Unraveling the Interplay between miR-146a, TP53, and Inflammation in DNMT3A CHIP Pathogenesis

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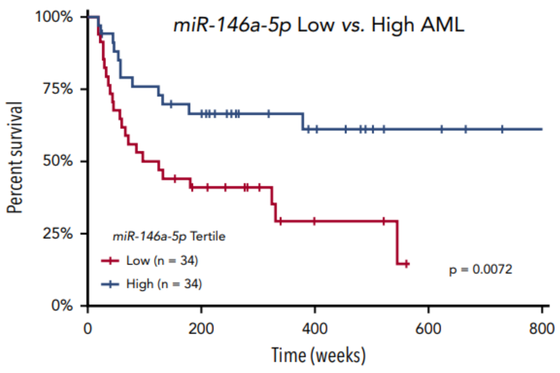
Introduction

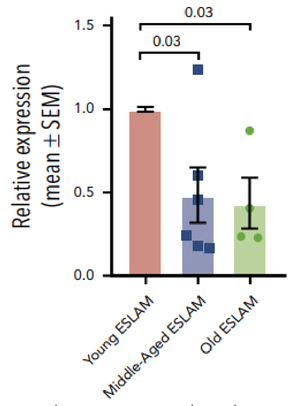
In our previous work*, we show that downregulation of miRNA, ***miR-146a*** **is involved in:**

1. Poor Prognosis in AML patients



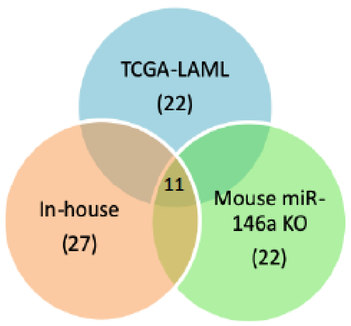
AML patients with lower miR-146a levels show poorer prognosis (PMP)

2. Ageing



Aged HSCs in mice show low miR-146a expression

3. Inflammation and *TP53* pathway activity



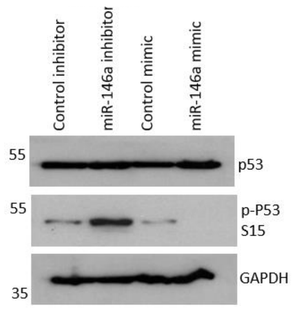
Common Pathways	Average Rank Score
TNFA_SIGNALING_VIA_NFKB	0.223401
INFLAMMATORY_RESPONSE	0.355892
COMPLEMENT	0.60202
INTERFERON_GAMMA_RESPONSE	0.661785
UV_RESPONSE_UP	1.067003
HYPOXIA	1.138552
ALLOGRAFT_REJECTION	1.166498
P53_PATHWAY	1.491751
KRAS_SIGNALING_UP	1.504209
COAGULATION	1.513131
IL2_STAT5_SIGNALING	2.121212

Inflammation and TP53 pathway gene-sets are upregulated in low miR-146a patients and miR-146a KO mice

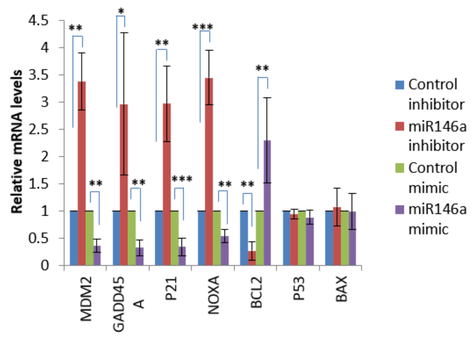
* J.Grants et.al 2020

Results

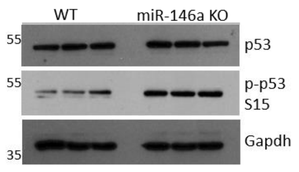
1. *miR-146a* targets *TP53* activity by regulating S-15 phosphorylation



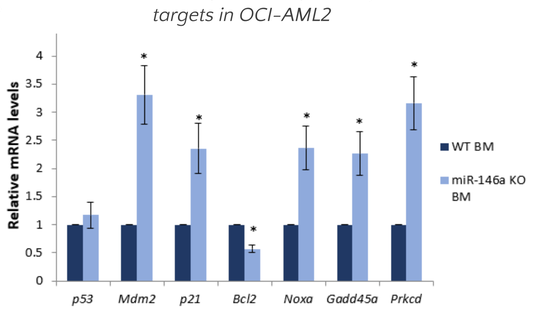
miR-146a impacts P53-S15 phosphorylation in AML cell line OCI-AML2



