Locus specific proteomics identify potential regulators of the *MYC* super enhancer in NOTCH-driven T-ALL

EXPERIMENTAL HEMATOLOGY / ONCOLOGY

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OBJECTIVES

T-ALL is an aggressive hematologic malignancy resulting from the transformation of immature T cell progenitor cells. Activating mutations in *NOTCH1* are found in over 60% of human T-ALLs and in about 80% of paediatric T-ALL cases. NOTCH1 is a transmembrane receptor that, once activated by the neighbouring cell membrane bound ligand, undergoes conformational changes and two successive proteolytic cleavages, resulting in the release of the intracellular domain (NICD). NICD subsequently translocates into nucleus, binds to the transcription factor RBPJ and regulates transcription of its target genes.

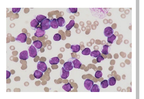
In the context of T-ALL, NOTCH regulates the expression of *MYC* through a downstream super-enhancer region, which is an essential hub for transmitting growth promoting signals for disease progression.

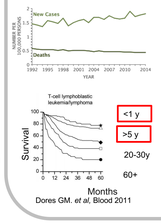
The complete composition of the NOTCH transcription complex (NTC) is still unknown. Moreover, it is currently unknown if the NOTCH regulated transcription factor compositions differ between disease driving super-enhancers and canonical promoter regulated Notch target genes e.g. *HES1 and pTa*.

The aim of this project is to identify, in a locus specific manner, the proteins regulating NICD dependent promoters and (super) enhancers.

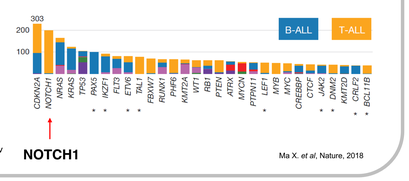
T - Acute Lymphoblastic Leukaemina (T-ALL)

- Acute lymphoblastic leukaemia (ALL) is the most common childhood cancer
- T-ALL is a malignant disorder that originates in T lymphocyte progenitors

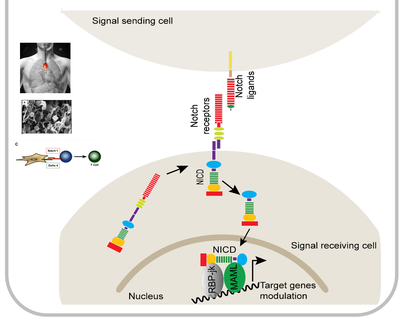




NOTCH1 is the Most Frequently Mutated Gene in Pediatric T-ALL

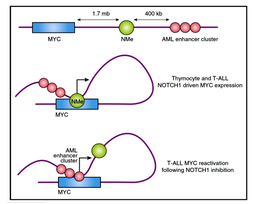


Persistent Notch Signalling Promotes T-ALL



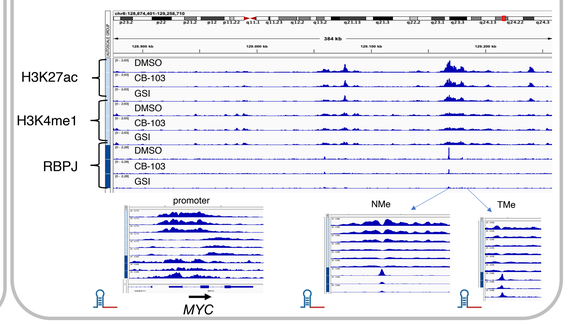
MYC is the main NOTCH1 direct target promoting T-ALL

Long-range enhancer activity determines *MYC* sensitivity to NOTCH inhibitors in T-ALL

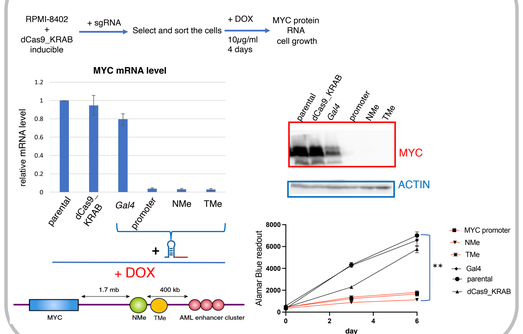


E4946–E4953 | PNAS | Published online November 4, 2014

MYC locus landscape in RPMI-8402 cells upon NOTCH inhibition: gRNA design



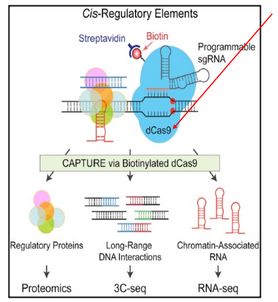
NMe and TMe are essentiel for *MYC* expression



