SUPPLEMENTARY DATA FOR

Live dynamic imaging of caveolae pumping antibody rapidly and specfically across endothelium in the lung

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Supplementary Data

Protein expression analysis. Monospecificity of our newly generated endothelial cell antibodies was confirmed by Western analysis of lung tissue subfractions, including caveolae (**Figure 1A**). To examine protein expression, we performed immunohistochemical staining of rat tissue sections (**Supplementary Figure 3A)**. TX3.833 and J310 (collectively mAPP) labeled selectively the blood vessels of lung but not other tissues, except for mild staining of proximal tubules in kidney. Skeletal muscle, thymus, intestine, stomach, thyroid, spleen, testes, and ovaries also stained negative for endothelial cell aminopeptidase P (APP) (data not shown). J120 (mCD34) stained only lung vessels. G278 (mPodo) recognizes rat podocalyxin which is a pan-endothelial marker and as expected strongly stained blood vessels in lung as well as all other organs tested. Tissues stained with secondary antibody alone were negative. Podocalyxin is also highly expressed in podocytes of renal glomeruli. Confocal fluorescence microscopy confirmed APP expression in lung endothelium by revealing significant triple co-localization of caveolin-1 antibodies, G278, and TX3.833 (**Supplementary Figure 3C**).

Two-dimensional (2D) gel (**Supplementary Figure 3D**) and Western analysis (**Supplementary Figure 3B**) also indicated restricted expression of APP in luminal endothelial cell plasma membranes isolated from lung but not other organs. APP was immunodetected as three adjacent spots in lung P. **(Supplementary Figure 3D)** Silver staining also showed these 3 spots in P from lung but not brain, heart, kidney, and liver. In agreement with the tissue immunostaining, J310 recognized a single band enriched in endothelial cell luminal membranes isolated from lung but not other organs. Other endothelial cell proteins including caveolin-1, ACE, and podocalyxin can easily be detected in endothelial cell membranes from more than one tissue. Some of the quality control for the membrane isolation is shown with enrichment for caveolin and actin while E-cadherin is depleted in the luminal endothelial cell membranes, as expected.

Identification of the TX3.833 antigen. To identify the antigen recognized by TX3.833, we immunopurified the protein for chemical amino acid sequencing of tryptic peptides. Because the resulting five derived peptide sequences (EFTGSTWQEK; LVETETYSPVMLIK; GTV(D)(E)FX(G)A(E)(L)I(A)(L)L; (S)LDEMYLVX(S)(G)(G)(Q)Y(Y); (S)LSAYIIPDTDAHMSEYIGK) did not match any known sequences in

GenBank at the time, we used them to design degenerate oligonucleotide probes for screening a rat lung cDNA library. A positive clone was isolated, sequenced, and used to identify corresponding ESTs, from which the entire open reading frame was assembled (**Supplementary Figure 2A**). A subsequent GenBank search using the new rat deduced amino acid sequence revealed considerable identity to human and mouse membrane-bound APP. At the time, we added this rat sequence to our rat database for mass spectrometric analysis and referred to it as rat membrane-bound APP. The full-length cDNA was expressed in CHO cells to confirm it as the TX3.833 antigen. Western analysis of cell lysates (**Supplementary Figure 2B**) showed that TX3.833 recognized the expected 85kD band for rat APP in the transfected cells but not in the control cells transfected with the parent plasmid. Immunofluorescence microscopy on intact cells probed with TX3.833 revealed an intense punctate signal on the CHO cells stably transfected with TX3.833 antigen (**Supplementary Figure 2C**) but not in non-transfected control cells (data not shown), indicating that the TX3.833 antigen is expressed and accessible on the cell surface. More recently, rat APP gene sequences which agree with our sequence have been added to the ever-growing rat genome.

Mass spectrometric analysis of antigens. To complete the identification process of the TX3.833 antigen, rat lung tissue lysates were immunoprecipitated using TX3.833 and the precipitated material was analyzed by mass spectrometry. Multiple distinguishable MS/MS spectra were measured representing 17 tryptic peptides, each of which were identical to the rat APP protein sequence for sequence coverage totaling 33%. Similar mass spectrometric analysis of J310 immunoprecipitations and the three 2D gel spots (**Supplementary Figure 3D**) in lung P also identified the excised protein as APP. The antigen for J120 was similarly analyzed and identified as CD34 from the rat database with 7 unique peptides. G278 was found to recognize rat podocalyxin by mass spectrometric analysis identifying 8 unique peptides.