Supplementary Information

Immunostaining protocol

Human amnion was embedded in OCT, snapped frozen in liquid nitrogen, and cut into5 µm-thick sections. Both tissue sections and HAECs cultured on plastic were subjected to immunostaining with appropriate dilutions of primary antibody and their respective secondary antibodies. Briefly, each sample was fixated in cold methanol for 10 min at –20 ^{o}C, permeabilized in 0.2% Triton X-100 for 15 min and blocked with 2% bovine serum albumin for 30 min at room temperature. Cells were then incubated with the primary antibody for 16 h at 4 ^{o}C. After 3 times of wash with PBS, specific binding was detected by a FITC-conjugated anti-mouse or anti-rabbit secondary antibody incubated for 30 min at room temperature. Finally, the sample was counterstained with Hoechst 33342 and mounted in anti-fading solution (Vector Labs, Burlingame, CA, USA). For Ki67, Musashi-1, and p63 immunostaining, a DAB kit (Dako, Carpinteria, CA) with an immunoperoxidase protocol (ABC kit Vectastain Elite, Vector Labs) was used. The negative control was performed by substitution of the primary antibody with PBS. For double immunostaining of both pan-cytokeratins and vimentin, tissue sections or cells were incubated with respective primary antibodies followed by sequential incubation for 30 min of FITC-conjugated anti-mouse and TRITC-conjugated anti-rabbit secondary antibodies, respectively.

Images were photographed with a NikonTe-2000u Eclipse epi-fluorescent microscope (Nikon, Tokyo, Japan). Positive immunostaining was counted at 200x magnification at 10 random fields in each sample.

Western blotting protocol

HAECs isolated immediately from amniotic epithelial sheets as well as from cultures of Passages 0, 1, and 6 in SHEM were quantitatively compared for their cytoskeleton protein expression using Western blots. Cultured cells were collected and solubilized in the lysis buffer containing 50 mM Tris–HCl (PH 8.0), 1% NP-40, 0.5% DOC, 0.1% SDS, 150 mM NaCl, 5 mM EDTA, 1 mM PMSF and a protease inhibitor cocktail. Total cell proteins were extracted from fresh amniotic sheets by gentle scraping with the same lysis buffer. Proteins in these lysates were denatured by boiling for 5 min with the equal volume of 2X Tris-glycine SDS sample buffer, separated by 4% to 15% gradient SDS-PAGE, and transferred to nitrocellulose membranes. Five percent nonfat milk was incubated for 1 h to block non-specific binding, followed by each of the three primary antibodies to vimentin, cytokeratin 18 (both at 1:500), and ß-actin (at 1:5000). The specific binding was then detected by respectively anti-mouse and anti-rabbit horseradish peroxidase (HRP)-conjugated antibodies (at 1:1000), and visualized by enhanced chemiluminescence method.