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

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Abstract

- 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 BMP4, A83-01 (an inhibitor of ACTIVIN), and PD173074 (an inhibitor of FGF2) .
- Following this treatment, the mononucleated trophoblast cells differentiate and fuse into multinucleated cells called syncytiotrophoblasts that upregulate genes specific to early trophoblast differentiation, as well as several genes with unknown function in trophoblast, notably *ACTC1*.
- ACTC1 is a cardiac actin abundantly localized in cardiac and skeletal muscle, but its role in 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.

Experimental Design

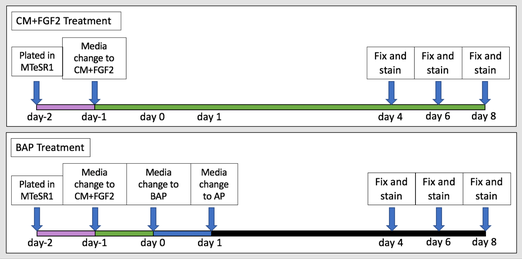
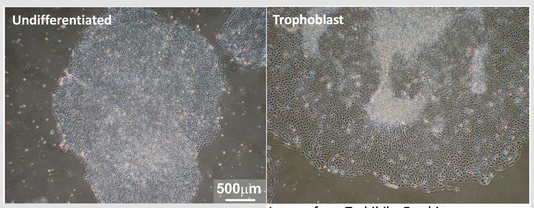


Figure 1: BAP treatment drives hESCs toward differentiated trophoblast. BMP4 (10 ng/mL), A83-01 (1 µM), PD173074 (1 µM). CM+FGF2 treatment maintains hESC in an undifferentiated state. FGF2 (4 ng/mL). Cells are cultured in 20% oxygen.



Images from Toshihiko Ezashi

Figure 2: Left shows hESC maintained in an undifferentiated state. Right shows hESC differentiated by BAP treatment into trophoblast. The dense, lighter colored areas are multinucleated masses, known as syncytiotrophoblast or syncytium.

Methodology

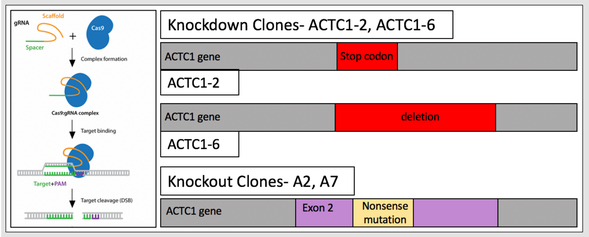


Figure 3a: CRISPR/Cas9 technology.
Figure 3b: ACTC1-2 and ACTC1-6 are monoallelic deletions. In clones A2 and A7, the nonsense mutation in exon 2 caused the clones to produce non-functional ACTC1 protein. KO clones were generated by WashU Genome Engineering and iPSC Center.

Results

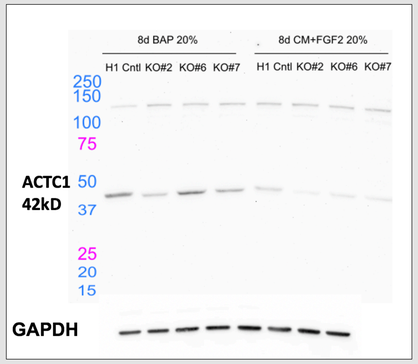


Figure 4: Western Blot analysis of ACTC1 knockdown. ACTC1-2 and ACTC1-7 clones have decreased ACTC1 protein expression.
(Karvas, unpublished)

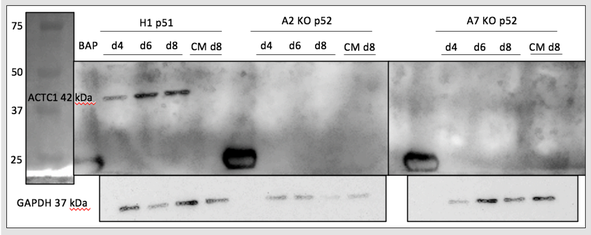


Figure 5: Western Blot analysis of ACTC1 knockout. KO clones A2 and A7 have complete absence of ACTC1. ACTC1 protein expression increases from day 4 to day 6 of BAP treatment in control H1 hESCs. ACTC1 protein is absent from the CM+FGF2 undifferentiated control treatment in all cell lines.

