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COUP-TFII Switches Responses of Venous Endothelium to Atherosclerotic Factors Through Controlling the Profile of Various Inherent Genes Expression

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ABSTRACT

Endothelial cells of arteries (AEC) have a strikingly greater responsiveness to atherosclerosis factors than venous endothelial cells (VEC). However, the reasons for this phenomenon remain unclear. Chicken ovalbumin upstream promoter-transcription factor II (COUP-TFII) plays an important role in regulating embryonic arterial-venous differentiation. We therefore investigate whether COUP-TFII is related to this different susceptibility between AEC and VEC. It is first confirmed that COUP-TFII is expressed in VEC but not in AEC in the adult. Using a siRNA strategy, we identified the expression of Jagged1 and Notch1 in cultured human VEC, which usually exist only in AEC, after knocking down of COUP-TFII. To further elucidate the role of COUP-TFII, we performed DNA microarrays in VEC transfected with the siRNA of COUP-TFII and subsequently stimulated with angiotensin II (AngII) and compared the expression profiles of 112 genes involved in various atherosclerosis-related pathways. The results indicated that expression of atherogenic genes was significantly upregulated after AngII stimulation in VEC transfected with COUP-TFII siRNA. Moreover, in vitro cell functional assay showed that knockdown of COUP-TFII in VEC increased not only basal but also AngII-induced cell adhesions. These results demonstrate that COUP-TFII suppresses the susceptibility of VEC to atherosclerosis through controlling the expression of various atherosclerosis-related molecules. J. Cell. Biochem. 112: 256–264, 2011.

KEY WORDS: ATHEROSCLEROSIS; ENDOTHELIAL CELL; ARTERY; VEIN; GENE

It is well known that atherosclerosis-inducing factors predominantly affect arteries but rarely veins in the normal anatomical locations although they are exposed to similarly systemic risk factors such as hyperlipidemia, diabetes mellitus, smoking, and hypertension. There have been a variety of reports demonstrating that hemodynamic factors contribute largely to the differences between arteries and veins in many aspects including atherosclerotic susceptibility [Zhang et al., 2004; Chatzizisis et al., 2007], however, accumulating evidences have shown that genetic variants in arteries and veins are also responsible for their initial differentiation [Torres-Vazquez et al., 2003; Swift and Weinstein, 2009; Lin et al., 2007]. It is therefore hypothesized that molecular divergences between arteries and veins might associate with their unlike susceptibilities to atherosclerosis.

Chicken ovalbumin upstream promoter-transcription factor II (COUP-TFII; also called NR2F and ARP-1) is an orphan receptor of the steroid/thyroid hormone receptor superfamily. Recently, You [You et al., 2005] have shown that COUP-TFII is specifically expressed in

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venous but not arterial endothelium in the embryonic vasculature, and ablation of COUP-TFII in endothelial cells (EC) induces transdifferentiation of vein to artery. Furthermore, they discovered COUP-TFII plays a critical role in maintaining vein identity through repressing Notch signaling during embryonic vasculature development. On the other hand, COUP-TFII has also been reported to be a regulator for lipids, glucose, and insulin metabolisms, which are involved in the process of atherosclerosis in diverse kinds of tissues [Zhang et al., 2002; Tian, 2003; Myers et al., 2006]. We hereby supposed that COUP-TFII associates with the susceptibility to atherosclerosis.

EC line the inner surface of the vascular wall and mediate a large number of physiological processes in maintaining vascular functions. Dysfunctions of EC are considered to be the initiation of atherosclerosis. Additionally, venous and arterial EC are quite different in embryonic origin and serve dissimilar vessel functions [Torres-Vazquez et al., 2003; Swift and Weinstein, 2009]. Collectively, it is reasonable to postulate COUP-TFII affects susceptibilities of EC to atherosclerotic factors. In the present study, we demonstrate that COUP-TFII is specifically expressed in Venous EC (VEC) and controls expressions of various inherent genes relative to atherosclerosis. Inhibition of COUP-TFII expression in VEC enhances the susceptibility of the cells to the atherosclerotic stimulation.

METHODS

CELL CULTURE AND TRANSFECTION OF A SIRNA

VEC were enzymatically isolated from human umbilical veins and cultured as previously described [Polikandriotis et al., 2005]. All protocols were approved by the local ethics review board of Fudan University. According to protocols by the manufacturer, VEC (50% confluence) were transfected with either human COUP-TFII small interfering RNA (siRNA, sc-38818; SantaCruz Biotechnology, Inc., California) or its scramble RNA (scRNA, sc-36869; SantaCruz) at the optimized concentrations, or with the same volume of vehicle alone (siRNA transfection reagent, sc-29528; SantaCruz). Forty-eight hours later, whole cell lysates were subjected to Western blotting using an antibody against COUP-TFII (sc-6578; SantaCruz) to confirm the suppressive effect of siRNA on COUP-TFII expression. In separate experiments, cells with or without transfection were incubated with medium containing 0.5% fetal serum for 24 h and subsequently treated with angiotensin II (AngII, 10^{-7} mol/L; Sigma, St. Louis, MO) for 4 h. Total RNA was harvested with TRIzol reagent (Invitrogen, Carlsbad, CA) for microarray and quantitative real-time PCR (RT-PCR) analysis.

