Differential expression of leukocyte β2 integrin signal transduction-associated genes in patients with symptomatic pulmonary embolism

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Abstract. Whole human genome oligo microarrays were employed to systematically investigate the differential expression characteristics of associated mRNAs, which were found in the signal transduction pathway of β2 integrins in peripheral blood mononuclear cells (PBMCs) between patients with symptomatic pulmonary embolism (PE) and controls. A total of 20 cases of PE patients and twenty gender- and age-matched controls were recruited for the study. Human cDNA microarray analysis was used to detect the differences in mRNA expression between the two groups and a random variance model corrected t-test was used to analyze the statistical data. A total of 80 associated mRNAs were detected. The mRNA expression of chemokines, ligands, inside-out and outside-in signaling pathway-associated proteins were upregulated significantly in the PE group, compared with the controls. In five subunit-associated mRNAs, the mRNA expression of ITGAL, ITGAM, ITGAX and ITGB2, which encode for the subunits of αL, αM, αX and β2, were upregulated in the PE group and the differences, with the exception of ITGB2, were statistically significant ($P<0.05$). The mRNA expression of ITGAD was downregulated; however, there was no significant difference ($P>0.05$). The expression of Fgr mRNA was significantly downregulated ($P<0.01$). Thus, in PE patients, bilateral signal transduction pathways of β2 integrins in neutrophils and monocytes were activated, enhancing innate immunity.

Introduction

Pulmonary embolism (PE), together with deep venous thrombosis (DVT), is termed venous thromboembolism (VTE). Acute venous thrombosis is a type of red thrombus. A large number of cells gather in red thrombus irregularly and the majority are neutrophils. Smeeth *et al* observed that VTE was associated with infection, and the risks of DVT and PE were significantly raised in the first two weeks of diagnosis (1). The current study observed that compromised immunity is associated with the occurrence of VTE (2). In 2009, it was reported (3) that the mRNA expression of natural killer (NK) cells and T lymphocytes in PE patients were significantly downregulated. It has recently been reported that the immunity of $CD3^+$ and $CD8^+$ T cells in patients with acute PE were reduced (1) and the immunity of $CD3^+$ and $CD8^+$ T cells in patients with chronic thromboembolic pulmonary hypertension were also reduced (2). These observations indicate that integrins are the core proteins in VTE and β2 integrins, which are distributed in the leukocyte and are involved in the occurrence of VTE.

Integrins are type I transmembrane glycoproteins. In humans, there are 24 integrins formed by specific non-covalent associations of 18 α and 8 β subunits (4). A type of integrin may be distributed in a number of cells and a number of integrins are expressed in signal cells.

Numerous integrins are specially expressed on specific types of cells. For example, β2 integrins are only expressed on the cytomembrane of leukocytes, thus, β2 integrins are also known as leukocyte integrins.

A recent study suggested that the associated mRNA expression of integrins, which were distributed in leukocytes and platelets, were upregulated significantly (5). It is unclear how the integrin-mediated signal transduction pathway and signaling proteins function. To investigate the differential expression of associated mRNAs, which were found in the signal transduction pathway of β2 integrins in PE patients, the whole human genome oligo microarray was employed to systematically investigate the expression differences of associated mRNAs.

Materials and methods

Patient information. A total of 20 patients were enrolled in the PE group and were admitted to Tongji Hospital (Shanghai, China) during 2007, including 11 males and 9 females, with an average age of 70±14 years (44-89 years old). All patients were diagnosed with PE on the basis of a minimum of two of

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Table I. mRNA expression of genes related to the subunits of β2 integrins.

Subunit	Gene	PE	Control	P-value	P-value
β2	ITGB2	17.9±0.40	17.88±0.29	-	0.834460
αL	ITGAL	14.00±0.64	13.51±0.67	<0.05	0.024773
αM	ITGAM	16.89±0.60	16.34±0.31	<0.01	0.000830
αX	ITGAX	13.91±0.60	12.84±0.43	<0.01	0.001798
αD	ITGAD	9.118±0.82	9.38±1.13	-	0.446025

P-values denote PE vs. control groups. PE, pulmonary embolism.

Table II. mRNA expression of genes related to the ligand of β2 integrins.

