Audrey Wind

Chesterfield, MO

Faculty Mentor: Dr. R. Michael Roberts, Biochemistry; Dr. Toshihiko Ezashi, Animal Sciences

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Most efficient BMP4 exposure and concentrations of inhibitors for pluripotent stem cell differentiation to trophoblast

Audrey Wind, Yuchen Tian, R. Michael Roberts, and Toshihiko Ezashi

Diseases of pregnancy, like preeclampsia, are believed to arise from placental trophoblast dysfunction. Human embryonic stem cells (hESCs) can be coaxed to differentiate into placental trophoblast cells resembling those in early pregnancy using BAP protocol. This involves addition of Bone Morphogenetic Protein 4 (BMP4), inhibitors of Activin/Nodal (A83-01), and FGF2 (PD173074) signaling. Our goal was to determine the minimum time hESCs need exposure to BMP4 and optimal concentrations of A83-01 and PD173074 for complete differentiation to trophoblast. To test BMP4 exposure time, BAP medium was replaced with control medium, i.e. minus BMP4, but still containing the inhibitors, at different times during a 7-day culture. The hESC (5×10^5 cells per d35 per well) were cultured under BAP conditions for either 6 h, 12 h, 24 h, or seven days (control). After each pre-determined period of BMP4 exposure, the medium only contained A83-01 and PD173074 (AP), without BMP4. These experiments were run in triplicate, with medium changed daily for seven days. The medium was collected on days 5, 6, and 7 to assess the production of human chorionic gonadotropin (hCG). We also collected photomicrographic images of the cells on these days to compare with control cells. By using the production of hCG and colony morphology as an indication of trophoblast differentiation, it appears that an exposure of 24h is sufficient to prime hESC for progression to syncytiotrophoblast. In on-going experiments, hESCs are cultured with 10 ng/ml BMP4 for 24 h and continued in culture with 1.0 M of A83-01 and adjusted concentrations of PD173074 (0.02 µM, 0.5 µM, and 2.5 µM with the control at 0.1 M). Future experiment will be done with varying concentrations of A83-01 (0.2µM, 5µM, and 25µM) with the PD173074 control at 10µM. In these experiments we expect to define conditions that favor differentiation of particular trophoblast sub-lineages.