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Supplemental Data

Preparation of endothelial cell cultures

Arterial tissue specimens were obtained from patients undergoing elective surgical reconstruction for an infrarenal AAA (Abdominal Aortic Aneurysm), with a protocol approved by the institutional review board of the Bioethical Committee of Tor Vergata Hospital.

Human aortic sections from abdominal aneurysm were washed in antibiotic/antimycotic solution and maintained in sterile normal saline solution. Vascular endothelial cells (ECs) strains were obtained from primary explant techniques as described (Mason et al. 2007; Hewett et al., 2009) with some modifications. Briefly, the tissue was cut longitudinally to expose the surface of the luminal endothelium and the adventitia and atherosclerotic media were dissected from each tissue. The remaining tunica intima was minced into 2-mm^2 pieces. Individual pieces were placed into separate chambers of a 100 mm tissue culture dish (Costar, Cambridge, MA) and allowed to attach to the plastic surface before tissue culture medium was added. The primary vascular endothelial cells were obtained and subcultured using Chang medium (Irvine Scientific) and used until the 6th passage (Xu et al., 2009).

FACS analysis

FACS analysis was used to characterize primary vascular EC cells, using a FACSAria flow cytometer (BD). Unstained and IgG FITC stained cells were used to optimize parameters for the analysis of Mab MEM-229 (Abcam) directed against CD105, a specific endothelial cell marker and Mab AC136 (Miltenyi Biotec) anti-CD34, a progenitor endothelial marker. ECs were gated on the basis of light scatter and 7-amino actinomycin-D (7-AAD) dead cell discrimination. 10,000 gated events were acquired for each sample and gates were set to eliminate debris. As shown in figure S1 the majority of cells (≥95%) derived from the intima were immuno-positive for anti-CD105 antibody (panels d-e-f) and ≥90% were positive for anti-CD34 (panels g-h-i). It is worth noting that the fluorescence intensity for CD105 antigen (panel f) was markedly higher than observed with CD34 (panel i), indicating that the isolated population is formed by differentiated endothelial cells.

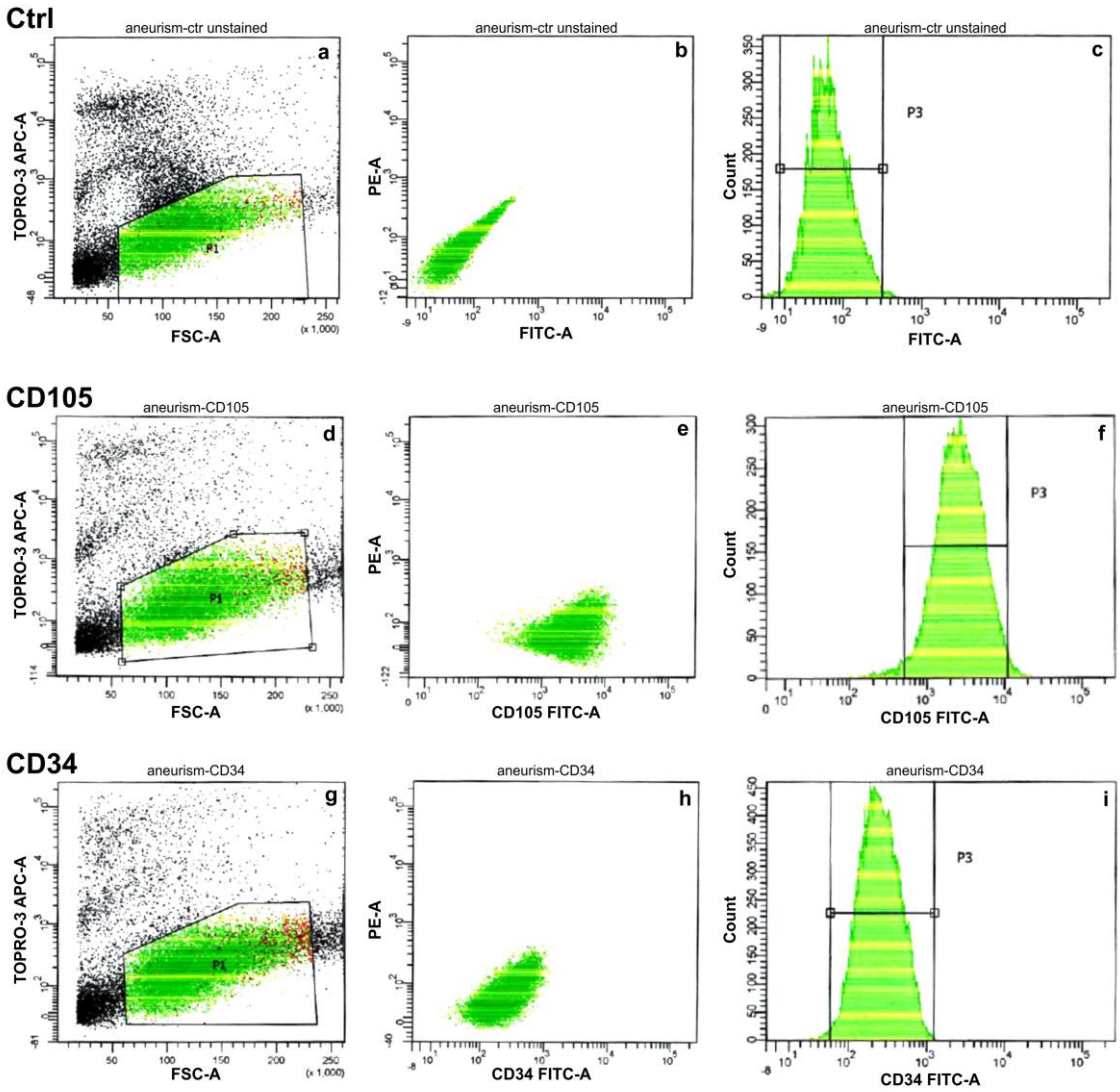
References

Hewett PW (2009) Vascular endothelial cells from human micro- and macrovessels: isolation, characterization and culture. *Methods Mol Biol* 467:95-111.

Mason JC, Lidington EA, and Yarwood H (2007) Isolation and analysis of large and small vessel endothelial cells. In Methods Mol Med.;135:305-321.

Xu S, Fu J, Chen J, Xiao P, Lan T, Le K, Cheng F, He L, Shen X, Huang H, and Liu P (2009) Development of an optimized protocol for primary culture of smooth muscle cells from rat thoracic aortas. *Cytotechnology* 61:65-72.

Figure S1



FACS analysis. Fluorescence associated with unstained isotype-matched controls (panels a, b and c), with anti-CD105 (panels d, e and f) and anti-CD34 (panels g, h and i) antibodies. FACS analysis were repeated at least three times for each antigen.