Ligand	Gene	PE	Control	P-value	P-value
ICAM-1	ICAM1	13.45±0.82	12.88±0.48	<0.05	0.01456
ICAM-2	ICAM2	15.13±0.60	15.42±0.36	-	0.07313
ICAM-3	ICAM3	17.84±0.43	17.43±0.37	<0.01	0.00253
ICAM-4	ICAM4	9.18±0.51	9.09±0.61	-	0.61033
ICAM-5	ICAM5	7.78±0.59	7.60±0.77	-	0.30345
JAM-1	F11R	15.64±1.04	15.5±0.56	-	0.45364
JAM-3	JAM3	9.9±0.88	9.47±0.74	<0.05	0.03685
RAGE	AGER	14.90±0.37	14.37±0.28	<0.01	1.1E-05
Fibrinogen	FGA	4.65±0.66	4.96±0.93	<0.05	0.01806
	FGB	4.44±0.45	4.82±0.70	<0.05	0.04980
	FGC	4.31±0.38	4.75±0.67	<0.05	0.01541
uPAR	PLAUR	14.45±0.71	13.96±0.57	<0.05	0.01965
Laminin 8	LAMC1	9.23±0.82	8.72±0.75	<0.05	0.04718
VCAM-1	VCAM1	5.83±1.49	5.64±1.17	-	0.65764

P-values denote PE vs. control groups. PE, pulmonary embolism; uPar, urokinase-type plasminogen activator receptor.

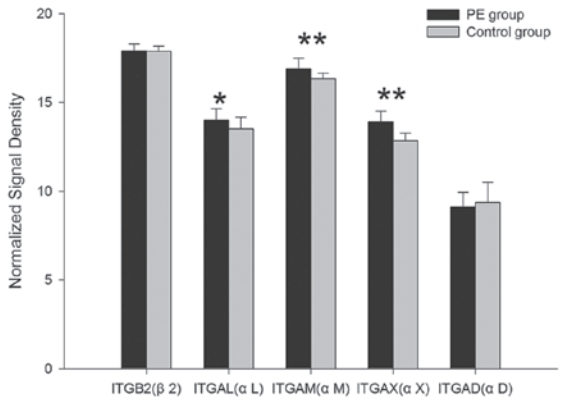

Figure 1. mRNA expression of genes associated with the subunits of β2 integrins. *P<0.05; **P<0.01. PE, pulmonary embolism.

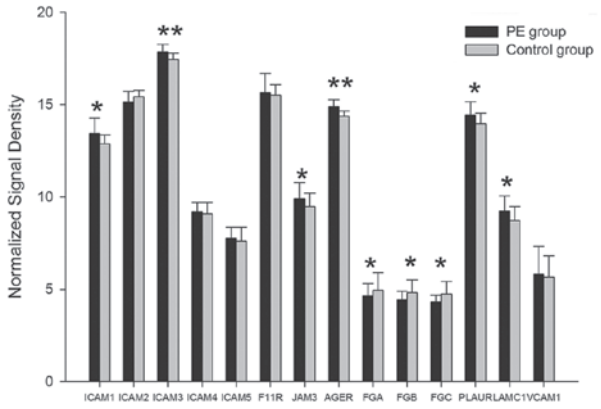

Figure 2. mRNA expression of genes associated with the ligand of β2 integrins. *P<0.05; **P<0.01. PE, pulmonary embolism.

the following criteria: i) selective pulmonary arteriography showing a filling defect or blockage; ii) pulmonary ventilation perfusion scanning exhibiting single or multiple blood flow perfusion defects with normal or abnormal ventilation and mismatched ratio of ventilation/perfusion and iii) other clinical characteristics, including a typical manifestation of PE. Arterial blood gas analysis, D-dimer test, ultrasound cardiogram and chest computerized tomography were used to support the diagnosis and exclude other cardiac and pulmonary disorders. A further 20 patients (11 males, 9 females; 44-91 years of age with a mean age of 72±14) with ischemic heart disease admitted during the same period, without PE, DVT and other congenital

Table III. mRNA expression of genes related to the chemokines.

Chemokine	Gene	PE	Control	P-value	P-value
SDF-1α	CXCL12	5.76±0.43	5.56±1.22	<0.01	0.00459
BLC	CXCL13	4.65±0.40	5.01±0.69	-	0.05251
SLC	CCL21	8.12±0.27	8.11±0.50	-	0.93293
RANTES	CCL5	16.37±0.63	16.69±0.36	-	0.06193
fMLP	FPR1	17.85±0.63	17.71±0.62	-	0.48139
IL-8	IL8	11.27±1.86	12.17±1.62	-	0.11033
PAF	PAF1	11.96±0.54	11.35±0.43	<0.01	0.00028

P-values denote PE vs. control groups. PE, pulmonary embolism.

Table IV. mRNA expression of Rap1-related signals in inside-out activation.

