PP2A recruits TRIM28 on the chromatin leading to transcriptional repression

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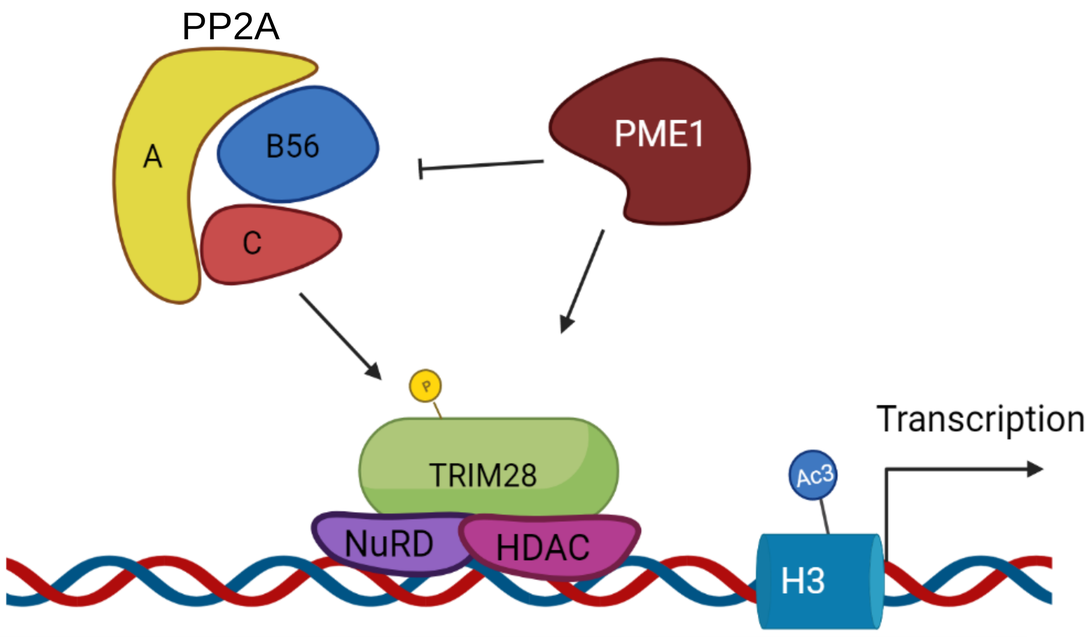
MOLECULAR CELL BIOLOGY

Introduction

TRIM28 is a transcriptional repressor that mediates its activity through the recruitment of NuRD complex proteins. This leads to transcriptional repression through the post translational modification of histone tails such as a decrease in H3K9 and/or H3K14 acetylation. PME1 is an inhibitor of the tumour suppressor PP2A. 5 (five) phosphomutants of TRIM28 were created based on phosphosites regulated by PP2A. These mutants' properties may shine light to how cancer operates.

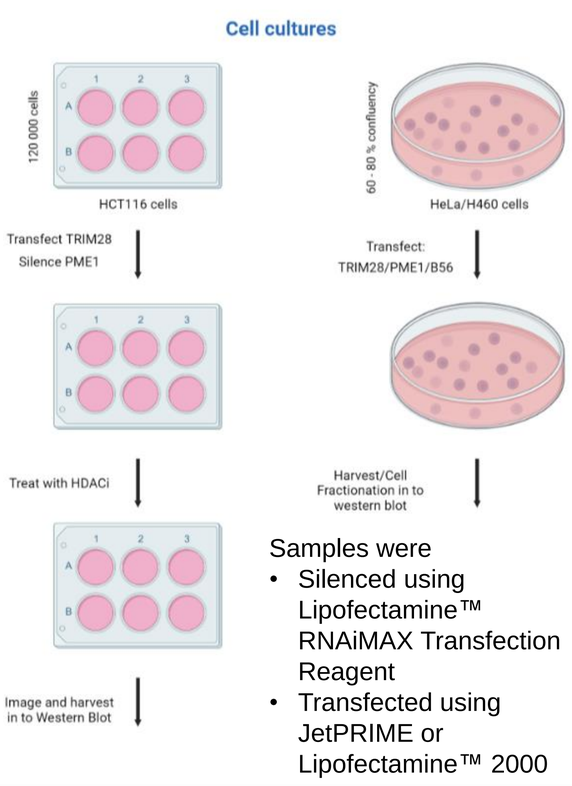


Aims

Study the regulation of TRIM28 by PP2A:

