Nuclear F-actin Formation Regulates Decidualization of Human Endometrial Stromal Cells

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Human endometrial stromal cells (ESCs) undergo cyclic changes during the menstrual cycle in response to changing levels of steroid hormones. Especially, ESCs morphologically and functionally change their cellular states for preparing pregnancy, referred to as decidualization. Decidualization is essential for implantation and maintenance of pregnancy. During decidualization, ESCs dramatically change their fibroblast-like morphology into the epithelial-like state with the dynamic rearrangement of cytoplasmic actin. Interestingly, this cytoskeletal actin dynamics not only morphologically, but also functionally regulate decidualization. Recent reports have suggested that actin dynamically alters its polymerized state (filamentous actin; F-actin) upon external stimuli not only in the cytoplasm, but also in the nucleus. However, nuclear actin dynamics during decidualization of human ESCs have not been elucidated. This study investigated the nuclear actin dynamics and its role in decidualization of human ESCs. For visualizing nuclear actin dynamics, ESCs expressing nuclear actin-GFP probe were established. Cells were treated with cAMP (0.5 mM) to induce decidualization. Time-lapse imaging revealed a dynamic formation of nuclear F-actin during decidualization. This was disassembled following the withdrawal of the decidualization stimulus, suggesting its reversible process. To investigate whether nuclear F-actin formation is involved in the regulation of decidualization, nuclear F-actin formation was inhibited by overexpressing the nuclear actin mutant (actin^{R62D}). This significantly reduced the number of cells exhibiting the nuclear F-actin induced by decidualization and suppressed the expressions of decidualization markers (IGFBP-1 and PRL). Therefore, nuclear F-actin formation was essential event for decidualization. In order to investigate how the nuclear F-actin formation is involved in decidualization, we performed RNA-sequence analysis. Among the 618 genes that should be repressed in the course of decidualization, the downregulation of 304 genes was not observed when cells were overexpressed actin^{R62D}. These genes were defined as nuclear actin-regulated genes and were related to the regulation of cell proliferation. Overexpression of actin^{R62D} suppressed the decidualization-induced decrease in cell number. Considering that ESCs have to exit the cell cycle for accomplishing their differentiation process towards the decidualized state, nuclear F-actin formation contributes to decidualization through the suppression of cell proliferation. Furthermore, upstream analysis was performed on nuclear actin-regulated genes to identify factors regulating nuclear F-actin formation, which predicted

C/EBPβ as an upstream factor. Knockdown of C/EBPβ suppressed nuclear F-actin formation, cell cycle arrest, and expression of decidualization markers, indicating that C/EBPβ induces the cell cycle arrest through the regulation of nuclear F-actin formation during decidualization. In conclusion, **w**e revealed that actin exists in the nucleus of human ESCs and nuclear F-actin formation is induced by C/EBPβ during decidualization. This induces cell cycle arrest to differentiate into decidualized ESCs, which is a novel mechanism for the regulation of decidualization.