Protein	Gene	PE	Control	P-value	P-value
PLCγ	PLCG1	10.23±0.96	10.1±0.55	-	0.48225
	PLCG2	15.36±0.44	14.91±0.39	<0.01	0.00142
SLP-76	LCP2	16.49±0.34	16.16±0.26	<0.01	0.00142
ADAP	FYB	16.68±0.35	16.44±0.37	<0.05	0.04653
DalDAG-GEFI	RASGRP1	12.31±0.53	12.39±0.38	<0.05	0.03376
SPA1	SIPA1	16.23±0.46	15.75±0.38	<0.01	0.00089
RIAM	APBB1IP	15.83±0.44	15.32±0.38	<0.01	0.00068
RAPL	RASSF5	14.16±0.95	13.28±0.77	<0.01	0.00039
Mst1	MST1	11.35±0.51	11.55±0.49	-	0.22278

P-values denote PE vs. control groups. PE, pulmonary embolism Rap1, Ras-related protein 1.

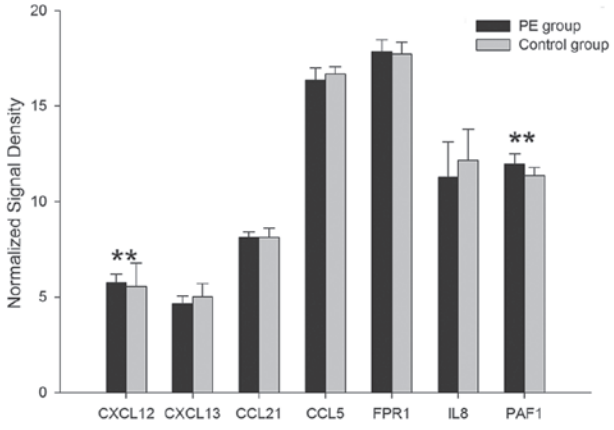

Figure 3. mRNA expression of genes associated with chemokines. **P<0.01, PE vs. control groups. PE, pulmonary embolism.

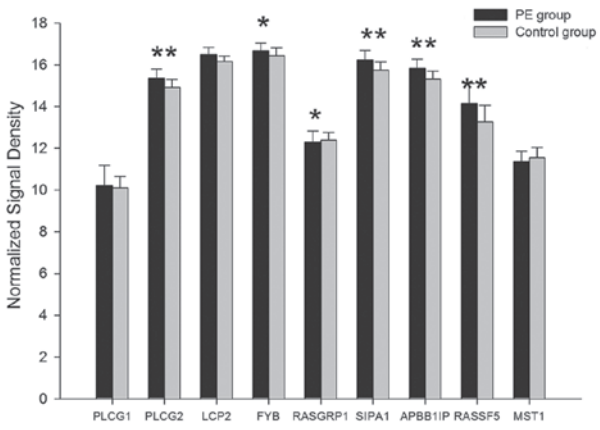

Figure 4. mRNA expression of Rap1 related signals in inside-out activation. *P<0.05; **P<0.01, PE vs. control groups. PE, pulmonary embolism; Rap1, Ras-related protein 1.

bleeding and thrombosis diseases, with comparative clinical presentation were enrolled in the control group. The study was approved by the Ethics Committee of Tongji University (Shanghai, China) and informed consent was obtained from all patients in accordance with the Declaration of Helsinki.

Total RNA isolation. A total of 5 ml of peripheral blood samples anti-coagulated with EDTA were drawn from patients suspected of having PE and from those without PE, immediately following admission to the hospital. Leukocytes were obtained by density gradient centrifugation with Ficoll solution and the remaining red blood cells were destroyed by erythrocyte lysis buffer (Qiagen, Hilden, Germany). Total mononuclear cell RNA was extracted with TRIzol (Invitrogen Life Technologies, Carlsbad, CA, USA) and purified with

Table V. mRNA expression of other proteins in inside-out activation.

Protein	Gene	PE	Control	P-value	P-value
Talin	TLN1	13.28±1.18	11.87±1.19	<0.01	0.00056
	TLN2	6.166±0.43	5.667±0.64	<0.05	0.01142
Vinculin	VCL	14.18±0.66	13.32±0.85	<0.01	0.00109
Dok1	DOK1	12.17±0.4	11.5±0.3	<0.01	0.00208
14-3-3ζ	YWHAZ	15.67±0.41	15.19±0.41	<0.01	0.00080
Filamin A	FLNA	16.16±0.74	15.42±0.82	<0.01	0.00447
Migfilin	FBLIM1	7.334±1.13	7.206±1.3	<0.05	0.01134
α-actinin	ACTN1	16.62±0.61	15.95±0.51	<0.01	0.00059
Radixin	RDX	10.81±0.41	10.59±0.33	-	0.07200
Calpain	CAPN1	13.8±0.98	12.62±0.96	<0.01	0.00044
	CAPN3	12.45±0.48	11.96±0.52	<0.01	0.00421
	CAPN10	12.7±0.62	12.42±0.46	<0.05	0.04066
	CAPN11	6.494±1.13	5.344±1.05	<0.01	0.00184
	CAPN13	6.303±1.58	5.59±1.13	<0.05	0.01328

P-values denote PE vs. control groups. PE, pulmonary embolism.