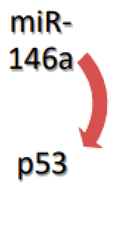
miR-146a impacts P53 pathway targets in OCI-AML2



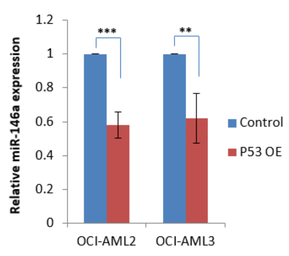
miR-146a KO mice have higher pS15



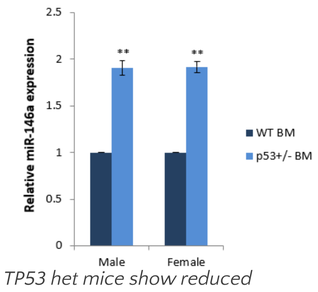
miR-146a KO mice have higher P53 pathway target gene expression



2. *TP53* inhibits *miR-146a* expression, forming a positive feedback loop

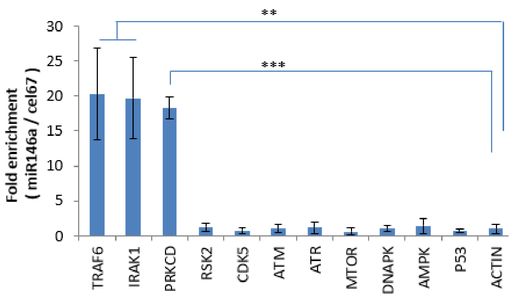


TP53 OE in AML cell lines, inhibits miR-146a expression

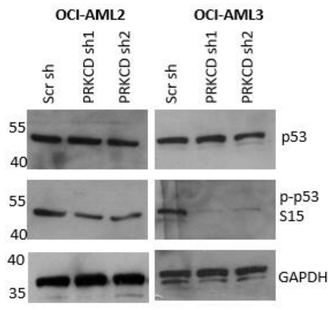


TP53 het mice show reduced expression of miR-146a

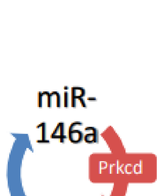
3. *miR-146a* targets P53 Kinase, *PRKCD*, thereby inhibiting *TP53* activity



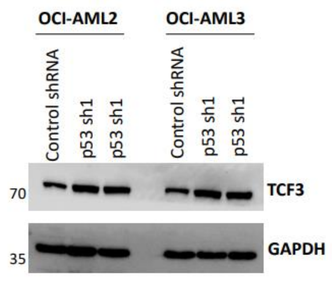
miR-146a ChIP qPCR analysis of putative TP53 kinases reveal PRKCD binding



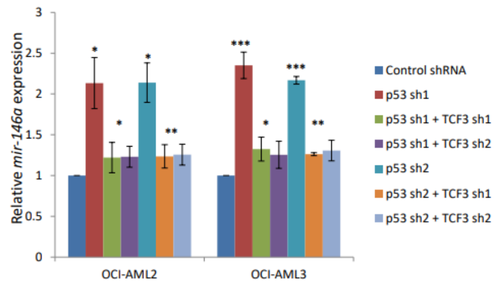
PRKCD inhibition in AML cell lines causes reduced TP53 S-15 phosphorylation



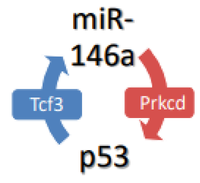
3. *TP53* downregulates *miR-146a* expression by suppressing *miR-146a* Transcription Factor, *TCF3/E2A*



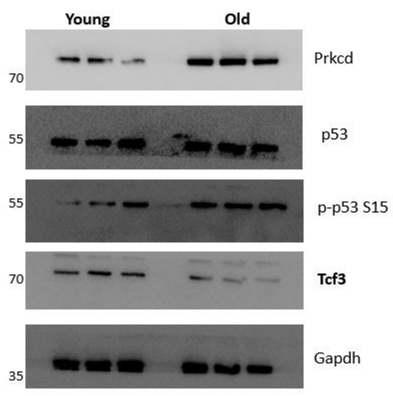
TP53 inhibition in AML cell lines causes lower expression of TCF3



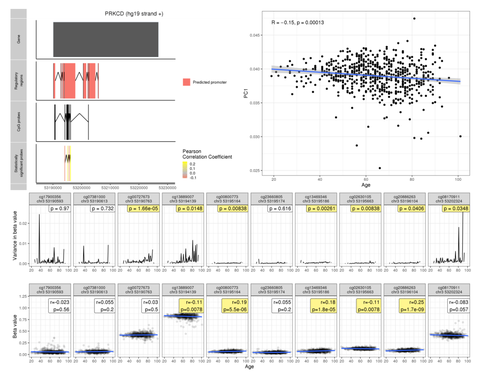
Double KD models in cell lines show that P53 targets miR-146a expression via TCF3



