Endometrial Expression and Secretion of Insulin-like Growth Factor 2 Facilitates Uterine Angiogenesis and Trophoblast Differentiation during Placenta Development

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During early pregnancy, the uterine stromal cells, under the influence of the ovarian steroid hormones, undergo a dramatic transformation to form the decidua, a secretory tissue that surrounds the growing fetus. The decidual cells control diverse functions within pregnant uterus, including the formation of an extensive maternal vascular network that supports embryo development. Proper differentiation and migration of the trophoblast cells, critical for forming functional placenta, are also influenced by yet unknown factors secreted by the decidual cells. A major challenge is to identify the signaling molecules emanating from the decidual cells that communicate with the endothelial and trophoblast cells within the implantation chamber to ensure successful pregnancy establishment and maintenance. We have recently developed a unique mouse model in which conditional knockout (cKO) of the gene encoding the transcription factor Runx1 in the uterus led to severe subfertility due to deficient decidual angiogenesis and an impairment in trophoblast cell lineage development and subsequent invasion into decidua during placentation. Interestingly, we found that expression of insulin-like growth factor 2 (IGF2), a protein known to influence both angiogenesis and trophoblast differentiation, was significantly reduced in decidual cells in the absence of Runx1. In this study, we investigated the role Runx1 plays in coordinating IGF2 expression and secretion in human endometrial stromal cells (HESC) undergoing decidualization. We found that Runx1 controls the expression of the *Igf2* gene by directly interacting with a distinct upstream regulatory region. Notably, decidualizing HESC secrete extracellular vesicles (EVs) which are taken up by various cell types within the uterus to influence critical functional events during pregnancy. Mass spectrometry analysis revealed that EVs secreted by HESC harbor IGF2 as a protein cargo. Upon treatment of these cells with Runx1-specific siRNA, IGF2 was absent from the EV cargo. Functional studies showed that, while delivery of control HESC-derived EVs into human endothelial cells HUVEC promoted vascular network formation, similar treatment with EVs obtained from Runx1- or IGF2-depleted HESC failed to do so. When Runx1 or IGF2-depleted EVs were added to human trophoblasts stem cells, their differentiation into extravillous trophoblast lineage was also reduced compared to the addition of control EVs. To examine the role of decidual IGF2 *in vivo*, we have recently created an IGF2 cKO mouse model in which *Igf2* gene is conditionally deleted in the uterus. Our initial results indicate that female IGF2 cKO mice exhibit marked subfertility, presumably due to post-implantation uterine defects. Collectively, these findings suggest that Runx1-driven expression of IGF2 in decidual cells plays a critical role in mediating cell-cell communication during uterine angiogenesis and trophoblast differentiation to ensure proper placenta formation.

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