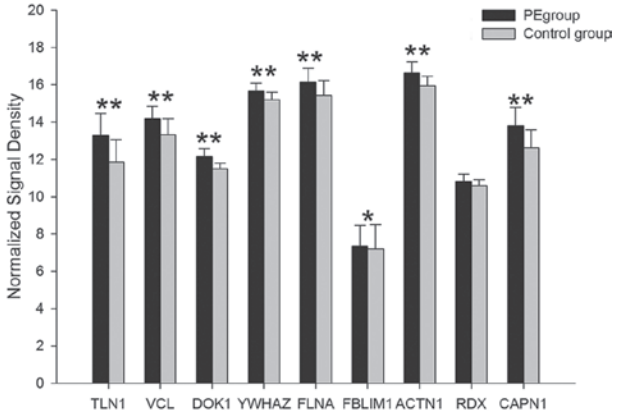

Figure 5. mRNA expression of other proteins in inside-out activation. *P<0.05; **P<0.01, PE vs. control groups. PE, pulmonary embolism.

RNeasy column (Qiagen), according to the manufacturer's instructions. The isolated total RNA was tested and quantified using a Nanodrop ND-1000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA).

Gene expression clip. Agilent G4112A Whole Human Genome Oligo Microarrays were purchased from Agilent Technologies Inc. (Santa Clara, CA, USA). The genes or transcripts included 314 negative control spots, 1,924 positive control spots and 359 blank spots. The functions of >70% of the genes in the microarray have been previously identified. All patients were subjected to clip analysis.

Target preparation and microarray hybridization. The RNA samples of patients with confirmed diagnosis of PE and controls were labeled using the indirect labeling method. Briefly, 1 μg of total RNA was reverse transcribed. Second strand cDNA was produced and purified, followed by *in vitro* transcription (IVT) with T7 RNA Polymerase. During IVT, the modified nucleotide, 5-(3-aminoallyl)-UTP (aaUTP) was incorporated into the cDNA. Subsequently, the fluorescent Cy3 was chemically coupled with the aaUTP, which contains a reactive primary amino group on the C5 position of uracil. The dye incorporation rate was assessed with a Nanodrop ND-1000 spectrophotometer and was found to be between 1.2 and 1.4 pmol/μl. Hybridization was performed using the Agilent Oligonucleotide Microarray *in situ* Hybridization Plus kit (p/n 5,184-3,568), according to the manufacturer's instructions. Briefly, 750 ng of Cy3-labeled sample cDNA was subjected to fragmentation (30 min at 60˚C) and hybridization on 44K Human Whole-Genome 60-mer oligo-chips (G4112F, Agilent Technologies) was performed in a rotary oven (10 rpm, 60˚C, 17 h). Slides were disassembled and washed in solutions I and II, according to the manufacturer's instructions.

Reverse transcription-polymerase chain reaction (RT-PCR). Three differential genes were selected and their expression was confirmed by RT-PCR. Among the genes with differential expression, seven genes were randomly selected and the house keeping gene [glyceraldehyde 3-phosphate dehydrogenase (GAPDH)] was subjected to RT-PCR. The relative expression levels were indicated as the expression of the target genes normalized to the expression of GAPDH ($2^{-\Delta\Delta Ct}$). A melting curve and the $2^{-\Delta\Delta Ct}$ method were used to compare the differences in the expression between the control and PE group. The results from RT-PCR were consistent with the microarray analysis.

Statistical analysis. Measurement data are expressed as the mean ± SD. The Agilent Feature Extraction software was used to collect the original data from the microarray, followed by an analysis with a robust multichip average. The gene intensity data between the PE and control group were compared with a random variance model-corrected Student's t-test by SPSS 14.0 software packet (SPSS, Inc., Chicago, IL, USA).

Table VI. mRNA expression of SFK and PKC in outside-in activation.

