Signal integration at the exit from ES cell self-renewal

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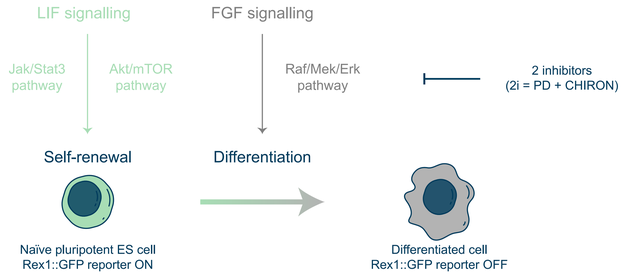
Introduction

The naïve pluripotent state of mouse embryonic stem cells (mESCs) is maintained by a precise balance of key set of signalling pathways. The Jak/Stat3 as well as the PI3K/Akt/mTOR pathway, both activated by LIF (Leukemia Inhibitor Factor), are essential to sustain a network of pluripotency-associated transcription factors. Although the Jak/Stat3 pathway has been intensively studied, little attention has been focused on the PI3K/Akt/mTOR pathway.

In order to exit the pluripotent state and enter differentiation, the network of transcription factors needs to be dismantled. Notably, molecular events acting in this context are still poorly understood. Several genome-wide loss-of-function screens have been performed by my lab and by others to shed light on this early differentiation process. Interestingly, among high confidence hits were found two negative regulators of the PI3K/Akt/mTOR pathway, Pten and Tsc2. A function for those factors in regulating the exit from naïve pluripotency has been already reported, although molecular mechanisms underlying their action have not been elucidated yet.

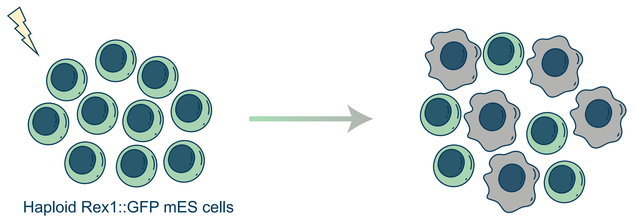
Taking advantage of knock-out (KO) ES cell lines, we observed that cells lacking Pten or Tsc2 display a differentiation defect. Intriguingly, Tsc2 KO cells show a stronger phenotype compared to Pten KO cells. Consistently, Tsc2 absence determines a stronger impact on ES cell transcriptome. By performing cellular, molecular and biochemical assays, as well as genetic screens, we aim to dissect the actual mechanistic role of Pten and Tsc2 in regulating the exit from naïve pluripotency. This will provide a better understanding of molecular events orchestrating the early differentiation process of mESCs.

1 | Major signalling pathways in mouse ES cells



2 | Genetic screen to identify key players for the exit from naive pluripotency

Gene trap mutagenesis



- Components of the FGF/ERK pathway
- Negative regulators of the PI3K/Akt pathway

Leeb et al., Cell Stem Cell 2014

Hypothesis:

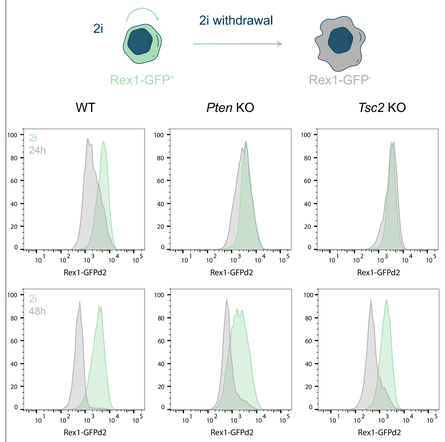
The modulation of the PI3K/Akt pathway enables ES cells to properly initiate differentiation

Aims:

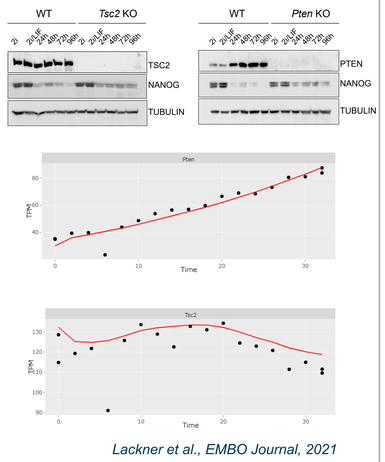
1. Dissect how the PI3K/Akt pathway coordinates the exit from naïve pluripotency
2. Identify key players of the PI3K/Akt pathway-dependent regulation of the exit from naïve pluripotency