MICROARRAY AND QUANTITATIVE RT-PCR ANALYSIS

Oligo GE Array Human Atherosclerosis Microarray systems (OHS-038; SuperArray Bioscience Corporation, Bethesda, MD) were used in analysis of microarray. Each array consists of 112 genes known to be related to lipid transport and metabolism, cell adhesion, cell growth, extracellular molecules, blood coagulation, and thrombosis. Microarray analysis was performed according to the manufacturer's instructions. Briefly, total RNA was reversely transcribed into cDNA and synthesized into cRNA. After the cRNA was labeled with Biotin-16-dUTP (Roche, Indianapolis, IN) and hybridized under precisely specified conditions to a positively charged nylon membrane containing the arrayed DNA, the arrays were visualized using the chemiluminescent detection system (SuperArray Bioscience). All experiments were repeated three times. Data were analyzed using the GE Array expression analysis suite and the Genes upregulated or downregulated by more than twice ($P < 0.05$) were considered significant in the selection criteria.

For confirmation of microarray results, quantitative RT-PCR was used to measure gene expression in aliquots of the same RNA preparations used for the microarray experiment according to the manufacturer's instructions (Rotor-Gene, Corbett Research). The oligonucleotide primers used were as follows: MMP1, sense, 5′-TCCTGCTTTGTCCTTTGATG3-′, antisense, 5′-AGCCCAGAATT-GATTTCCTTTA-3′; CCL5, sense, 5′-ATCCTCATTGCTACTGCCCTC-3′, antisense, 5′-GCCACTGGTGTAGAAATACTCC-3′; OLR1, sense, 5′-ACCTA TTTTCCTCGG GCTCA-3′, antisense, 5′-GGGGCAT-CAAAGGAGAACC-3′; vWF, sense, 5′-CCTGCCGCTCTCTCTCTTAC-3′, antisense, 5′-AGCACATGGTGTGATGGTCTG-3′; GAPDH, sense, 5′-AAGAAGG TGGTGAAGCAGGC-3′, antisense, 5′-TCCAC-CACCCTGTTGCTGTA-3′. SYBR Green (Molecular Probes) fluorescence was monitored after each cycle. Amplification of specific transcripts was confirmed by melting curve profiles at the end of each PCR. Levels of mRNA expression were quantified by using the second-derivative maximum method of Rotor-gene 6.0 Software (Corbett Research) and normalized to GAPDH expression.

WESTERN BLOTTING

Protein expression of COUP-TFII, Notch1, and Jagged1 in VEC was analyzed by Western blotting. Briefly, total cell lysates were separated by electrophoresis on the SDS–polyacrylamide gel and transferred to the PVDF membrane (Roche, Basel, Switzerland). The membranes were immunoblotted with antibodies against COUP-TFII, Notch1 (sc-6014) or Jagged1 (sc–6011, 1:1,000; SantaCruz) at 4°C overnight. Immunoreactivities were detected using the enhanced chemiluminescence reaction (ECL) system (Amersham Pharmacia Biotech). Expression of GAPDH was used as a loading control. Expression levels of proteins were quantified by scanning densitometries and normalized by GAPDH.

FLUORESCENCE-ACTIVATED CELL SORTER (FACS) ANALYSIS

Expression of Jagged1 and Notch1 on VEC was quantitatively evaluated by a flow cytometry. Briefly, VEC transfected with siRNA of COUP-TFII or its scRNA were harvested and incubated with primary antibodies (goat anti-human Jagged1, goat anti-human Notch1, SantaCruz) or with phosphate-buffered saline (PBS) as a negative control for 1 h, and then with a Cy3 conjugated antibody (Sigma) for 30 min. Cells were fixed with 1% paraformaldehyde and immediately analyzed by a flow cytometry (FACS Calibur, Becton Dickinson).

FLUORESCENCE IMMUNOCYTOCHEMISTRY

Expression of COUP-TFII, Notch1, and Jagged1 in VEC and AEC was examined by the fluorescence immunocytochemistry method. Briefly, VEC and AEC were enzymatically isolated and cultured from human umbilical veins and arteries respectively as previously described [Polikandriotis et al., 2005]. After 60%–70% of confluence, the cells were fixed with 4% paraformaldehyde, incubated with primary antibodies (goat anti-human COUP-TFII,

goat anti-human Jagged1, goat anti-human Notch1, 1:100, Santa-Cruz) or PBS as a negative control overnight at 4°C, and then with a Cy3 conjugated antibody (1:100; Sigma) at room temperature for 30 min. After staining the nuclei with 4′,6′-diamidino-2-phenylindole (DAPI, 5 μg/ml, Sigma) for another 30 min, cells were examined by a fluorescence microscope (Leica DMIRB, Germany).

ADHESION ASSAY

VEC-THP1 cell adhesion assay was performed as previous described with some modifications [Hiraoka et al., 2004; Kawakami et al., 2007]. VEC transfected with siRNA of COUP-TFII or its scRNA were treated with or without AngII (10^{-7} mol/L, 4 h). THP1 cells (ATCC, Manassas, VA) were then labeled with DAPI (5 μg/ml; Sigma), plated on a confluent EC monolayer in a 24-well plate (1×10^5 THP1 cells per well), and allowed to adhere for 10 min. Non-adherent cells were removed and adhered DAPI positive cells were counted in five randomly selected optical fields (20× lense) and averaged. Number of the adhered cells was expressed as cells/field.

