Examination of the Role of MAPK Activity on Placental Formation in Vitro Using Stem Cells

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Introduction

Mitogen-activated protein kinase kinase kinase 4 (MAP3K4) has been demonstrated to promote fetal and placental growth by regulating the insulin-like growth factor 1 receptor (IGF1R), insulin receptor (IR), and Akt signaling pathway [1]. MAP3K4 kinase inactivation (KI) reduces IGF1R/IR expression and activity as well as Akt activation, resulting in reduced fetal and placental growth [1]. MAP3K4 KI also leads to fetal skeletal defects [2]. The labyrinth placental layer enables gas, nutrient, and waste exchange between the maternal and fetal blood [3]. The placenta is formed by differentiation of trophoblast stem (TS) cells, and in vitro differentiation of these cells by removal of factors that promote the stem cell state leads to formation of the trophoblasts found in the labyrinth [1]. MAP3K4 kinase activation also promotes the activation of other proteins, including the p38 mitogen-activated protein kinase (MAPK) signaling pathway, in TS cells [4]. However, the role of MAP3K4 kinase activity in regulation of the p38 signaling pathway in differentiated trophoblasts is unknown. The goals of this project are to (1) define the role of MAP3K4 kinase activation in control of the p38 pathway in labyrinth trophoblasts and (2) determine the impact of TS cell differentiation to labyrinth trophoblasts on the activation of this pathway.

Materials & Methods

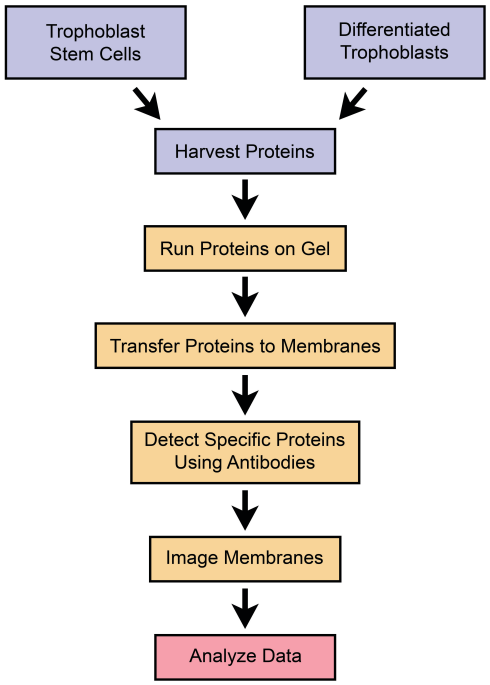

Fig. 1. Diagram of methods used to measure the p38 pathway in WT and KI TS cells and differentiated trophoblasts.

Results

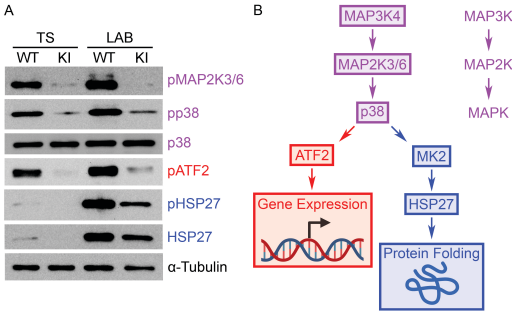

Fig. 2. (A) Images show Western blots from 3 biologically independent experiments performed on protein harvested from WT and KI TS cells and labyrinth (LAB) trophoblasts to measure p38 pathway activation. (B) Activation of the p38 pathway promotes gene expression and proper protein folding. Images were created using biorender.com.

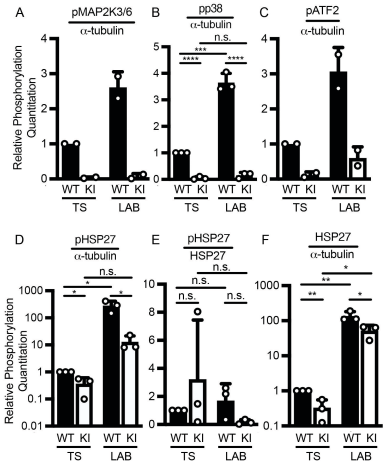

Fig. 3. Densitometry and statistical results from blots shown to the left. *,**,***,****; $p < 0.05$

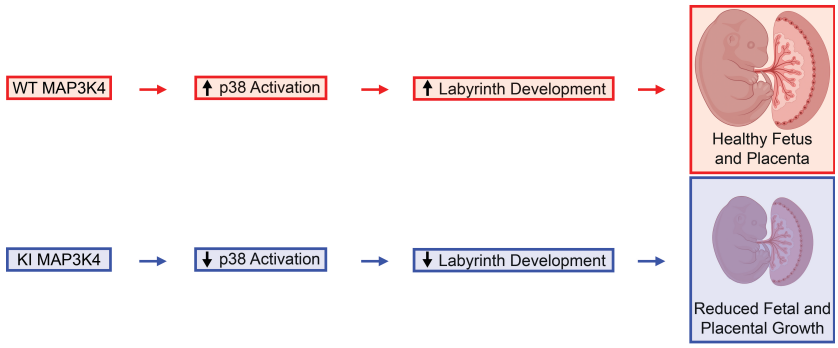

Fig. 4. Impact of KI mutation on fetal and placental growth. Images were created using biorender.com.

Conclusions

- The p38 pathway is induced upon trophoblast differentiation to the labyrinth, indicating a potential role for this pathway in the formation of these cells.
- MAP3K4 KI disrupts p38 signaling pathway activity in both TS and labyrinth cells, suggesting this pathway is MAP3K4-dependent in these cells.
- MAP3K4 KI may inhibit the formation of the labyrinth placental layer.
- Future work will include (1) assessing the activation of the p38 MAPK pathway in vivo during placental development and (2) comparing the effects of MAP3K4 activity on the activation of this pathway in placental WT and KI cells.

References

[1] Perry, C. et al. (2022). "MAP3K4 promotes fetal and placental growth by controlling the receptor tyrosine kinases IGF1R/IR and Akt signaling pathway." *J Biol Chem* **298**(9): 102310.
[2] Abell, A. et al. (2005). "Ablation of MEKK4 kinase activity causes neurulation and skeletal patterning defects in the mouse embryo." *Mol Cell Biol* **25**(20): 8948-8959.
[3] Marsh, B. and R. Blelloch (2020). "Single nuclei RNA-seq of mouse placental labyrinth development." *Elife* **9**.
[4] Shendy, N. and Abell, A. (2021). "MAP Kinase Cascades." *Encyclopedia of Molecular Pharmacology*.

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