The Increased Proliferation Rate of Kinase-Inactive MAP3K4 Trophoblast Stem Cells Is Associated with a Shortened G1 Phase

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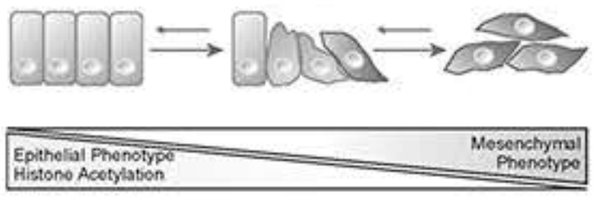
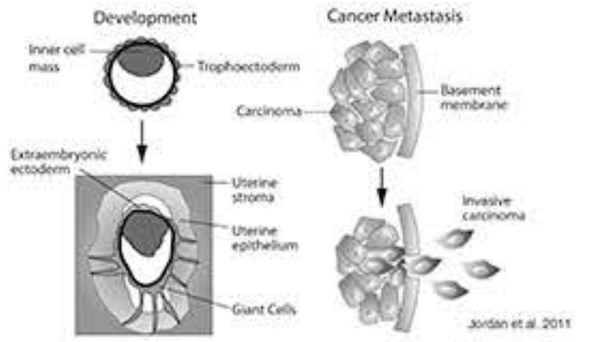
Introduction

Epithelial to mesenchymal transition (EMT) is a process where epithelial cells with tight cell-to-cell adhesion change phenotypically to become mesenchymal and invasive. EMT occurs normally during embryonic development and wound healing, and it is reactivated in organ fibrosis and cancer metastasis[1].



Trophoblast stem (TS) cells provide a model system for studying EMT. Wild-type trophoblast stem (TS^{WT}) cells display an epithelial phenotype and form tight, round colonies[2]. Trophoblast stem cells with MAP3K4 kinase-inactivation (TS^{KI4}) have mesenchymal properties with increased invasiveness and loss of some epithelial markers[2]. Importantly, TS^{KI4} cells share changes in gene expression with claudin-low breast cancer cells[2]. Our preliminary data show that the average time per cell division of TS^{KI4} cells is lower than TS^{WT} cells, indicating that TS^{KI4} cells have a higher proliferation rate.

Our hypothesis is that Ki-67 protein expression is altered in TS^{KI4} cells. Ki-67 is an established marker of cell proliferation and cell cycle[3]. Cells in G0 do not express Ki-67 while cells in stages G1 through M express high levels of Ki-67[3]. Importantly, cells in each stage of the cell cycle display distinct Ki-67 distribution[3].



Methods

- TS^{WT} and TS^{KI4} cells were cultured.
- Phase contrast microscopy
- Immunofluorescence staining was performed to examine Ki-67 protein expression and localization.
- Automated counting of Ki-67 positive cells was performed using Gen5 software.

Conclusions and future studies

- Mesenchymal TS^{KI4} cells have a shortened cell division time.
- Ki-67 expression is not significantly different in TS^{KI4} cells compared to TS^{WT} cells.
- Cell cycle dependent Ki-67 patterning is altered in TS^{KI4} cells compared to TS^{WT} cells.
- Higher cell division rates in TS^{KI4} cells is associated with a shortened G1 phase.
- These data support previous work that JNK activity inhibits G1/S transition and promotes G2/M transition.
- Future studies will use fluorescence-activated cell sorting to assess cell cycle fractions in TS^{WT} and TS^{KI4} cells.

References

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3. Braun, Norbert, Thomas Papadopoulos, and Hans Konrad Müller-Hermelink. Cell cycle dependent distribution of the proliferation-associated Ki-67 antigen in human embryonic lung cells. Virchows Archiv B 56.1 1988: 25-33.
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Results