IMMUNOHISTOCHEMISTRY IN VESSELS

Healthy male Sprague-Dawley rats (250 g) were purchased from Fudan University. Animals were anesthetized and the aorta and vena cava were harvested after perfusion fixation with 4% paraformaldehyde in PBS at 100 mmHg for 15 min. Vessels were then fixed overnight in4% paraformaldehyde in PBS and paraffin embedded. Segments were sectioned in 5 μm thick and mounted, and expression of Jagged1, Notch1, and COUP-TFII was examined by immunohistochemistry using antibodies against Jagged1, Notch1, and COUP-TFII, respectively (1:100, SantaCruz) [You et al., 2005]. The investigation conforms with the *Guide for the Care and Use of Laboratory Animals* published by the US National Institutes of Health (NIH Publication No. 85–23, revised 1996) and all protocols were approved by the Institutional Animal Care and Use Committee of Fudan University.

STATISTICAL ANALYSIS

All experiments were repeated at least three times on different occasions. Data were expressed as mean ± SEM and analyzed by independent *t*-test or by one-way ANOVA. Values of $P < 0.05$ were considered significant.

RESULTS

EXPRESSION OF COUP-TFII IN ADULT RAT VESSELS

Since COUP-TFII has been reported to contribute to the vein identity during embryonic vasculogenesis by suppressing Notch signaling [You et al., 2005], using immunohistochemistry method, we initially investigated the expression of COUP-TFII and Notch1/Jagged1 in the endothelium of aorta and vena cava isolated from adult rats. The results showed that Notch1/Jagged1 expressed in endothelium of the artery but not in the vein, whereas COUP-TFII was observed in endothelium of the vein rather than that of the artery (Fig. 1). The expression pattern of COUP-TFII in adult vessels is similar to that observed in embryonic development, indicating that COUP-TFII is a conserved gene.

EXPRESSION OF NOTCH1/JAGGED1 IN VEC AFTER INHIBITION OF COUP-TFII

To further examine the roles of COUP-TFII, we cultured VEC and AEC from human umbilical veins and arteries, respectively. Immunofluorescence staining showed that VEC in culture expressed COUP-TFII but not Notch1 or Jagged1 whereas AEC expressed both Notch1 and Jagged1 but not COUP-TFII (Fig. 2A), which was similar to the results observed from in vivo vascular endothelium. We next employed a siRNA to suppress the expression of COUP-TFII and examined whether this suppression induce expression of Notch1/Jagged1 in VEC. Specific suppression of COUP-TFII by siRNA was

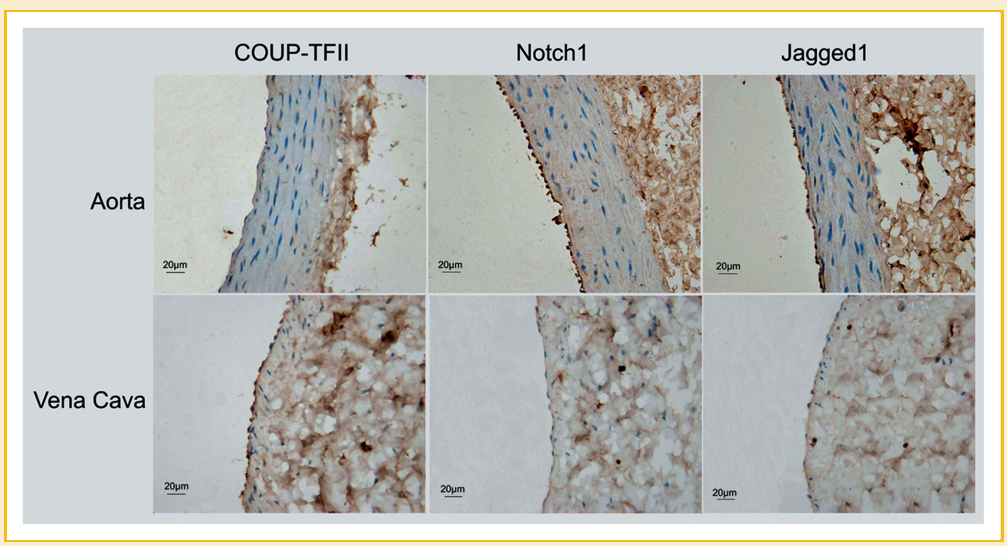


Fig. 1. Differences in expression of COUP-TFII, Notch1, and Jagged1 in the endothelium of aorta and vena cava. Sections of aorta and vena cava from adult Sprague–Dawley rats were immunohistological stained with anti-COUP-TFII, Notch1, or Jagged1 antibodies. Representative photomicrographs from three independent experiments are shown. Expression of COUP-TFII appeared only in the endothelium of vein but not aorta whereas Notch1 and Jagged1 expressed in the endothelium of aorta but not vein.