SFK	Gene	PE	Control	P-value	P-value
Hck	HCK	16.49±0.73	15.79±0.54	<0.01	0.00128
Fgr	FGR	13.63±1.05	12.55±0.68	<0.01	0.00044
Lyn	LYN	17.47±0.52	17.06±0.45	<0.05	0.01608
Lck	LCK	15.72±0.56	15.95±0.37	-	0.34016
PKC	PRKCA	7.127±0.67	6.268±0.59	<0.01	0.00011
	PRKCB1	15.82±0.58	15.36±0.38	<0.01	0.00511
	PRKCBP1	7.599±0.48	6.902±0.39	<0.01	0.00473
	PRKCD	16.44±0.67	15.71±0.44	<0.01	0.00015
	PRKCDBP	8.591±0.84	8.226±0.68	<0.01	0.00653
	PRKCE	6.308±0.41	6.212±0.22	-	0.3829
	PRKCG	6.209±1.11	6.179±1.16	-	0.93199
	PRKCH	8.351±0.5	8.237±0.34	<0.05	0.01567
	PRKCI	11.39±0.46	11.51±0.3	-	0.18020
	PRKCQ	12.53±0.53	12.69±0.35	-	0.26876
	PRKCSH	12.4±0.82	11.55±0.69	<0.01	0.00111
	PRKCZ	9.199±0.44	9.379±0.41	-	0.70588

P-values denote PE vs. control groups. PE, pulmonary embolism; SFK, Src family kinase; PKC, protein kinase C.

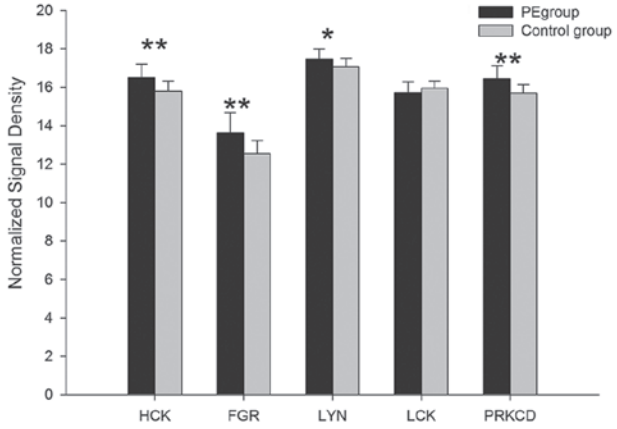

Figure 6. mRNA expression of SFK and PKC in outside-in activation. *P<0.05; **P<0.01, PE vs. control groups. PE, pulmonary embolism; SFK, Src family kinase; PCK, protein kinase C.

Differentially expressed genes were identified from whole genomes. P<0.05 was considered to indicate a statistically significant difference.

Results

A total of 80 associated mRNAs were detected (Tables I-VIII, Figs. 1-8). 14-3-3ζ occurred in bilateral signal transduction pathways so was detected twice; once in inside-out activation, once in outside-in activation.

mRNA expression of α/β integrins. Among the 5 subunit-associated mRNAs, the mRNA expression of ITGAL, ITGAM, ITGAX and ITGB2, which encode for the subunits of αL, αM, αX, β2, were upregulated in the PE group and the differences, with the exception of ITGB2, exhibited statistical significance (P<0.05). Compared with the controls, the mRNA expression of ITGAD in the PE group was downregulated, but there was no significant difference (P>0.05) (Table I; Fig. 1).

mRNA expression of 12 ligands of β2 integrins. Among the 14 associated mRNAs of 12 ligands, the gene expression of 10 ligands, ICAM-1, ICAM-3, ICAM-4, ICAM-5, JAM-1, JAM-3, RAGE, urokinase-type plasminogen activator receptor (uPAR), laminin 8 and VCAM-1, were upregulated in the PE group and the differences of ICAM-1, ICAM-3, JAM-3, AGER, PLAUR and LAMC1 exhibited statistical significance (P<0.05). Compared with the controls, the mRNA expression of ICAM-2 and fibrinogen were downregulated. The differences of FGA, FGB and FGG mRNA exhibited statistical significance (P<0.05); however, there was no significant difference for ICAM-2 (P>0.05) (Table II, Fig. 2).

mRNA expression of 7 chemokines for β2 integrins. Among the 7 associated mRNAs of 7 chemokines for β2 integrins, the gene expression of 6 chemokines, SDF-1α, SLC, RANTES, fMLP, IL-8 and PAF, were upregulated in the PE group and the differences of CXCL12 and PAF1 mRNA exhibited statistical significance (P<0.01). Compared with the controls, the mRNA expression of CXCL13 in the PE group was downregulated; however, this was not observed to be statistically significant (P>0.05) (Table III; Fig. 3).

mRNA expression of 33 signal transduction proteins. A total of 55 associated mRNAs of 33 signal transduction proteins which related to the β2 integrins were detected:

i) Inside-out activation of the β2 integrins. Among the 23 associated mRNAs of 17 inside-out signal proteins which related to β2 integrins, the mRNA expression of 15 proteins,

Table VII. mRNA expression of SYK-related proteins in outside-in activation.