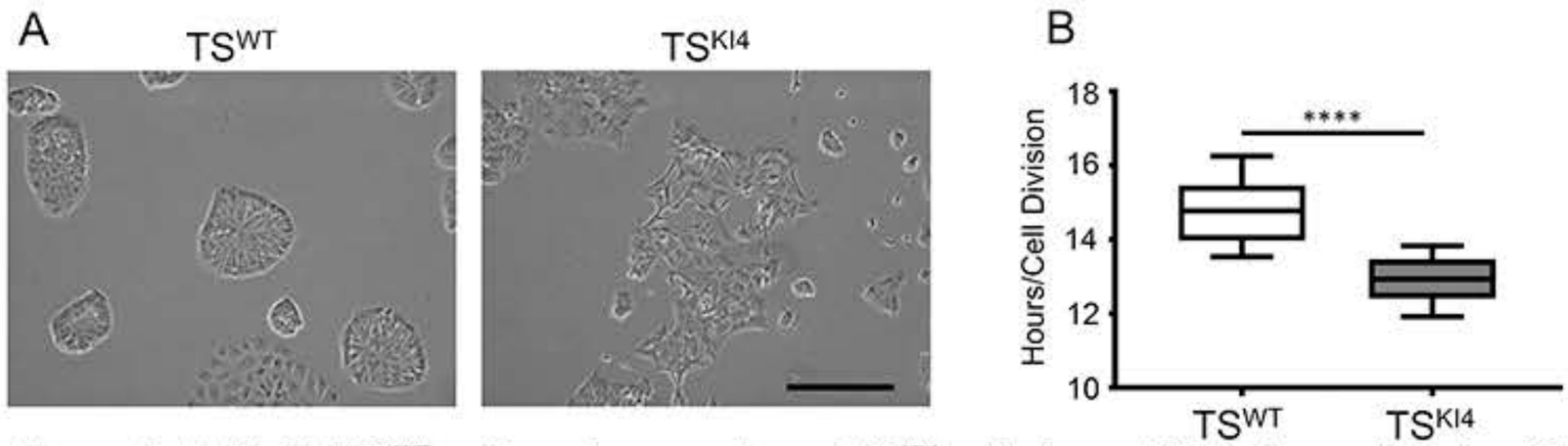

Figure 1: Epithelial TS^{WT} cells and mesenchymal TS^{KI4} cells have different growth rates. (A) Phase contrast microscopy was used to examine morphology of TS^{WT} and TS^{KI4} cells. **(B)** Cells division rates were calculated based on cells set and cells harvested after 96 hours of culture (n=11). ****p<0.0001. Bar represents 300 µm.