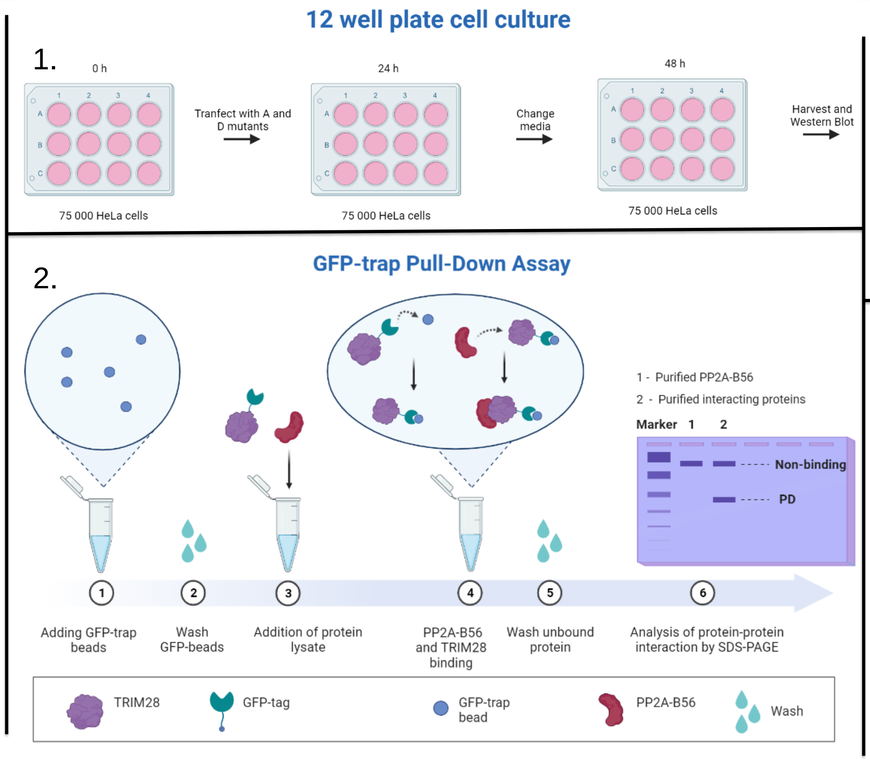
1. PP2A regulated phosphosites on TRIM28
2. Interaction of PP2A and TRIM28 with Pull-Down
3. Image analysis of overexpressing TRIM28 and silencing PME1
4. Phosphosites role in chromatin recruitment of TRIM28 using Cell Fractionation