Name	Gene	PE	Control	P-value	P-value
Syk	SYK	15.70±0.49	15.34±0.37	<0.05	0.01133
Vav1/3	VAV1	15.93±0.45	15.49±0.33	<0.01	0.00130
	VAV3	10.71±0.47	10.14±0.43	<0.01	0.00176
c-ab1	ABL1	12.18±0.48	11.85±0.41	<0.05	0.02491
	ABL2	8.336±0.56	7.952±0.50	<0.05	0.02867
c-cb1	CBL	8.773±0.36	7.921±0.40	<0.01	0.00066
FAK	PTK2	10.59±0.49	10.08±0.48	<0.01	0.00632

P-values denote PE vs. control groups. PE, pulmonary embolism; SYK, spleen tyrosine kinase.

Table VIII. β2 mRNA expression of proteins which mediate cytoskeletal remodelling in outside-in activation.

Name	Gene	PE	Control	P-value	P-value
14-3-3ζ	YWHAZ	15.67±0.41	15.19±0.41	<0.01	0.0008
Rac	RAC1	14.37±0.41	13.72±0.30	<0.01	0.00104
cdc42	CDC42	13.80±0.41	13.69±0.24	-	0.56060
RhoA	RHOA	15.22±0.37	14.86±0.22	<0.01	0.00579
ROCK1/2	ROCK1	13.11±0.53	12.64±0.32	<0.01	0.00739
	ROCK2	10.73±0.55	10.27±0.47	<0.05	0.01867
mDia	DIAPH1	14.76±0.35	14.31±0.35	<0.01	0.00107
	DIAPH2	12.07±0.47	11.78±0.50	<0.05	0.03168
	DIAPH3	5.382±0.94	5.332±0.98	-	0.29674

P-values denote PE vs. control groups. PE, pulmonary embolism.

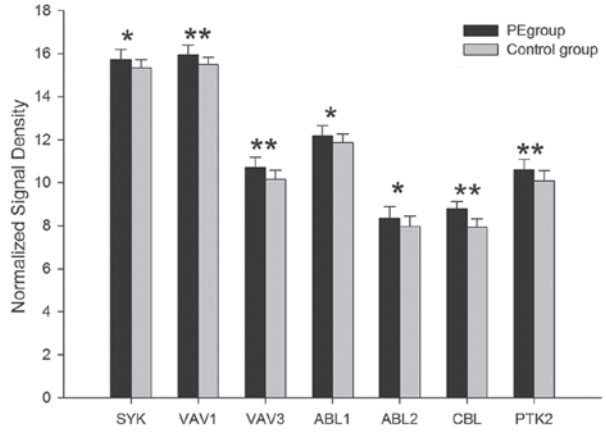

Figure 7. mRNA expression of SYK related proteins in outside-in activation. *P<0.05; **P<0.01, PE vs. control groups. PE, pulmonary embolism; SYK, spleen tyrosine kinase

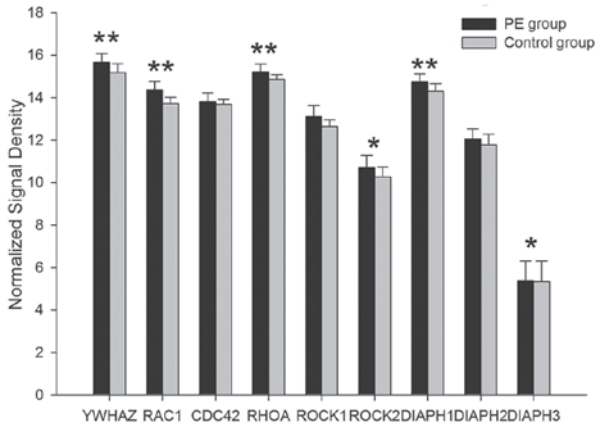

Figure 8. mRNA expression of proteins which mediate cytoskeletal remodelling in outside-in activation. *P<0.05, **P<0.01, PE vs. control groups. PE, pulmonary embolism.