3 | *Pten* and *Tsc2* regulate ES cell exit from naïve pluripotency

A *Pten* KO AND *Tsc2* KO ES CELLS SHOW A DELAY IN DIFFERENTIATION



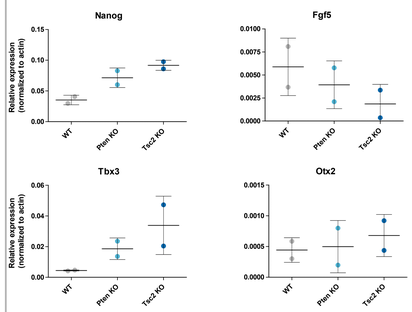
B PTEN EXPRESSION INCREASES DURING ES CELL DIFFERENTIATION



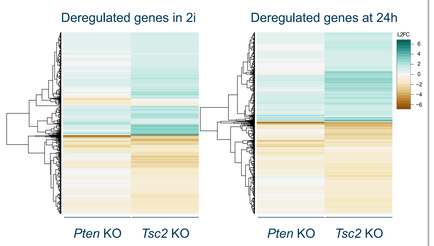
Lackner et al., EMBO Journal, 2021

Naïve markers at 24h

Early differentiation markers at 24h

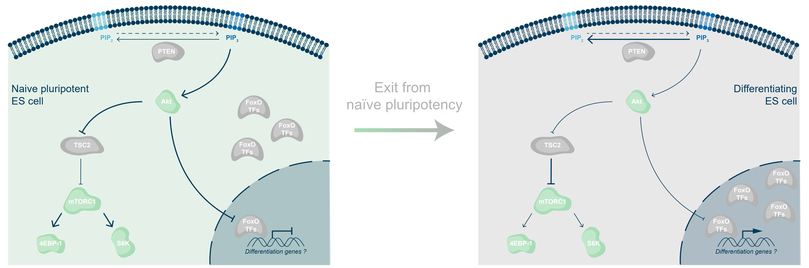


C LOSS OF *Pten* OR *Tsc2* SIMILARLY AFFETCS ES CELL TRANSCRIPTOME



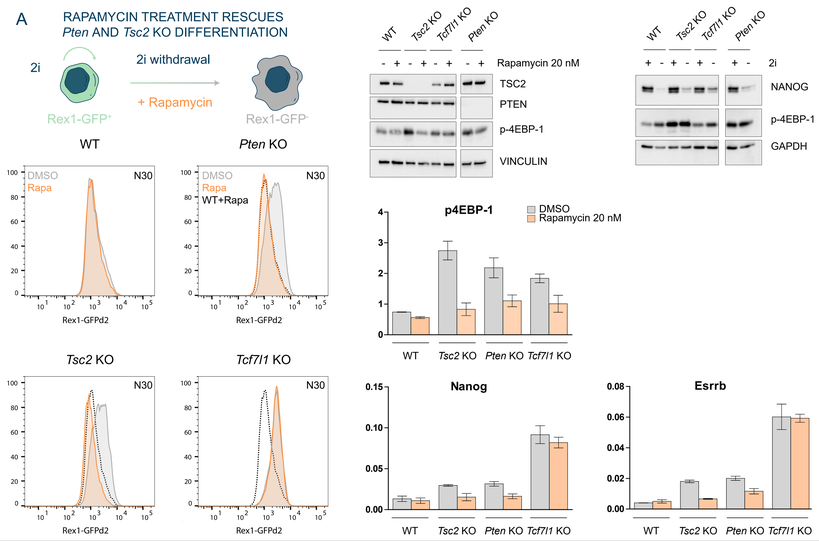
Lackner et al., EMBO Journal, 2021

Graphic Summary



4 | PTEN signalling is (partially) integrated through mTORC1

A RAPAMYCIN TREATMENT RESCUES *Pten* AND *Tsc2* KO DIFFERENTIATION



Conclusions

1. The modulation of the PI3K/Akt/mTOR pathway enables ES cells to properly initiate differentiation.
2. TSC2 regulate ES cell differentiation by acting through the mTORC1 pathway.
3. PTEN signal is also integrated into the mTORC1 pathway.

Future perspectives

1. Evaluate the potential involvement of additional signalling cascades (GSK3 pathway, FoxO pathway) downstream PTEN.
2. Dissect the specific contribution of each cascade through which PTEN and TSC2 act to coordinate ES cell differentiation.
3. Identify key downstream targets of the PI3K/Akt/mTOR pathway involved in the regulation of ES cells early differentiation process.
4. Obtain a clear and detailed picture on how Pten and Tsc2 coordinate the exit from naïve pluripotency at the molecular level.