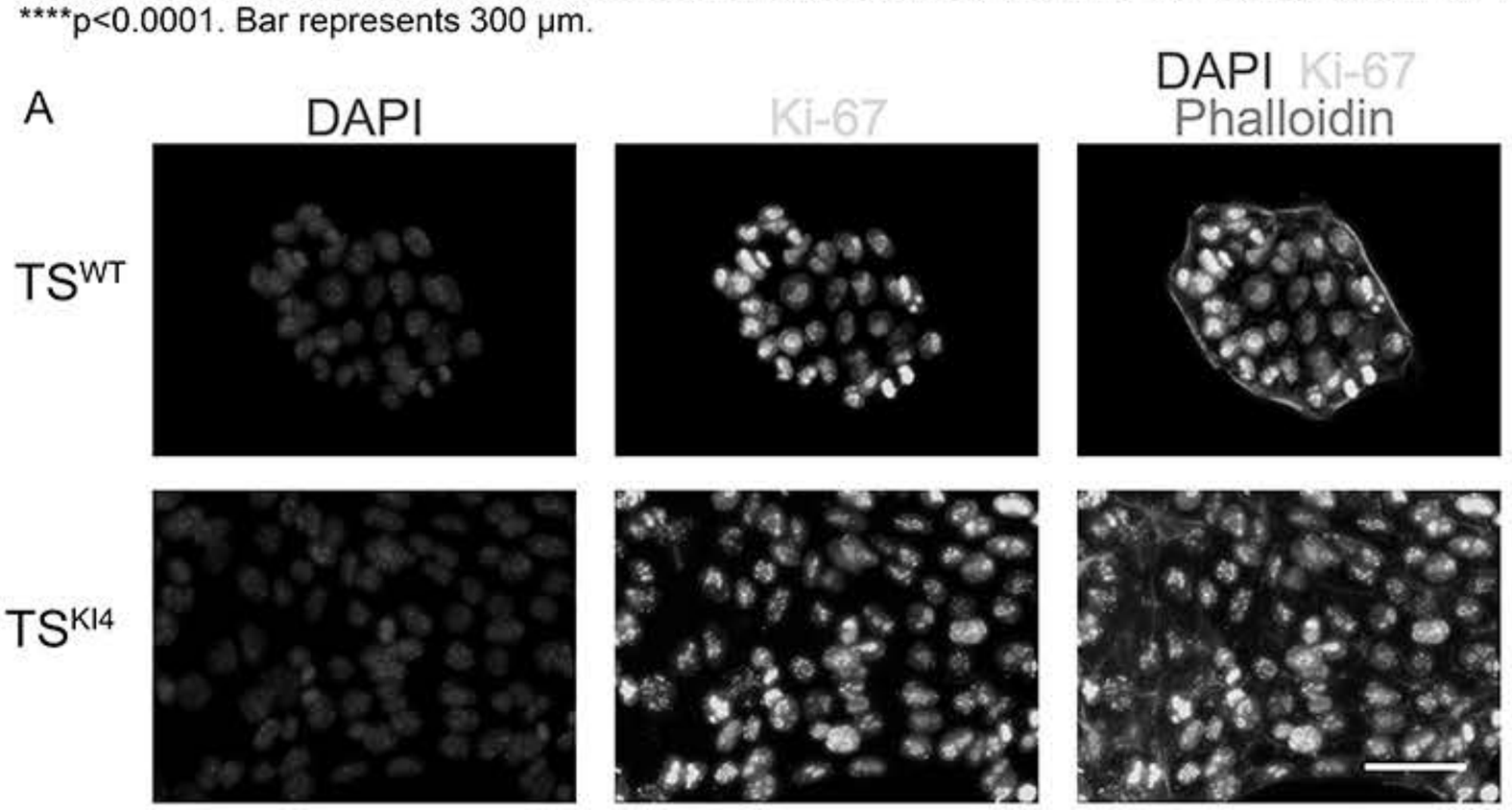

Figure 2: TS^{WT} and TS^{KI4} cell populations express similar levels of Ki-67. (A) Immunofluorescence was used to examine Ki-67 expression and localization in TS^{WT} and TS^{KI4} cells (DAPI nuclear staining in blue, Phalloidin actin staining in red). **(B)** Automated cell counting was used to determine percentage of $Ki\text{-}67^+$ cells in TS^{WT} and TS^{KI4} cell images. Bar represents 50 µm.

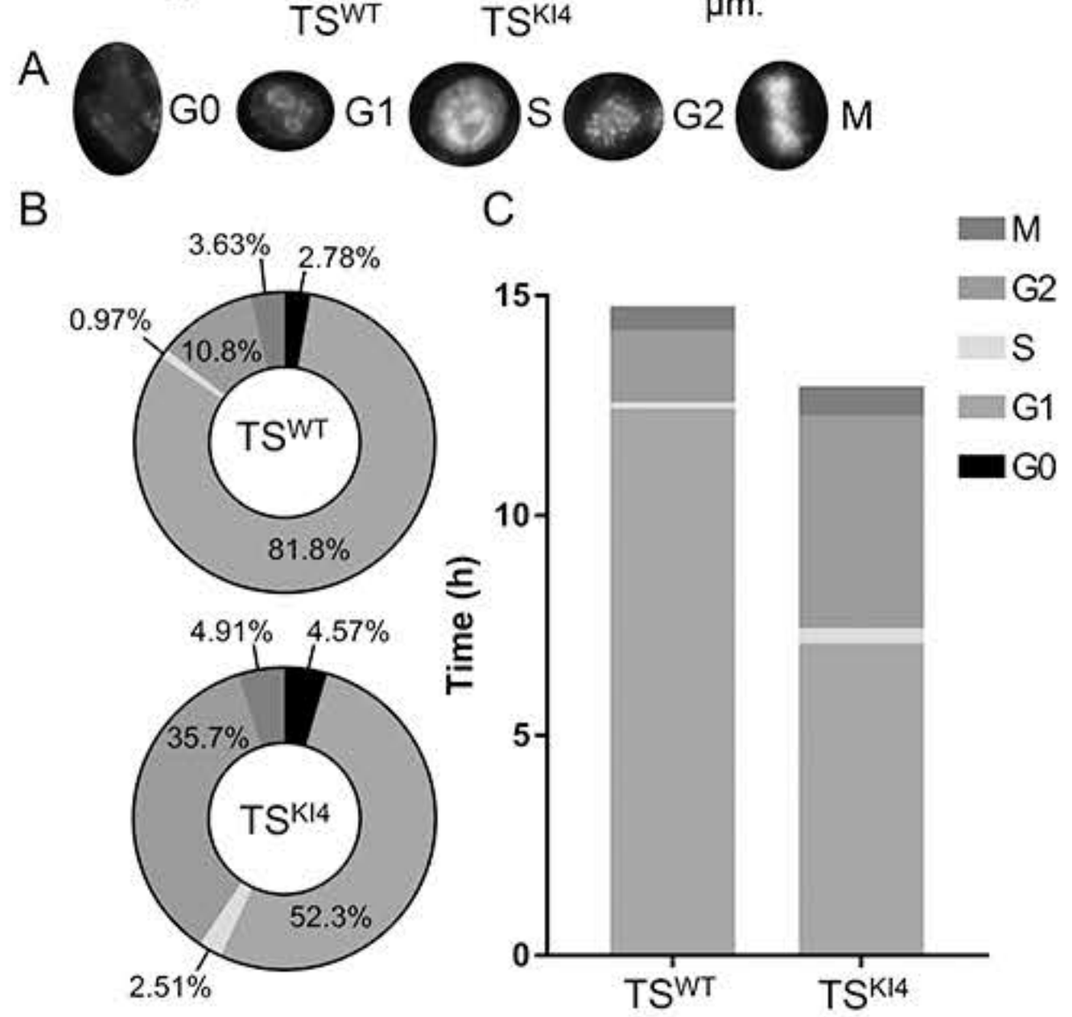

Figure 3: TS^{KI4} cells have a shortened G1 phase relative to TS^{WT} cells.
(A) Representative cell cycle dependent Ki-67 staining shown using immunofluorescence staining as in Figure 2A. **(B)** Quantification of cell cycle stages from immunofluorescence images of TS^{WT} and TS^{KI4} cell Ki-67 distribution. **(C)** Cell cycle stages normalized to cell division rates of TS^{WT} and TS^{KI4} cells.

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