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Cellular dynamics of human uterine epithelial RL95-2 cells during trophoblast binding: a high resolution confocal microscopy study on Rho protein regulation

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Embryo implantation requires attachment of the blastocyst to the uterine epithelium. The mechanisms regulating trophoblast adhesion to the uterine epithelium are hardly understood. Monolayer-cultured endometrial RL95-2 cells are an established *in vitro*-model for the receptive human uterine epithelium. We reported previously that binding of RL95-2 cells to spheroids of human trophoblast-like JAR cells is dependent on Rho GTPases, most likely RhoA, as measured by a centrifugal force-based adhesion assay. In this study, we investigated changes in expression and distribution of RhoA in both the apical and basal pole of RL95-2 cells caused by binding of JAR spheroids. Correlations between changes in the subcellular localisation of Rho proteins and a re-distribution of actin filaments induced by RhoA were determined and compared in areas of JAR-cell contact vs. non-contact areas using high resolution fluorescence confocal microscopy. In the basal region of non-contact RL95-2 cells, significant higher fluorescence signals of Rho protein (37 ± 6 grey scale values) and of F-actin (41 ± 3 grey scale values) were found as compared to the apical pole (RhoA: 24 ± 3 grey scale values, F-actin: 28 ± 2 grey scale values). Binding of JAR spheroids to RL95-2 cells dramatically altered RhoA and F-actin localisation within RL95-2 cells leading to an inverted pattern of RhoA and F-actin along the apico-basal cell axis. High intensities of RhoA (53 ± 12 grey scale values) and F-actin (108 ± 17 grey scale values) were found in the apical region, i.e. at the newly formed binding sites. At the basal pole of the same cells intensity of RhoA signal was reduced (31 ± 14 grey scale values). In contrast, the F-actin signal increased (60 ± 20 grey scale values) but less than in the apical cell pole. These results demonstrate that uterine epithelial RL95-2 cells respond to trophoblast contact with an overall increase of F-actin and a re-distribution of RhoA protein which may be part of a general modification of their architecture. Thus, not only the apical but also the basal pole of uterine epithelial cells may be involved in regulation of adhesiveness for trophoblast during early embryo implantation *in vivo*.