Abstract
The study of lymphatic endothelial cells and lymphangiogenesis has, in the past, been hampered by the lack of lymphatic endothelial-specific markers. The recent discovery of several such markers has permitted the isolation of lymphatic endothelial cells (LECs) from human skin. However, cell numbers are limited and purity is variable with the different isolation procedures. To overcome these problems, we have transfected human dermal microvascular endothelial cells (HDMVECs) with a retrovirus containing the coding region of human telomerase reverse transcriptase (hTERT), and have produced a cell line, hTERT-HDLEC, with an extended lifespan. hTERT-HDLEC exhibit a typical cobblestone morphology when grown in culture, are contact-inhibited, and express endothelial cell-specific markers. hTERT-HDLEC also express the recognized lymphatic markers, Prox-1, LYVE-1 and podoplanin, as well as integrin alpha9, but do not express CD34. They also form tube-like structures in three-dimensional collagen gels when stimulated with vascular endothelial growth factors -A and -C. Based on these currently recognized criteria, these cells are [...]
(A) Immunohistochemistry was performed as previously described. Consecutive sections of paraffin-embedded human skin were incubated O/N at 4°C with 0.6 µg/ml of purified anti-podoplanin 201853. Immunolabelling was revealed with a peroxidase-conjugated secondary antibody and visualized using diaminobenzidine (Envision +; DAKO) before counterstaining with hematoxylin (left panel). Experiments performed in parallel with anti-LYVE or anti-podoplanin (from D.K.) antibodies resulted in the same staining pattern (data not shown). Neutralizing experiments in which purified anti-podoplanin 201853 was pre-incubated for 1 hour on ice with a 100-fold molar excess of each peptide alone or all possible combinations of two out of three peptides or all three peptides together revealed that only peptide RN16CT was able to block antibody reactivity (right panel). Arrows indicate blood vessels, arrow with asterisk indicates a lymphatic vessel. Bar = 100 µm. (B) Podoplanin and LYVE-1 expression was assessed by RT-PCR as described in Materials and Methods. Podoplanin and LYVE-1 mRNAs are expressed in hTERT-HDLEC and in HMEC-1 but not in SkHep-1 cells. (C) Western blot analysis was performed as previously described. Protein extracts from hTERT-HDLEC and SKHep-1 were electrophorezed in a 12% SDS polyacrylamide gel under non-reducing conditions and transferred to a polyvinylidene difluoride (PVDF) membrane. Membranes were incubated with 0.5 µg/ml of purified anti-podoplanin. Primary antibody staining was revealed with horseradish peroxidase-conjugated goat anti-rabbit IgG (Santa Cruz, sc-2004) and detected with the enhanced chemiluminescence ECL™ detection system (Amersham) followed by exposure to autoradiographic films (Kodak). The upper part of the gel was removed and stained with Coomassie blue to assess
protein loading. Anti-podoplanin 201853 recognizes a ∼ 38 band which corresponds to the reported size of human podoplanin \(^3\); as predicted by RT-PCR, SkHep-1 cells were negative for podoplanin. (D) FACS analysis was performed as described in Materials and Methods. HMEC-1 were incubated with 1 µg/ml of purified anti-podoplanin alone or with the same antibody pre-incubated for 1 hour with a 100-fold molar excess of peptide alone (RN16CA, RN16CT, RN16CG), or all possible combinations of two out of three peptides or all three peptides together. Neutralizing experiments in which purified anti-podoplanin 201853 was pre-incubated for 1 hour on ice with a 100-fold molar excess of all peptide combinations revealed that only peptide RN16CT was able to block antibody reactivity (right panel). As predicted by RT-PCR and Western blot, SkHep-1 cells were negative for podoplanin (data not shown).