Endothelial Cells Cytoskeleton Reorganization during Functional Monolayer Formation *in vitro*

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Endothelial cell (EC) monolayer located on the inner surface of the blood vessel wall is the semi-selective barrier for molecules and cells of immune system. Cytoskeletal components consisting of intermediate filaments (IF), microfilaments and microtubules (MTs) as well as adhesive structures are key elements involved in EC barrier integrity maintenance. To study EC barrier function we used EC isolated from vessels and cultured *in vitro*. These cells are able to divide, spread and form VE-cadherin based contacts. Confluent monolayer of these cells has barrier properties similar to EC *in vivo*. To examine EC reorganization leading to EC monolayer formation we used two lines of human EC: human pulmonary artery EC (HPAEC) and hybrid line EA.*hy*926 derived from human umbilical vein EC (HUVEC) and lung carcinoma cell line (A549). Vimentin filaments are the major part of IFs for both cell lines. Vimentin evenly distributed through the entire EC cytoplasm excluding lamellae. Cell area occupied by vimentin filaments slightly increased upon formation of monolayer due to the disappearance of free lamellae. Actin formed two different cytoskeletal structures in both cell types such as cortical actin and stress fibers bundles. Cortical actin, which is located at the zone free cell edge, is tipical for single EC and EC with small area (up to 30%) of intercellular contacts. Stress fibers are present on all stages of confluent monolayer formation and their amount is independent upon cell-cell contacts. In contrast, MT system undergoes major changes upon monolayer formation. In the single EC spread on the surface of substrate MT system is radial, but upon formation of intercellular contacts it becomes asymmetric (average amount of MTs in the area of intercellular contacts is always larger compare to the area of free lamellae and this difference depends upon the length of contact). With intercellular cell contact length up to 10% of the cell perimeter average amount of MTs in the contact region is ~1.5 fold higher in HPAEC compare to free lamellae region (9,70±1,69 vs 6,96±2,09, respectively). Upon monolayer formation the amount of MTs at the edge of the cell increased, but the difference between two groups sustained (for EC with contact area 70-80% average amount of MTs in the area of contact is 12,93±2,22, compare to 8,20±2,64% in the free lamellae area). Symmetrical disposition of MT ends can be achieved only in fully formed monolayer with mature contacts. Asymmetry of MT system during EC monolayer formation is also depends upon the centrosome position. In EC with extension of contacts to 50%-75% of the cell perimeter the centrosomes relocated toward the area of new contacts, but with extension of contacts above 75% it is equiprobably shifts toward free lamellae zone or cell-cell contacts area. Therefore, the centrosome is involved into EC monolayer formation and its position determines symmetrical state of radial MT and may have an effect on the amount of dynamic MT ends in the area of intercellular contacts. Supported by RFBR (Grant # 12-04-00488) to I.B.A. and NIH (HL101902).