Leukemia Inhibitory Factor Signaling in Human Trophoblast Cell Development

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In the early stages of human pregnancy, cells from the developing placenta, termed extravillous trophoblast (**EVT**) cells, enter the uterus. The entry of EVT cells into the uterus results in remodeling of uterine arteries to meet the increased demand of blood flow and oxygen during pregnancy. Dysregulation of the molecular controllers responsible for this structural uterine remodeling can lead to pregnancy complications such as early pregnancy loss, preeclampsia, intrauterine growth restriction, pre-term birth, or placenta accreta. Human trophoblast stem (**TS**) cells provide a powerful in vitro model to dissect regulatory processes controlling trophoblast cell lineage development. Previously, a uterine-derived cytokine called leukemia inhibitory factor (**LIF**) was implicated as a candidate regulator of early placentation in the mouse. In the present study, we performed in-situ hybridization, RT-qPCR, and bulk RNA-sequencing to help discern LIF-signaling in human first trimester placental tissue and in human TS cells. In situ hybridization revealed EVT cell columns were positive for *LIF* receptor ***(LIFR)***, and its downstream signaling partners, janus kinase 1 (***JAK1***) and signal transducer and activator of transcription 3 (***STAT3***). *LIFR* and *JAK1*, but not *STAT3*, transcript levels increased as TS cells transitioned from the stem to EVT cell state. We also examined the effects of LIF on human TS cells. Acute exposure to LIF resulted in an increase in phosphorylated STAT3. The effects of chronic LIF on EVT cell differentiation was examined morphologically followed by RT-qPCR and bulk RNA-sequencing. At the end of differentiation into EVT cells, LIF-treated TS cells failed to fully differentiate and instead exhibited features similar to TS cell stem state. We then used RT-qPCR to quantify stem and EVT cell state markers within control or LIF-treated cells following differentiation. LIF treatment corresponded to an increase in selected stem markers (*NPPB* and *PEG10*) and a decrease in EVT cell markers (*FLT4* and *FSTL3*). Bulk RNA-sequencing assisted in expanding the list of additional stem and EVT cell markers. In summary, LIF-signaling has prominent restraining effects on development of the EVT cell phenotype. This may reflect a pathway utilized by the uterine compartment to restrain excessive intrauterine EVT cell invasion and a regulatory mechanism susceptible to dysregulation in disease states characterized by abnormalities in intrauterine EVT cell invasion. This research was supported by K-INBRE P20 GM103418 (S.L.S), Lalor Foundation (S.L.S., A.M.), and NIH grants (HD020676, HD099638, HD105734).