PUBLIC ABSTRACT

Pregnancy has the seemingly impossible task of building a whole baby from a single cell. The embryo goes through innumerable incremental changes on its journey. Some of the most crucial steps of development occur before a person even knows they are pregnant. Defects in these crucial steps can lead to early miscarriage or severe birth defects. We can use mouse embryos as models for human embryo development, which allows us to better understand the genes and proteins that are crucial for normal development at these early stages and eventually possibly prevent early pregnancy loss. This dissertation focuses on the factors and signals that start and maintain development during the first and second cell fate decision when the mouse embryo has just 16 cells. Though the embryo has 16 cells it has only two cell types, inside and outside cells. We wanted to know what happens when a cell cannot decide if it is an inside or outside cell. We found that an on/off switch called HIPPO can help to resolve this cellular confusion. Furthermore, we studied embryos at the second cell fate decision, when the inside cells become two distinct groups. This leaves the embryo with three cell types total. We hoped to discover more about how two proteins that are expressed at this stage, OCT4 and GATA6 impact the development of these three cell types. We turned off OCT4 and GATA6 and evaluated the changes that occurred. We found that in addition to their known roles in promoting inside cell fate, they are actively preventing inside cells from becoming outside cells. Additionally, in the outside cells, OCT4 and GATA6 are required for early maintenance of outside cell factors, and prevention of inside cell factors.

ABSTRACT

Preimplantation embryo development is highly complex. Cells must manage changes to potency, cell positioning, and lineage specific gene expression. The embryo must first differentiate between inside and outside cells, where inside cells remain pluripotent (able to become any cell within the organism), and the outside cells lose potency to become multipotent stem cells (able to become only a few cell types). The differentiation of outside cells relies partially on the inactivation of the HIPPO signaling pathway that drives expression of the protein CDX2 in outside cells. Inside cells have active HIPPO signaling, maintain pluripotency and express SOX2. However, it is unknown how specific HIPPO pathway members like YAP1/WWTR1 regulate expression of these lineage-specific proteins. The second cell fate decision differentiates the inside cells into pluripotent epiblast and multipotent primitive endoderm. Transcription factor OCT4 has previously been shown to be necessary for development of both cell types. This dual requirement of OCT4 in two distinct lineages raises the question, how can OCT4 drive two cell different cell fates simultaneously. OCT4 has previously been shown to work with cofactors like SOX2, which is specific to pluripotent epiblast, so I hypothesized that OCT4 may also work with the primitive endoderm specific factor GATA6. My transcriptome analysis showed that OCT4 and GATA6 repress trophectoderm gene transcripts in the inner cell mass (ICM), which may help indirectly drive primitive endoderm fate. Additionally, I noted that loss of GATA6 and OCT4 caused reduced expression of trophectoderm specific factor CDX2 in early blastocyst and mid-blastocyst stages, respectively. *Gata6* null mid-blastocyst stage embryos also exhibited prolonged OCT4 expression in the trophectoderm, indicating a novel and stage specific role for

OCT4 and GATA6 in regulating trophectoderm gene expression.