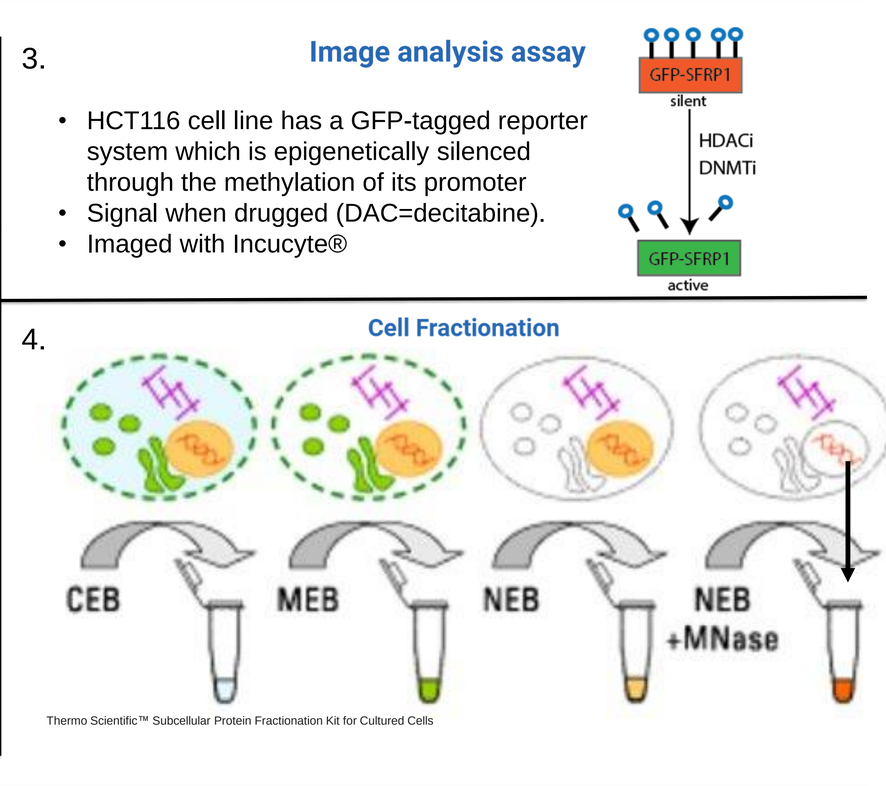
Materials and methods



Samples were

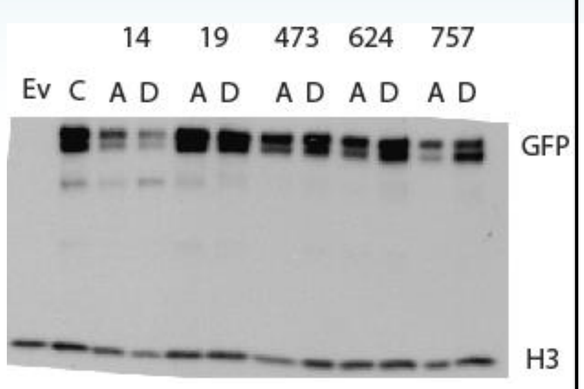
- Silenced using Lipofectamine™ RNAiMAX Transfection Reagent
- Transfected using JetPRIME or Lipofectamine™ 2000





Results and discussion

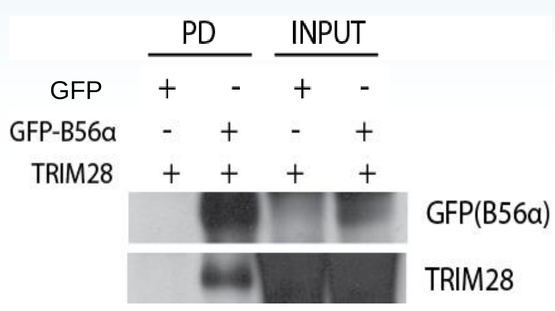
1. Differences between TRIM28 phosphomutants



› A-mutants mimic dephosphorylation, D-mutants rescue, opposite result on phosphosite 19

› Can be blotted for H3K9ac/ H3K14ac mark

2. TRIM28 is interacting with PP2A-B56

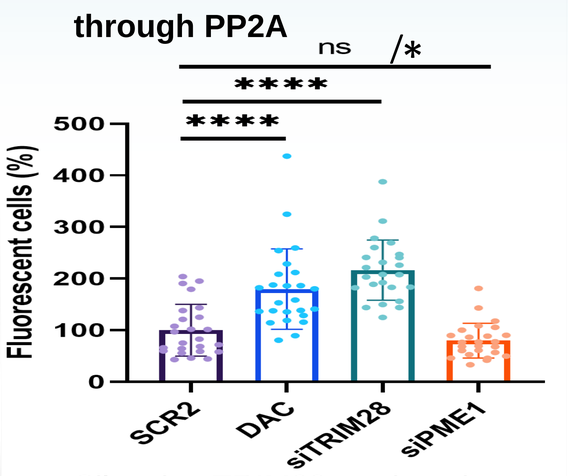


› Band when both TRIM28 and B56 are present, none when either is missing

› B56 was able to Pull-Down TRIM28

› Interaction between PME1 and TRIM28 couldn't be found

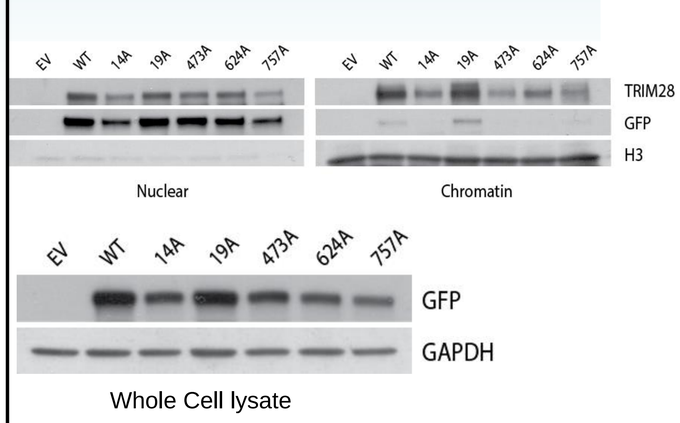
3. TRIM28 is a gene repressor, PME1 regulates TRIM28 through PP2A



› Silencing TRIM28 produced more fluorescence than adding DNMT1i

› Silencing PME1 produced non-significant results, using t-test small drop in fluorescence

4. All phosphomutants are binding to the chromatin



› 19A was binding more than TRIM28 WT, others less

Preliminary data suggests that:

- ***TRIM28 is regulated by PP2A(-B56) on the chromatin***
- ***PME1 regulates TRIM28 through PP2A inhibition***
- ***TRIM28 is a contributor of gene regulation***

References

- Pokharel, Y., Saarela, J., et al. Relevance Rank Platform (RRP) for Functional Filtering of High Content Protein–Protein Interaction Data. MCP **14**, 12 (2015)
- Thermofisher™, products: 78840, 11668019, and 13778030
- Chromotek™, GFP-Trap agarose

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