carcinoma tissue homogenates. KYSE70 human esophageal carcinoma conditioned medium is different from the esophageal carcinoma tissue of patients. qRT-PCR was further used in induced HUVECs by human esophageal carcinoma tissue homogenates. The results of qRT-PCR showed that IL6, VEGFA, S1PR1, TYMP mRNA increased in the induced HUVECs by esophageal carcinoma tissue homogenates compared with induced HUVECs by peri-carcinoma tissue homogenates ($p<0.001$).

Discussion

Esophageal carcinoma is one of the most common cancers in China. When the tumor grows beyond 1-2 mm in diameter, angiogenesis is indispensable for supporting the expansive growth. It has been proved that the tumor microenvironment governs tumor-associated angiogenesis in multiple ways, and there are great differences between tumor vascular endothelial cells and normal vascular endothelial cells (6,16). Herein, for the first time, we found a significant alteration of gene expression in induced HUVECs by tumor microenvironment which was simulated by esophageal cancer cell supernatant (KYSE70). This study introduces a new prospect that contributes to understanding the influence of tumor microenvironment on angiogenesis, and has an important clinical significance in inhibiting the developing of neovascularization, which would be a promising therapeutic strategy for cancer treatment.

In the present study, we used gene chip and bioinformatics technology to study the gene expression profile of induced HUVECs by KYSE70 supernatant. Further statistical analysis of differentially expressed genes revealed 3769 differentially expressed genes in induced HUVECs, including 1609 upregulated genes and 2160 downregulated genes (Fig. 1A). These genes were classified according to their cellular component, molecular function, biological process and pathway. The results indicate that there are different molecular mechanisms governing the process of angiogenesis affected by tumor microenvironment.

The analysis of gene annotations in this study implied that the differently expressed genes mainly play important roles in cell differentiation and angiogenesis, and are involved in the MAPK signaling pathway, JAK-STAT signaling pathway, Toll-like receptor signaling pathway, Hematopoietic cell lineage, Cytokine-cytokine receptor interaction, VEGF signaling pathway, mTOR signaling pathway, and Wnt signaling pathway. These pathways or related genes have been previously confirmed to have great relation with angiogenesis and cell differentiation (6,17,18).

VEGF signaling pathway is involved in proliferation, survival, migration and integrity of endothelial cells, which play a key role in cancer-induced angiogenic processes (6). Moreover, mTOR signaling pathway has equal importance in tumorigenesis, development and the differentiation of tumor stem cells (19). In this research, VEGFA which was associated with these pathways was confirmed highly expressed in induced HUVECs by qRT-PCR and the fold-change was

consistent with the microarray data. Further study indicated that induced HUVECs by esophageal carcinoma tissue homogenate also expressed VEGFA at a high level. These results may illustrate that tumor microenvironment has influence on tumor angiogenesis by the way of normal endothelial cell differentiation toward tumor endothelial cells, and VEGFA may play an important role in this process. Although many kinds of agents based on VEGF or its receptor have been created, there is a need for further in-depth study (17).

Another upregulated gene, IL6, which is an angiogenic member of the CXC chemokine family was also identified by qRT-PCR. It has been proved that IL6 produced by endothelial cells with the tumor contributes to tumor development through neovascularization (20). IL6 was also involved in the JAK-STAT signaling pathway and Toll-like receptor signaling pathway which were significantly changed and play an important role in angiogenesis (21). As the expression level of IL6 is higher in induced HUVECs, we predict that there may be some pro-angiogenic constituents in the tumor microenvironment promoting normal endothelial cells differentiation toward tumor endothelial cells.

Another pathway that was significantly changed was Wnt signaling pathway. It has been report that the Wnt signaling promotes neovascularization of the retina in patients with diabetic retinopathy, and has an important role in angiogenic activity of endothelial cells (18). The Wnt signaling pathway controls the proliferation, migration and differentiation of vascular cells as well as the expression of angiogenic factors, such as VEGF and IL8 (18,22). The Wnt signaling pathway may promote neovascularization by promoting normal endothelial cell differentiation into tumor vascular endothelial cells.

Another gene involved in angiogenesis and endothelial cell differentiation is S1PR1 (sphingosine 1-phosphate receptor 1) which is one of the five G protein-coupled receptors for S1P1 (23). Previous studies have suggested that S1PR1 is required for stabilization of nascent blood vessels during embryonic development (24). Further study using the Levis lung carcinoma model of tumor growth showed that microvessels within the tumor expressed S1PR1. Immunofluorescence analysis with the S1PR1 antibody also confirmed that S1PR1 is induced in endothelial cells during tumor angiogenesis (25). In this study, the high S1PR1 expression level in induced HUVECs illustrated that the HUVECs already have the characteristic of tumor vessel endothelial cells. S1PR1 may have the potential to be a new biomarker in antitumor angiogenesis.

TYMP is an enzyme involved in pyrimidine nucleoside metabolism. It can catalyze the reversible phosphorolysis of thymidine, deoxyuridine and their analogs to their bases and 2-deoxyribose-1-phosphate (26). It has been reported that the TYMP expression is significantly higher in the patient with endometriosis, which indicates it may play a key role in angiogenesis (27). Another study demonstrated that TYMP and VEGF expression were correlated to patient with colorectal cancer, and TYMP plays an important role in angiogenesis, ECM remodeling, and in the prognosis of patients with colorectal cancer (28). Further studies are needed to define its role, but it is clear that TYMP has a potential role as a new biomarker in anti-angiogenesis.

In conclusion, the comparison of the gene expression profiles of induced HUVECs by KYSE70 supernatant and normal HUVECs suggested that the gene expression of endothelial cells were altered according to their microenvironment. We believe that the results pave the way to more functional studies that are needed to elucidate their possible role in tumor angiogenesis. Specifically, major signaling pathways particularly the VEGF signaling pathway, mTOR signaling pathway, cytokine-cytokine receptor interaction and Wnt signaling pathway play key roles in angiogenesis induced by tumor microenvironment. Moreover, several genes such as VEGFA, IL6, TYMP, S1PR1, especially TYMP and S1PR1 are suggested to serve as new biomarkers of angiogenesis, and can be used as targets to against esophageal carcinoma.

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