PLCγ, SPA1, SLP-76, ADAP, RIAM, RAPL, Talin, Vinculin, Dok1, 14-3-3ζ, Filamin A, Migfilin, α-actinin, Radixin and Calpain, were upregulated in the PE group. The differences in PLCG2, SIPA1, LCP2, FYB, APBB1IP, RASSF5, TLN1, TLN2, VCL, DOK1, YWHAZ, FLNA, FBLIM1, ACTN1 and CAPN1 mRNA were observed to be statistically significant (P<0.05). Compared with the controls, the mRNA expression of DalDAG-GEFI and Mst1 in the PE group were downregulated. The difference of DalDAG-GEFI encoded by RASGRP1 mRNA exhibited statistical significance (P<0.05), however there was no significant difference for Mst1 mRNA (P>0.05) (Tables IV and V; Figs. 4 and 5).

ii) Outside-in activation of the β2 integrins. Among the 32 associated mRNAs of 16 outside-in signal proteins which are related to β2 integrins, the gene expression of 14 proteins,

Table IX. The expression, ligands and functions of β2 integrins.

Integrin	Other names	Expression	Ligands	Function	Ref.
αLβ2	CD11a/CD18 LFA-1	All leukocytes	ICAM-1, ICAM-2, ICAM-3, ICAM-4, ICAM-5, JAM-1	Mediates leukocyte infiltration; involved in immune synapse formation	8 9,10
αMβ2	CD11b/CD18 Mac-1	Monocytes, neutrophils macrophages, NK cells and γ δ T-cells	ICAM-1, ICAM-2, ICAM-4, JAM-3, RAGE, laminin 8, fibrinogen and more	Mediates leukocyte phagocytosis; mediates leukocyte adhesion; involved in immune tolerance	11 12,13
αXβ2	CD11c/CD18 p150,95	Monocytes, NK cells, macrophages and dendritic cells	ICAM-1, ICAM-4, fibrinogen, LPS and more	Mediates the adhesion between monocytes and endotheliocytes	
αDβ2	CD11d/CD18	Macrophages and eosinophils	ICAM-3, VCAM-1 and more	Involved in the phagocytosis of senescent erythrocyte; promotes eosinophil infiltration	14

Hck, Lyn, PKC, Syk, Vav1/3, c-ab1, c-cb1, FAK, 14-3-3ζ, Rac, cdc42, RhoA, ROCK1/2 and mDia, were upregulated in the PE group and the differences, with the exception of cdc42, exhibited statistical significance (P<0.05). Compared with the controls, the mRNA expression of Fgr and Lck1 in the PE group was downregulated. The difference of Fgr encoded by FGR mRNA exhibited statistical significance (P<0.01), however, alterations in LCK mRNA were not observed to be statistically significant (P>0.05) (Tables VI-VIII; Figs. 6-8).

Discussion

Neutrophils and monocytes are indispensable parts of innate immunity. During an inflammatory response, neutrophils and monocytes destroy invading pathogens by phagocytosis. In 2009, it was observed (3) that the associated mRNA expression of neutrophils and monocytes in PE patients were upregulated significantly (P<0.01). β2 integrins are the primary adhesion molecules for leukocyte adhesion in the inflammatory response and are also significant in the phagocytosis of neutrophils and monocytes.

The leukocyte-restricted β2 integrins include four members: αLβ2, αMβ2, αXβ2 and αDβ2 (Table IX) (7-13). The exudation of neutrophils or monocytes includes margination, rolling, stable adhesion and transendothelial migration. The stable adhesion occurs mainly through the interaction between αLβ2 and αMβ2 and their ligands on the surface of cytomembrane. When inflammation occurs, the expression of chemokines is upregulated. The inside-out signal transduction is activated following chemokine binding with the receptors on the surface of neutrophils and monocytes. Following G-protein signaling, activated phospholipase C (PLC) mediates the activation of DalDAG-GEFI. Ras-related protein 1 (Rap1) is a small GTPase, which is a key protein in the inside-out signaling. SPA1 may convert activated Rap1 to inactive Rap1. DalDAG-GEFI may convert inactive Rap1 to activated Rap1 and subsequently activate the downstream effectors of Rap1, including RIAM, RAPL and Mst1. The effectors may regulate the ligand-binding affinity of the β2 integrins via cytoskeletal proteins which bind to the integrin β cytoplasmic tail, including Talin, Dok1, 14-3-3ζ, Filamin A, Migfilin and Radixin, eventually improving the affinity of the β2 integrins and promoting the adhesion of leukocytes.