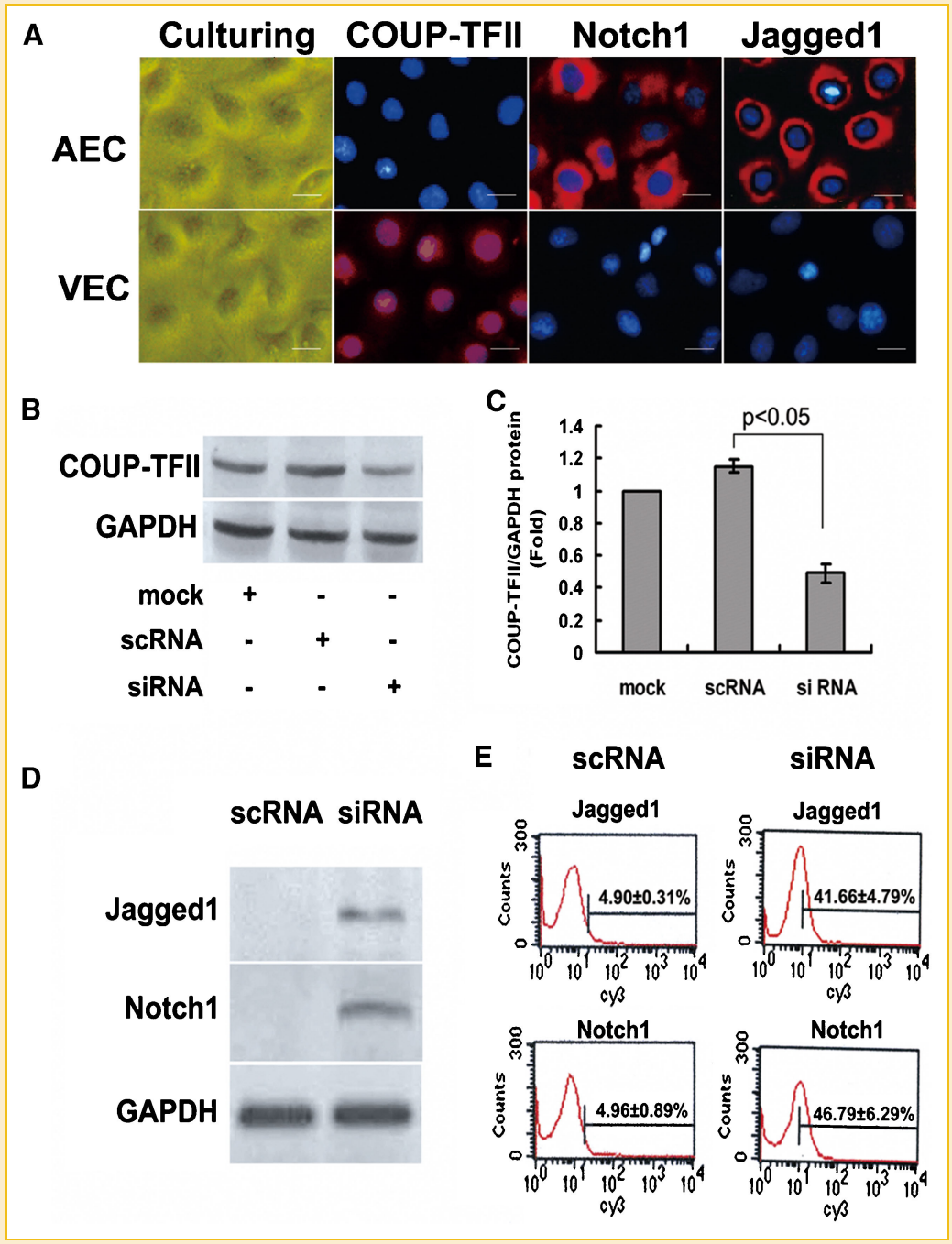


Fig. 2. Induction of Jagged1 and Notch1 expression by knockdown of COUP-TFII in cultured VEC. VEC and AEC from human umbilical veins and arteries were cultured as described in Methods Section. A: Representative photomicrographs of the cultured cells and the staining of COUP-TFII, Notch1, and Jagged1. COUP-TFII, Notch1, or Jagged1 staining was merged with DAPI staining. Red indicates positive signals. Nuclei of VEC but not AEC were positively stained with a COUP-TFII antibody. Notch1 could stain both the membrane and the cytoplasma while Jagged1 antibody could only stain the membrane of AEC but not VEC. Bar = 20 μm. B: Analysis of COUP-TFII expression by Western blotting. siRNA of COUP-TFII (siRNA) or its scramble sequence (scRNA) were added to cultured VEC as described in Methods Section. Expression of GAPDH served as a loading control. Mock, without transfection. Expression of COUP-TFII was significantly suppressed (>50%) by siRNA but not by scRNA. C: Quantification of COUP-TFII expression. Expression of COUP-TFII was densitometrically analyzed and expressed as folds of GAPDH expression. Data are presented as mean ± SEM (n = 3). D: Expression of Jagged1 and Notch1 analyzed by Western blotting in VEC transfected with siRNA or its scRNA. Expression of GAPDH served as a loading control. E: FACS analysis. Preparation of VEC for FACS analysis of Jagged1 and Notch1 expression was described in Methods Section. Jagged1 and Notch1 positive cells were expressed as % of total cells (n = 3).

