

Supplemental Methods

Real time PCR

TM and EPCR genes expression was assessed in unstimulated and 50 ng/ml TNF- α activated HIMEC. The cells were washed with cold PBS twice and total RNA was isolated by TRIzol Reagent (Invitrogen) and quantified by optical density. One microgram of RNA was reverse transcribed using MultiScribe kit (PE Applied Biosystems, Foster City California) in a total reaction volume of 50 μ l. RT-PCR was based upon the TaqMan fluorogenic detection system (TaqMan $^{\circledR}$, Roche), using a fluorogenic oligonucleotide probe designed to hybridize to the specific target sequence. The TaqMan probes were labeled at the 5' with the fluorescent reporter dye FAM (6-carboxyfluorescein) (R) and at the 3' with the quencher dye TAMRA (6-carboxytetramethylrhodamine) (Q). The sequences for gene-specific forward and reverse primers and the probes were designed using Probe Library $^{\text{TM}}$ primer design software (Roche Diagnostic, Milan, Italy). The following sequences of primers and number of the probe were used for RT-PCR of TM mRNA: 5' AATTGGGAGCTTGGGAATG 3' (forward); 5' TGAGGACCTGATTAAGGCTAGG 3' (reverse); (GeneBank, Accession No. NM_000361.2). The following primers and number of the probe were used for RT-PCR of EPCR mRNA: 5'GTAGCCAAGACGCCTCAGAT 3' (forward); 5' GATAGGGGTCGCGGAAGTA 3' (reverse); (GeneBank, Accession No. NM_006404.3). The mRNA of GAPDH was used for RT-PCR as the house keeping gene with following primers and number of probe, 5' TCCACTGGCGTCTTCACC 3' (left primer), 5' GGCAGAGATGACCCTTTT 3'(right primer).

Quantitative real-time PCR was performed using the FastStart Taqman probe Master(Rox) (Roche).

Briefly, 2 μ l reaction product of RT reaction was used in 25 μ l real time PCR reaction with a probe concentration of 100nM and 200nM for each primer. The conditions of RT-PCR were as follows: 40 cycles of 95 $^{\circ}$ C/15s and 60 $^{\circ}$ C/ 1 min. Triplicate RT-PCR reactions were prepared for each sample and amplified by using BIO-RAD Chrom4

detector RT-PCR. Data were analyzed by software version Opticon Monitor 3. Data were presented by the relative amount of mRNA with the formula $2^{(-\Delta CT)}$, which stands for the difference of threshold cycle (CT) between a gene of interest and the housekeeping gene GAPDH.

The point at which the PCR product is first detected above a fixed threshold, termed cycle threshold (Ct), was determined for each sample, and the average Ct of triplicate samples was calculated. For the negative controls the H₂O was included in the real time PCR reaction.

Measurement of *in vitro* capacity for PC Activation

HIMECs monolayers were incubated an additional 24 hours in the presence or absence of 50 ng/ml TNF- α (R&D Systems, Minneapolis MN). The cells' capacity for supporting PC activation was then measured essentially as previously described (60). In brief, cells were washed thoroughly with PBS before addition of serum-free medium containing 400 nM human PC (Sigma), 10 nM human thrombin (Sigma, specific activity \sim 100 NIH units/nmole) and 2 mM CaCl₂. Activation reaction was performed at room temperature for 15 min, at which time an aliquot was removed, diluted into a 96-well plate containing a 4-fold excess of hirudin (4 NIH units hirudin/1 NIH unit thrombin) and incubated 5 min to allow hirudin inhibition of thrombin. The amidolytic activity of "generated" aPC was then measured by adding chromogenic substrate S-2366 (Chromogenix) to an initial concentration of 500 μ M and the change in absorbance at 405 nm (mOD/min) was monitored at room temp using a ThermoMax plate reader (Molecular Devices). In all experiments, the PC/thrombin solution was also incubated in wells lacking HIMEC and any background activation by "free" thrombin (non-TM-bound) was subtracted. Conversion of mOD/min to amounts of aPC generated was calculated using the specific activity of fully-activated PC under identical amidolytic activity assay conditions and, under these conditions, less than 50% of initial PC substrate was activated.