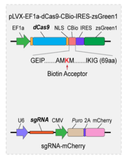
METHODS

The method of choice is based on the CRISPR technology and is termed CAPTURE (**C**RISPR **A**ffinity **P**urification *In Si**tu*** of **R**egulatory **E**lements). Briefly, sgRNA design ensures the specificity of the chromatin purification while overexpressed Cas9 protein enables its purification. Namely, Cas9 is engineered to be enzymatically inactive and to be *in vivo* biotinylated. Hence, the chromatin region of interest is purified by Streptavidine affinity chromatography and the DNA bound proteins are identified by LC-MS/MS.

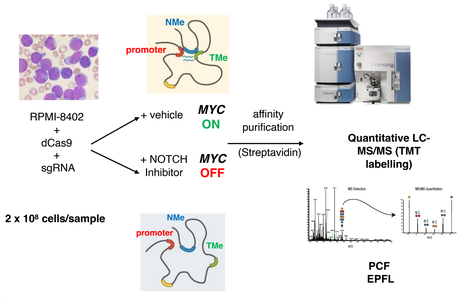
CRISPR **A**ffinity **P**urification *In Si**tu*** of **R**egulatory **E**lements (**CAPTURE**)



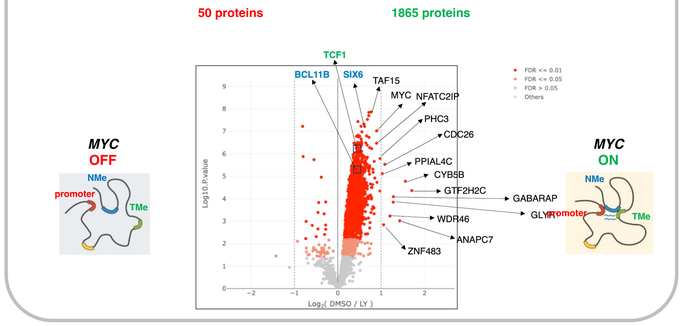
dCas9
- enzyme dead
- Biotin acceptor site C-terminus



Experimental set-up

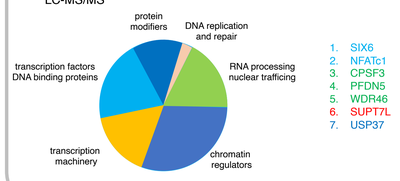


Protein composition at the *MYC* super enhancer upon NOTCH inhibition



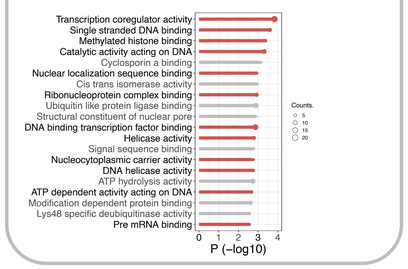
What are the potential MYC regulators?

1. Candidates are in the top 100 enriched in TMe
2. The candidates are enriched in NMe
3. The candidates are not detected in the ACTIN ctrl by CAPTURE LC-MS/MS

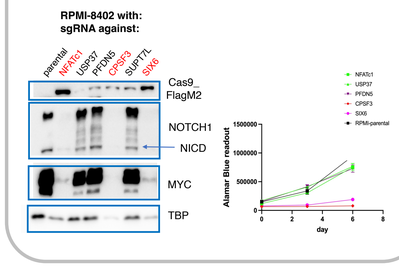


1. SIX6
2. NFATc1
3. CPSF3
4. PFDN5
5. WDR46
6. SUPT7L
7. USP37

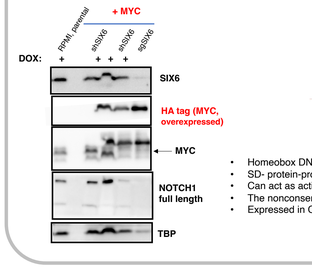
ORA GO Molecular Function of proteins at *MYC* TMe super enhancer