The results of the current study showed that the mRNA expression of associated chemokines were upregulated in the PE group, compared with the controls. The differences of SDF-1α and PAF1 mRNA were statistically significant (P<0.01). Among the 23 associated mRNAs of the 17 inside-out signal proteins which related to the β2 integrins, the mRNA expression of 15 proteins was upregulated significantly in the PE group (P<0.05). Among the 5 subunit-associated mRNAs, the mRNA expression of ITGAL, ITGAM, ITGAX and ITGB2, which encode for the subunits of αL, αM, αX and β2, were upregulated in the PE group and the differences, with the exception of ITGB2, were observed to be statistically significant (P<0.05).

The current study suggested that the inside-out signal transduction pathway of the β2 integrins was activated in the neutrophils and monocytes of PE patients. Given the overexpression of activated Rap1, the expression of DalDAG-GEFI was downregulated as the result of negative feedback. Filamin may compete with Talin to bind the integrin tail (6). As a negative regulator of integrin activation, the mRNA expression of Filamin A was upregulated significantly for the overexpression of activated Rap1. The associated chemokines SDF-1α, RANTES, fMLP, IL-8 and PAF promote the migration of leukocytes through the endothelium (7). The differences of SDF-1α and PAF1 mRNA were statistically significant (P<0.01), suggesting that the exudation of neutrophils and monocytes is enhanced. The gene expression of Talin, Dok1, 14-3-3ζ, Filamin A, Migfilin and Skelemin were upregulated significantly (P<0.01). It suggested that the β2 integrins were activated, neutrophils and monocytes had high-affinity to the endothelium and the adhesive attraction was enhanced.

Neutrophils and monocytes associated with the phagocyte. The phagocytosis of neutrophils or monocytes includes recognition, adhesion, ingestion and degradation. Depending on the Fc receptor and C3b receptor (C3bi or αMβ2), neutrophils and monocytes may recognize and adhere the bacteria which are

coated with the antibody or complement and ingest the phagosome via the cytoskeletal reorganization.

Phagocytosis mediated by β2 integrins primarily depends on the outside-in signal transduction pathway of αMβ2. When the β2 integrins of neutrophils and monocytes bind with the ligand, the outside-in signaling events are initiated. Non-receptor tyrosine kinase and the PTK (receptor tyrosine kinase), including spleen tyrosine kinase (SYK) and FAK, are activated through the phosphorylation of Src family kinase (SFKs), including Hck, Fgr and Lyn. SFKs and SYK are required for the regulation of adhesion, spread and cytoskeletal reorganization in neutrophils and monocytes. Under the effect of Vav (a Rac GEF), cross-linking of the β2 integrins may induce the activation of RhoA. The activated RhoA regulates the cytoskeletal structures and promotes the phagocytosis of leukocytes. Protein kinase C (PKC) promotes the phagocytosis of leukocytes. uPAR is required for the adhesion reaction of endothelial cells with monocytes (14).

The results of the current study showed that among the 14 associated mRNAs of 12 ligands, the gene expression of 10 ligands was upregulated in the PE group. The differences of ICAM-1, ICAM-3, JAM-3, AGER, PLAUR and LAMC1 was statistically significant ($P<0.05$). Compared with the controls, the mRNA expression of ICAM-2 and Fibrinogen were downregulated. There were significant differences of FGA, FGB and FGG mRNA expression ($P<0.05$). Among the 32 associated mRNAs of 16 outside-in signaling proteins which related to the β2 integrins, the mRNA expression of 14 proteins were upregulated in the PE group and the differences, with the exception of cdc42, were statistically significant ($P<0.05$). Compared with the controls, the mRNA expression of Fgr and Lck1 in the PE group were downregulated. The difference of FGR mRNA was statistically significant ($P<0.01$).

It is hypothesized that the outside-in signal transduction pathway of β2 integrins was activated in the neutrophils and monocytes of PE patients. The mRNA expression of Hck, PKC, FAK and RhoA were upregulated significantly ($P<0.01$). The mRNA expression of Lyn and SYK were markedly upregulated ($P<0.05$). These observations suggest that the phagocytosis of neutrophils and monocytes is enhanced. Fgr negatively regulates the migration and adhesion via the signal transduction pathway of the β2 integrins. Compared with the controls, there was no negative feedback rise in the mRNA expression of Fgr, instead, it was downregulated significantly ($P<0.01$). Although the β2 integrins of leukocytes in PE patients were activated, the associated mRNA expression of Fgr is hypothesized to be inhibited.

The analysis of mRNA differential expression in leukocyte β2 integrins signal transduction suggested that the bilateral signal transduction pathways of β2 integrins were activated. The innate immune response in PE patients was enhanced. From the perspective of genomics, it is indicated that the occurrence of PE was apparently associated with the activation of β2 integrins in neutrophils and monocytes. The occurrence of PE and the inflammatory reaction are closely related.

References

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