Results

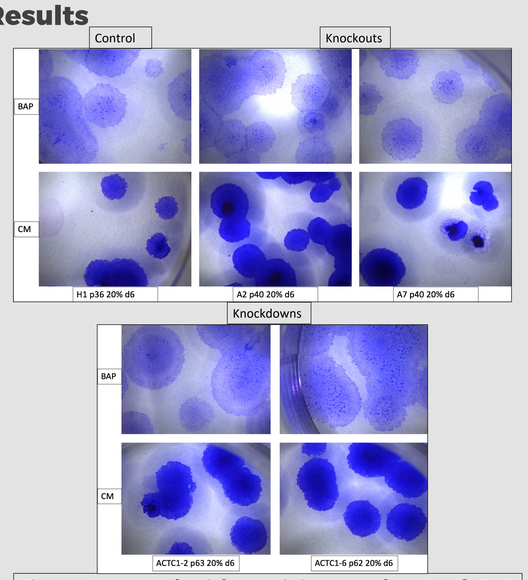


Figure 6: Crystal violet staining on day 6 of BAP treatment. Knockdown cell lines appear to have larger, more spread-out colonies.

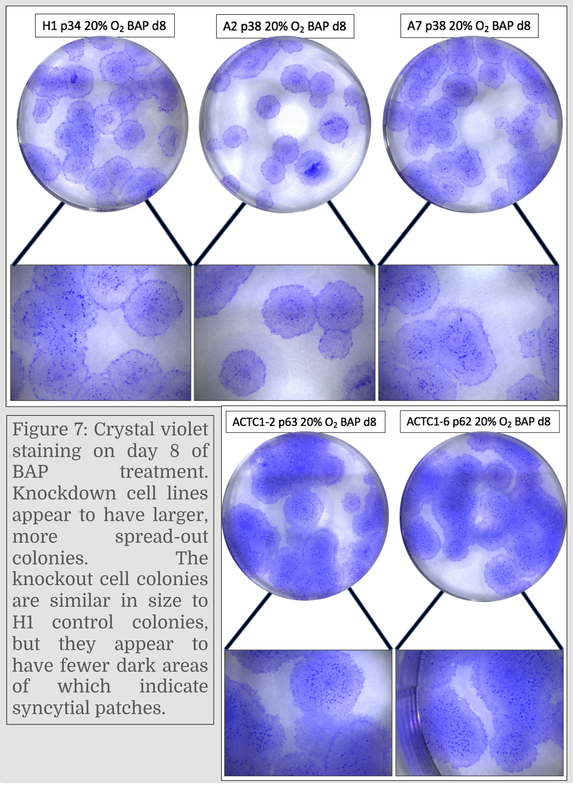


Figure 7: Crystal violet staining on day 8 of BAP treatment. Knockdown cell lines appear to have larger, more spread-out colonies. The knockout cell colonies are similar in size to H1 control colonies, but they appear to have fewer dark areas of which indicate syncytial patches.

Results

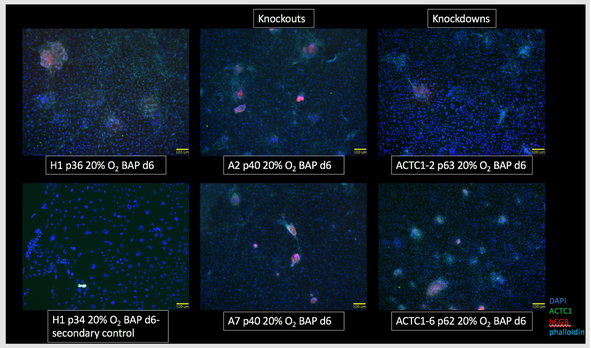


Figure 8: Immunofluorescence staining of ACTC1, hCG, phalloidin, and DAPI. Secondary control slide where no primary antibodies were added is completely negative.

Summary

- Western blot analysis indicates that ACTC1 protein expression increases from day 4 to 6 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.
- Preliminary immunocytochemistry suggests differences in size of syncytial areas, but not syncytiotrophoblast-specific hormone production.

Future Directions

- Perform quantification of cell colony diameter by analyzing stained crystal violet plates.
- Continue immunocytochemistry staining to quantify syncytialized area and syncytiotrophoblast-specific hormone production. Compare between cell lines.
- Conduct an hCG ELISA to quantify hCG production.

Acknowledgements

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