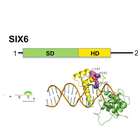


Knock Out/Down of potential MYC regulators



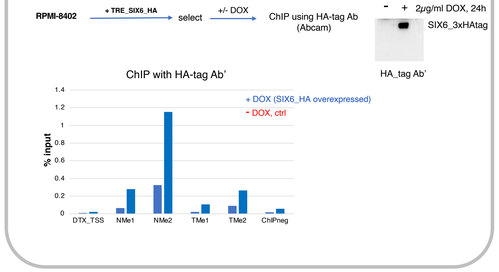
Ectopic *MYC* expression rescues cells after SIX6 knock down



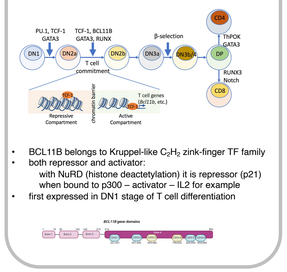


- Homeobox DNA binding domain (helix-turn-helix)
- SD- protein-protein interaction domain
- Can act as activator and repressor
- The nonconserved domain defines the specificity
- Expressed in CNS (eye), colon, liver, adipose tissue...

How is SIX6 regulating *MYC* expression in T-ALL?

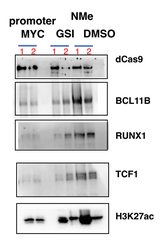


BCL11B is necessary for T cell commitment



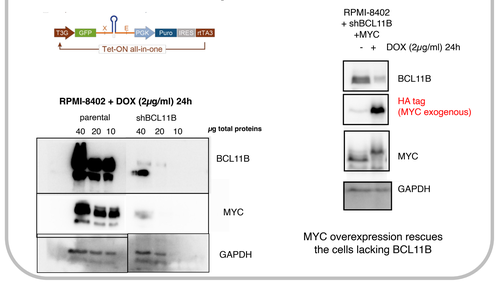
- BCL11B belongs to Kruppel-like C_2H_2 zink-finger TF family
- both repressor and activator:
  with NuRD (histone deactetylation) it is repressor (p21)
  when bound to p300 – activator – IL2 for example
- first expressed in DN1 stage of T cell differentiation

BCL11B binds to NMe as shown by CAPTURE



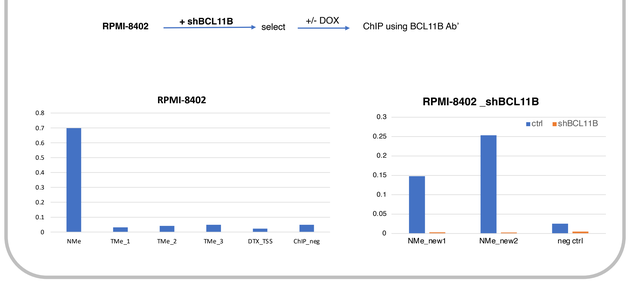
1, 2 – the first and second elution from the beads
GSI - gamma secretase inhibitor, NOTCH inhibitor

BCL11B knock down leads to decreased *MYC* expression



MYC overexpression rescues the cells lacking BCL11B

BCL11B binds to NMe region in RPMI-8402 cells



RESULTS

We have identified the proteins regulating two MYC super enhancer regions crucial for leukemic cell survival, NMe (NOTCH dependent MYC enhancer) and TMe (TCF1 dependent MYC enhancer). The ongoing efforts are focused on two transcription factors: BCL11B and SIX6.

CONCLUSION

These analyses aim at gaining new insights into molecular mechanisms of NOTCH and disease driving loci-specific transcriptional regulation. The ultimate goal will be to identify novel druggable therapeutic targets.

PERSPECTIVES

- Confirm by CAPTURE the SIX6 and BCL11B binding to MYC SE
- Establish the MYC SE conformation after SIX6 and/or BCL11B knock down
- Dissect the active transcription complexes in promoters and enhancers of other NOTCH driven genes.
- Investigate the possible role of the candidate proteins in other NOTCH driven malignancies
- Test identified factors for their therapeutic potential

Presented at SOHC 2025 from 19 – 21 November 2025