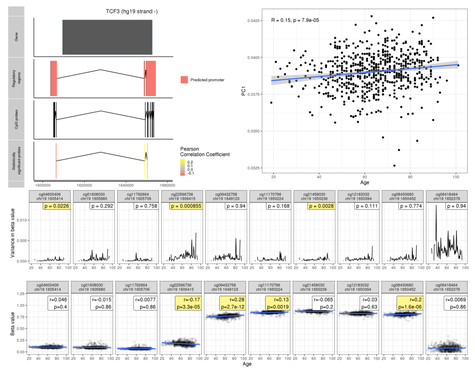
4. Ageing-related methylation changes likely triggers the activation of this self-perpetuating loop



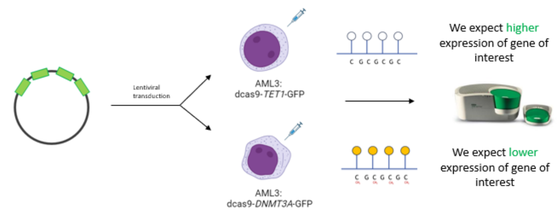
WT aged mice show activation of loop



PRKCD shows age-associated promoter-hypomethylation in a healthy cohort (n=649)

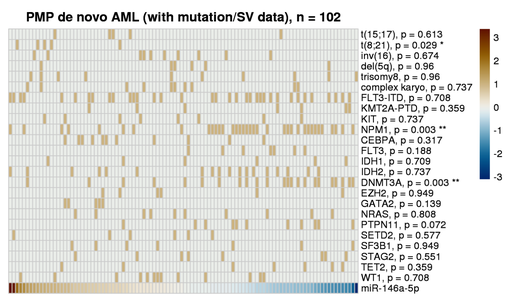


TCF3 shows age-associated promoter-hypermethylation in a healthy cohort (n=649)

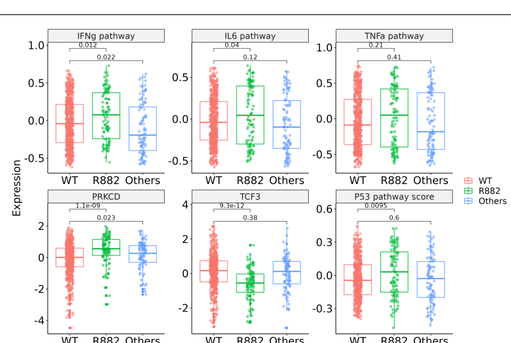


Work in Progress Pipeline: *We are currently using sgRNA mediated targeted methylation to probe the impact of promoter methylation of PRKCD and TCF3 on their expression. The sgRNAs were designed based on above figures.*

5. The positive feedback loop shows association with *DNMT3A*-mutated AML, particularly for the CHIP associated R882 variant



Low miR-146a associates with DNMT3A mutated AML and commonly co-occurring mutation, NPM1

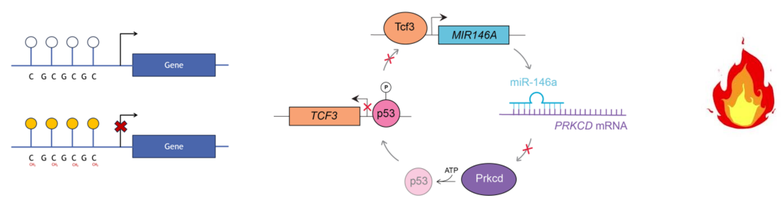


R882 bearing patients show loop activity and inflammation, in contrast to other DNMT3A mutations (n = 762 AML patients)

Conclusion

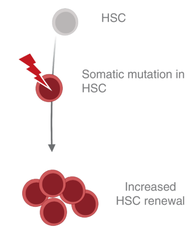
Our current data validates the following sequence of events:

Ageing-related methylation changes → Low *miR-146a* High *TP53* loop → Inflammation → *DNMT3A* CHIP



Classically, TP53 cancers have been associated with the loss of function of the protein. Here we demonstrate a model where high TP53 activity promotes oncogenesis.

Future Studies



Previous studies show pre-existing inflammation promotes *DNMT3A* CHIP.

We hypothesize high *TP53* activity associated inflammation will promote *DNMT3A* CHIP.

Acknowledgements