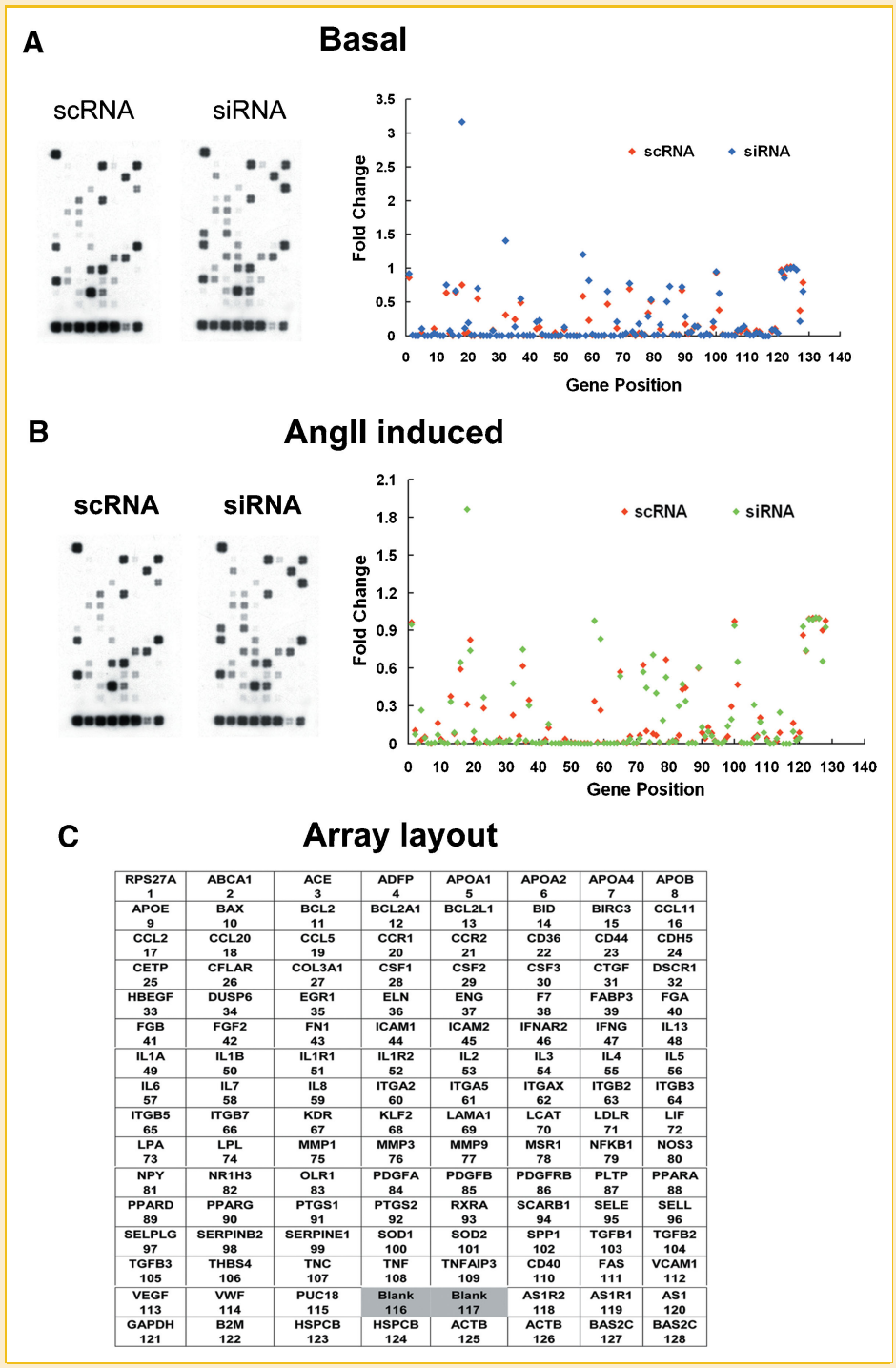
verified by Western blotting (Fig. 2B,C). As shown, expression of Notch1/Jagged1 emerged in the cells after inhibition of COUP-TFII (Fig. 2D,E). These results suggest that COUP-TFII controls expression of some specific genes to maintain the venous identity in adult veins.

COMPARISON OF GENE EXPRESSION PROFILES IN VEC WITH OR WITHOUT INHIBITION OF COUP-TFII

Inhibition of COUP-TFII induced expression of Notch1/Jagged1 in VEC, suggesting that COUP-TFII might determine susceptibility of

EC to atherosclerosis through controlling certain downstream genes. We therefore examined the change of gene expression profiles in VEC after suppression of COUP-TFII with its siRNA.

Among measured 112 genes, 11 genes including MMP1, MMP3, CCL5, CCL20, CCR1, vWF, LPA, apoA1, IL-8, IL-6, and DSCR1 were significantly upregulated while three genes including apoE, IL13, and IL-4 were downregulated in the VEC after knockdown of COUP-TFII (Fig. 3A; Table I). After administration of AngII, an atherogenic factor [Mazzolai and Hayoz, 2006], to the cells, expression of 10 atherosclerosis related genes including MMP1, MMP3, CCL5, CCL20,



