Toshihiko Ezashi, D.V.M., Ph.D.

Research Summary

I was trained as a veterinary pathologist and molecular biologist. Molecular cloning of both subunits of luteinizing hormone and prostaglandin $F_{2\alpha}$ receptor led my interests into reproductive biology. I subsequently studied transcriptional controls on interferon-tau (IFNT), a protein crucial for the maternal recognition of pregnancy in ruminants and identified the ETS2 transcription factor as a key component of trophoblast function and specification. Later I demonstrated a potential means whereby a pluripotent stem cell factor, POU5F1 also known as OCT4, might be able to silence transcription factors such as ETS2 by forming a soluble complex and silence expression of key signature trophoblast genes, including IFNT and the two encoding human chorionic gonadotropin (hCG). I have acquired a great deal of experienced in working on the growth and differentiation of embryonic stem cells (ESC) and induced pluripotent stem cells (iPSC). We found that culturing hESC under physiologic oxygen levels (2-5% O_2) prevented spontaneous differentiation, and increased the formation of embryoid bodies, which signify the greater ability of the hESC to develop into many different cell types of the body. This finding has significantly enhanced our ability to work with and maintain hESC in the undifferentiated state in culture. My research on the use of low oxygen to culture hESC was recognized as among the top 50 advances for 2006/7 by Scientific American.

Research Interest

My research interest has three goals and objectives.

1. Pluripotent human stem cells as models for normal and diseased trophoblast

Trophoblast differentiation is the first lineage specification event that occurs during early embryonic development and is critical for subsequent embryonic development, as trophoblast cells ultimately give rise to the placenta. Hence, I am interested in the use of hESC and iPSC to understand how the trophoectoderm lineage is specified during early embryonic development. While many diseases of poor placentation, including early pregnancy loss, intrauterine growth retardation and preeclampsia, have their origins in abnormal early placental development, such development cannot be studied *in vivo* in humans. The basis of preeclampsia is poorly understood and it remains a major cause of maternal and fetal morbidity worldwide. We establish and characterize a hESC-derived cell culture model, for the study of specified trophoblasts, syncytiotrophoblast and extravillous trophoblasts. Based on conditions of the hESC differentiation into trophoblasts, we re-create of the trophoblasts from infants born to mothers with preeclampsia by generating iPSC from discarded umbilical cord and converting these pluripotent cells to the specified trophoblasts. The properties of these cells will be compared with generated from cord cells from pregnancies not complicated by preeclampsia. The pluripotent stem cell-based trophoblast models will provide insight into a pathophysiology of early stage placentation. These models should help to define the pathogenesis of these and related diseases and direct development of novel diagnostic and treatment strategies.

2. Generation and characterization of porcine iPSC and induced trophoblast (iTR)

Derivation of porcine embryonic stem cells (pESC) continues to be problematic. As an alternative I and my colleagues derived induced pluripotent stem cells (iPSC) from pig fibroblasts with lentiviral vectors expressing four reprogramming genes, POU5F1, SOX2, KLF4, and c-MYC. The porcine iPSC (piPSC) are capable of dividing more or less indefinitely in culture without senescence and have high telomerase activity. However, a lack of silencing of the exogenous transgenes has invariably been observed in iPSC from ungulates, despite the concomitant up-regulation of endogenous pluripotency markers. We are testing improved reprogramming approach including integration-free episomal plasmids and modified culture method. Additionally we obtained induced trophoblast (iTR) cells during standard reprogramming of porcine fibroblasts to piPSC.

3. Cellular reprogramming to investigate intellectual disability and neurodevelopment disorders

Obvious limitations exist in accessing neuronal tissues of human studies of neurodevelopmental disorders. Disease-specific iPSC derived from neurogenetic disorder patients provide unprecedented accesses to neuronal cells bearing the specific genetic disorders, because iPSC retain the genetic composition of the parental somatic cells. This project is a collaborative project with Dr. Sarah Elsea, Associate Professor at Baylor College of Medicine. The cells have been converted into iPSC and some of the lines are differentiated into neural cells. The phenotypes of the cells will be compared with those of the control cells.