Investigating the effects of ACTC1 on cell fusion during early placental development

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Placental samples from early stages in human pregnancy are challenging to obtain; therefore, our laboratory generated a pluripotent human embryonic stem cell (hESC) model to study early placental trophoblast. These hESCs can be differentiated to trophoblast by BAP treatment, which consists of **B**MP4, **A**83-01 (an inhibitor of ACTIVIN), and **P**D173074 (an inhibitor of FGF2). Following this treatment, the mononucleated trophoblast cells differentiate and fuse into multinucleated cells called syncytiotrophoblasts. When treated with BAP, hESCs upregulate genes specific to early trophoblast differentiation, as well as several genes with unknown function in trophoblast, notably *ACTC1*. ACTC1 is an alpha cardiac actin abundantly localized in cardiac and skeletal muscle, but the role of ACTC1 in placental trophoblast is unknown. The goal of the present study is to generate hESC lines that lack ACTC1 in order to determine whether it is necessary for trophoblast differentiation. Our lab used CRISPR/Cas9 technology to knock down *ACTC1* in our hESC line, and we obtained hESCs where *ACTC1* was completely eliminated. Western blot analysis indicates that ACTC1 protein expression increases from day 4 to 8 of BAP differentiation in control hESCs but a complete absence of ACTC1 protein in two clones of the knockout cell lines. Crystal violet staining suggests differences in colony morphology and size between control cells and both knockdown and knockout cell lines. Ongoing experiments to analyze differentiation in these cell lines include quantification of cell colony diameter, syncytialized areas, and syncytiotrophoblast-specific hormone production.