RPS27A 1	ABCA1 2	ACE 3	ADFP 4	APOA1 5	APOA2 6	APOA4 7	APOB 8
APOE 9	BAX 10	BCL2 11	BCL2A1 12	BCL2L1 13	BID 14	BIRC3 15	CCL11 16
CCL2 17	CCL20 18	CCL5 19	CCR1 20	CCR2 21	CD36 22	CD44 23	CDH5 24
CETP 25	CFLAR 26	COL3A1 27	CSF1 28	CSF2 29	CSF3 30	CTGF 31	DSCR1 32
HBEGF 33	DUSP6 34	EGR1 35	ELN 36	ENG 37	F7 38	FABP3 39	FGA 40
FGB 41	FGF2 42	FN1 43	ICAM1 44	ICAM2 45	IFNAR2 46	IFNG 47	IL13 48
IL1A 49	IL1B 50	IL1R1 51	IL1R2 52	IL2 53	IL3 54	IL4 55	IL5 56
IL6 57	IL7 58	IL8 59	ITGA2 60	ITGA5 61	ITGAX 62	ITGB2 63	ITGB3 64
ITGB5 65	ITGB7 66	KDR 67	KLF2 68	LAMA1 69	LCAT 70	LDLR 71	LIF 72
LPA 73	LPL 74	MMP1 75	MMP3 76	MMP9 77	MSR1 78	NFKB1 79	NOS3 80
NPY 81	NR1H3 82	OLR1 83	PDGFA 84	PDGFB 85	PDGFRB 86	PLTP 87	PPARA 88
PPARD 89	PPARG 90	PTGS1 91	PTGS2 92	RXRA 93	SCARB1 94	SELE 95	SELL 96
SELPLG 97	SERPINB2 98	SERPINE1 99	SOD1 100	SOD2 101	SPP1 102	TGFB1 103	TGFB2 104
TGFB3 105	THBS4 106	TNC 107	TNF 108	TNFAIP3 109	CD40 110	FAS 111	VCAM1 112
VEGF 113	VWF 114	PUC18 115	Blank 116	Blank 117	AS1R2 118	AS1R1 119	AS1 120
GAPDH 121	B2M 122	HSPCB 123	HSPCB 124	ACTB 125	ACTB 126	BAS2C 127	BAS2C 128

Fig. 3. Atherosclerosis microarray analysis in VEC after suppression of COUP-TFII. Cultured VEC were transfected with siRNA of COUP-TFII or its scRNA and treated with or without AngII, atherosclerosis microarray of mRNA was performed as described in Methods Section. A,B: Representative photographs of microarray analysis in VEC with (B) and without (A) AngII incubation are shown. C: Array layout. There was a significant difference in expression of a variety of genes between siRNA and scRNA transfection or between incubation with and without AngII.

TABLE I. The Genes Changed After Inhibition of COUP-TFII in VEC Without AngII Stimulation

Accession no	Gene name	Gene symbol	Fold change	*P*-value
NM_002421	Matrix metalloproteinase 1 (interstitial collagenase)	MMP1	3.871	0.037
NM_002422	Matrix metalloproteinase 3 (stromelysin 1, progelatinase)	MMP3	2.836	0.000
NM_002985	Chemokine (C-C motif) ligand 5	CCL5	3.354	0.026
NM_004591	Chemokine (C-C motif) ligand 20	CCL20	4.113	0.031
NM_001295	Chemokine (C-C motif) receptor 1	CCR1	6.522	0.003
NM_000552	Von Willebrand factor	vWF	3.983	0.026
NM_005577	Lipoprotein, Lp(a)	LPA	3.584	0.043
NM_000041	Apolipoprotein E	apoE	0.416	0.009
NM_000039	Apolipoprotein A-I	ApoA1	3.669	0.003
NM_000584	Interleukin 8	IL-8	3.126	0.026
NM_000600	Interleukin 6 (interferon, beta 2)	IL-6	2.225	0.008
NM_002188	Interleukin 13	IL-13	0.142	0.018
NM_004414	Down syndrome critical region gene 1	DSCR1	4.052	0.040
NM_000589	Interleukin 4	IL-4	0.370	0.015

Numbers are median fold changes from three biological replicates.

LPA, IL-6, THBS4, OLR1, ADFP, and SERPINB2 were further elevated in VEC with siRNA of COUP-TFII (Fig. 3B; Table II). Four genes including MMP1, CCL5, vWF, and OLR1 were chosen to further confirmation by RT-PCR, and the results were consistent with the DNA array data (correlation was 0.84; Online supplement Table).

Among these genes, MMP1 and MMP3 belong to the matrix metalloproteinases family, which are capable of degrading the components of extracellular milieu. CCL5, CCL20, CCR1, IL-8, IL-6, IL13, IL-4, and DSCR1 are chemokines, the cell adhesion and inflammatory markers. vWF, THBS4, and SERPINB2 are involved in the fundamental processes of coagulation and thrombosis. apoA1, apoE, and LPA are related to lipid transport and metabolisms. These results collectively suggest that COUP-TFII contributes to control the expression of a variety of atherosclerosis-related genes in VEC.

ADHESION OF THP1 CELLS TO VEC INDUCED BY INHIBITION OF COUP-TFII

We finally asked whether inhibition of COUP-TFII in VEC would induce atherogenic responses. Adhesion and subsequent recruitment of monocytes to endothelium plays an initial role in atherogenesis and plaque instability [Hiraoka et al., 2004; Kawakami et al., 2007]. We therefore observed adhesion of the monocyte, THP1 cells to VEC after knockdown of COUP-TFII. Treatment of VEC with COUP-TFII siRNA significantly increased adhesion of THP1 cells to the VEC (5 ± 2 cells/field vs. 12 ± 3 cells/field, $n = 5$, $P < 0.05$; Fig. 4A,C), and addition of AngII to the cells significantly amplified the increase in adhesion induced by inhibition of COUP-TFII (19 ± 4 cells/field vs. 36 ± 6 cells/field, $n = 5$, $P < 0.05$; Fig. 4B,D).

DISCUSSION

Atherosclerosis usually develops in arteries but rarely in veins under normal conditions. In this study, we demonstrate that COUP-TFII is expressed in VEC but not in AEC in adult vessels, and knockdown of COUP-TFII in VEC alters expression profile of various atherosclerosis-related genes selectively including those of lipid transport and metabolism, extracellular matrix, thrombosis, inflammation and adhesion. These findings suggest COUP-TFII contributes to the differences in atherosclerotic susceptibility between AEC and VEC.

Accumulating data indicate that AEC and VEC are molecular distinct even before the output of the first embryonic heartbeat [Torres-Vazquez et al., 2003; Swift and Weinstein, 2009]. Recently, Chi [Chi et al., 2003] employed a microarray method by which 817 vein-specific genes and 59 artery-specific genes have been found. Among these genes, COUP-TFII has been reported to be the upstream transcription factor that regulates vasculature differentiation during embryo development. You [You et al., 2005] found that removing of COUP-TFII from embryonic VEC activates the expression of artery-specific genes such as NP-1, Jagged1, Notch1, and Efnb2, and leads to the acquisition of arterial features. We here observed that Notch1/Jagged1 but not COUP-TFII was expressed in AEC, while COUP-TFII but not Notch1/Jagged1 was expressed in VEC of adult vessels. Suppression of COUP-TFII in cultured VEC induces expression of

TABLE II. The Genes Changed After Inhibition of COUP-TFII in VEC With AngII Stimulation

Accession no	Gene name	Gene symbol	Fold change	*P*-value
NM_002421	Matrix metalloproteinase 1 (interstitial collagenase)	MMP1	8.673	0.036
NM_002422	Matrix metalloproteinase 3 (stromelysin 1, progelatinase)	MMP3	5.853	0.013
NM_002985	Chemokine (C–C motif) ligand 5	CCL5	9.743	0.039
NM_004591	Chemokine (C–C motif) ligand 20	CCL20	6.835	0.016
NM_005577	Lipoprotein, Lp(a)	LPA	5.306	0.028
NM_000600	Interleukin 6 (interferon, beta 2)	IL-6	2.946	0.002
NM_003248	Thrombospondin 4	THBS4	5.369	0.047
NM_002543	Oxidised low density lipoprotein (lectin-like) receptor 1	OLR1	6.156	0.040
NM_001122	Adipose differentiation-related protein	ADFP	4.843	0.002
NM_002575	Serine (or cysteine) proteinase inhibitor, clade B (ovalbumin), member 2	SERPINB2	2.645	0.009

Numbers are median fold changes from three biological replicates.

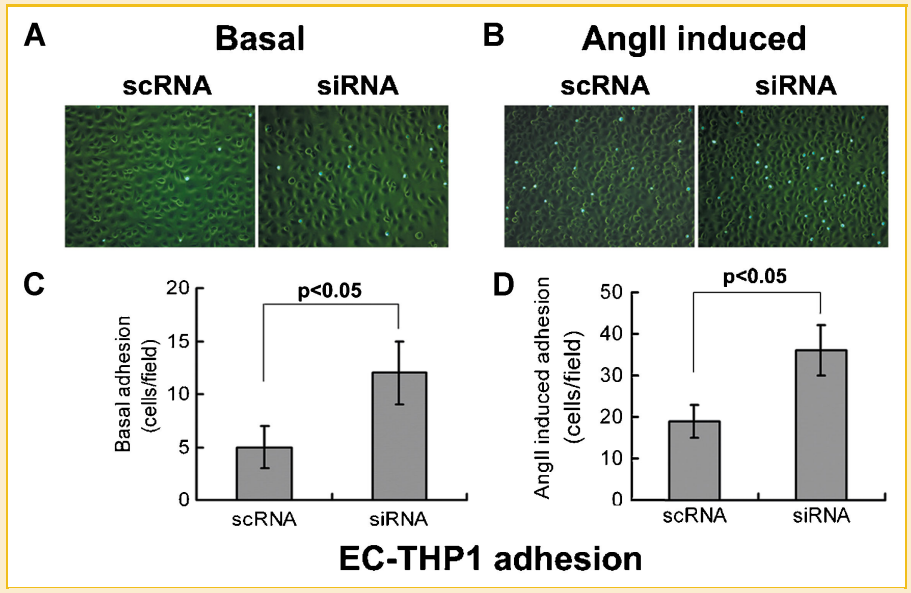


Fig. 4. Effects of COUP-TFII-knocking down in VEC on adhesion of THP1 cells. Cultured VEC were transfected with siRNA of COUP-TFII or its scRNA and treated with or without AngII for 4 h. THP1 labeled with DAPI were planted on the VEC monolayer and allowed to adhere for 10 min. Non-adherent cells were removed. A,B: Representative photomicrographs of adhesion of THP1 cells to VEC transfected with siRNA and scRNA without (A) or with (B) AngII stimulation are shown. C,D: Quantification of adhered THP1 cells. DAPI positive cells were counted in five randomly selected optical fields. Data are presented as mean ± SEM of cells/field ($n = 5$).

Notch1/Jagged1. The apparently conserved expression pattern of COUP-TFII in adult and embryonic vasculature highlights its potential importance in establishing and maintaining of proper arterial-venous demarcation.

Besides the function in arterial-venous differentiation, COUP-TFII regulates a variety of metabolic processes including β-oxidation, fatty acid synthesis, glucose homeostasis, and insulin sensitivity [De et al., 2004; Bardoux et al., 2005; Buholzer et al., 2005], and these are all relative to the development of atherosclerosis. By employing a microarray in VEC after inhibition of COUP-TFII, we found that, of the 112 atherosclerosis related genes, 11 genes were upregulated and 3 were downregulated. These genes have been reported to play diverse roles in endothelial biology, including lipid transport and metabolism (apoA1, apoE, LPA), extracellular matrix (MMP1, MMP3), thrombosis (vWF), inflammation and adhesion molecules (CCL5, CCL20, CCR1, IL-8, IL-6, IL13, IL-4, DSCR1). Importantly, most of the selectively upregulated genes induced by inhibition of COUP-TFII were pro-atherogenic ones such as MMP1, MMP3, LPA, vWF, CCL5, CCL20, CCR1, IL-6, and IL-8 whereas only few of them (DSCR1 and apoA1) were anti-atherogenic ones, and contrarily, downregulated genes were all atheroprotective ones (apoE, IL-4, and IL-13) [Curtiss and Boisvert, 2000; Catena et al., 2005; Barter and Rye, 2006; Blann, 2006; Liang et al., 2006; Liehn et al., 2006; Minami et al., 2006]. These data indicate that inhibition of COUP-TFII could reprogram expression of pro- and anti-atherogenic genes, which might be one of the reasons to explain why arteries are more prone to atherosclerosis than veins.

AngII is a defined risk factor for the development of atherosclerosis [Mazzolai and Hayoz, 2006]. To understand whether knockdown of COUP-TFII affect the responses to atherogenic stimuli of VEC, we further observed gene expression profile after treating VEC with both siRNA of COUP-TFII and AngII. Comparing with the treatment with AngII alone, AngII plus downregulation of COUP-TFII specifically elevated 10 of the 112 atherogenic genes related to the lipid transport and metabolism (LPA, OLR1, ADFP), extracellular matrix (MMP1, MMP3), thrombosis (THBS4, SERPINB2), inflammation and adhesion (CCL5, CCL20, IL-6). Among these reprogrammed genes, some overlap the results of knockdown of COUP-TFII alone such as MMP1, MMP3, CCL5, CCL20, LPA, and IL-6, while ADFP, OLR1, THBS4, and SERPINB2 required simultaneously inhibition of COUP-TFII and stimulation with AngII to change their expression. Although COUP-TFII was initially characterized as a transcriptional stimulator of the chicken ovalbumin promoter, it has recently been recognized to play a regulation role in the transcriptional control of several genes including AngII [Yanai et al., 1999]. We therefore speculated that COUP-TFII is an upstream regulator for the renin-angiotensin pathway, and thereby influences the gene expression of ADFP, OLR1, THBS4, and SERPINB2 which are regulated by AngII.

Arteries and veins are different in their local environment including oxygen tension and hemodynamic forces. Hypoxia has been shown to accelerate the development of atherosclerosis in many situations [Nakano et al., 2005; Sluimer and Daemen, 2009]. However, it is the oxygen tension in the vessel wall but not in the blood flow determines its influence on the development of atherosclerosis [Simanonok, 1996]. In contrast, there are significant evidences revealing that hemodynamic forces are major determinants of atherosclerotic plaque localization. Shear stress, by creating a force tangential to the vessel wall, and pressure, by creating a circumferential stretch, are biologically active in affecting the expression of atherogenic genes. It has been commonly noticed that high laminar shear stress is protective but the oscillatory blood flow or

low shear stress promotes the development of atherosclerosis. Our study, in a different way, showed that intrinsic molecular divergences contribute to the different susceptibilities to atherosclerosis between arteries and veins. COUP-TFII exerts its effects on controlling the susceptibility of VEC to atherosclerosis through either direct suppression of some atherogenic gene expression or interfering atherogenic factor-induced gene expression.

Adhesion of leukocytes to vascular endothelium is an essential process in the initiation and development of atherosclerosis [Boisvert, 2004; Liehn et al., 2006]. It has been indicated that cell adhesion pathways can be activated by atherogenic stimulators in AEC rather than in VEC [Amberger et al., 1997; Deng et al., 2006], which partially explains the differences in adhesive responses between AEC and VEC. The present study revealed that knockdown of COUP-TFII in VEC activates adhesion of the THP-1 cells to the VEC even at the absent of AngII. These data suggest that COUP-TFII plays a key role in regulating the effects of chemokines and inflammatory factors, thereby inhibiting the interaction of VEC with monocytes.

Although the heterogeneity of endothelium from different vessels has been reported for a long time [Gerritsen, 1987; Aird, 2007], its relation to atherosclerosis is indicated only in recent years [Aird, 2006a,b]. Deng [Deng et al., 2006] have reported quite recently that atheroprotective genes were more expressed in VEC than that in AEC. In the viewpoint of vascular development, our study focused on how the differences between AEC and VEC are acquired, and found that COUP-TFII can alter profiles of atherosclerosis-related gene expression in VEC at both basal and atherogenic factor-stimulated conditions. Although there are a variety of reasons including environment and hemodynamics for the different susceptibilities to atherosclerosis between the artery and vein, our study indicates that the intrinsic molecules such as COUP-TFII and its related genes may also contribute, at least in part, to the difference between the two vessels. Targeting these intrinsic molecules may be a potential strategy for atherosclerosis therapy.

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