ZMYM3 MUTATIONS DYSREGULATE HISTONE ACETYLATION AND IMPACT THE OUTCOME OF CHRONIC LYMPHOCYTIC LEUKEMIA PATIENTS

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1. INTRODUCTION

Recent large-scale DNA sequencing studies have identified recurrent mutations in *ZMYM3* in 2–4% of chronic lymphocytic leukemia (CLL) patients at diagnosis, gene that has been related with chromatin remodeling through histone acetylation. However, the clinical and functional implications of these mutations in CLL remain largely unknown.

2. OBJECTIVES

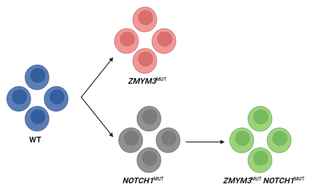
1. To assess the mutational profile of *ZMYM3* mutated patients and to define the clinical impact of these variants.

2. To analyze the functional and biological implications of *ZMYM3* mutations in a CLL cellular model designed by CRISPR/Cas9.

3. METHODS

1. Next generating sequencing (NGS) studies: the mutational status of 487 CLL patients was evaluated by targeted NGS in a panel with 54 CLL-related genes.

2. CLL cellular models and functional studies: the CRISPR/Cas9 system was used to introduce the most frequent truncating mutation in *ZMYM3* (*ZMYM3*^{MUT}) observed in our cohort in the HG3-CLL derived cell line with 2 genetic backgrounds: wild-type (WT) and *NOTCH1* mutated cells (*NOTCH1*^{MUT}), which harbor the most frequent mutation reported in CLL (p.P2514fs), to generate cells which simultaneously combine both mutations (*ZMYM3*^{MUT} *NOTCH1*^{MUT}).



These cellular models were used to evaluate the functional impact of these alterations in CLL transcriptome, DNA damage response and apoptosis.

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4. RESULTS

1 *ZMYM3* mutations are mostly loss-of-function, associate with *NOTCH1* mutations and shorter time to first treatment of CLL patients.

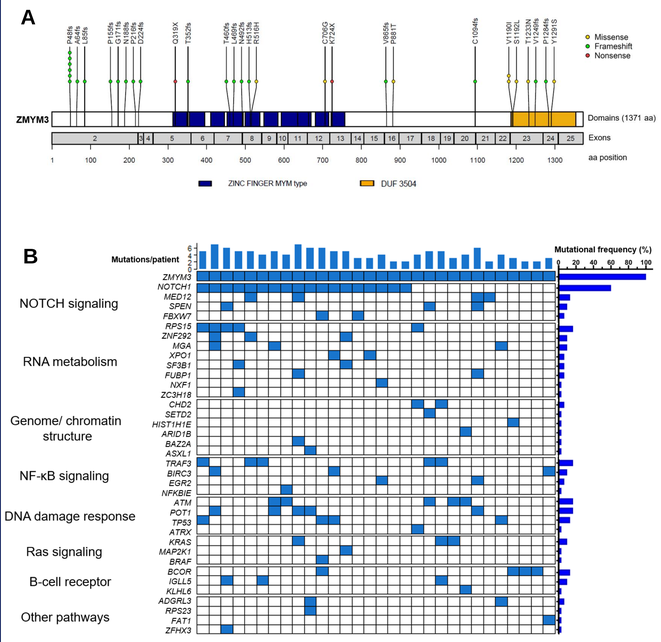

Figure 1. A. *ZMYM3* mutations identified in 30 CLL patients of the sequenced cohort. **B.** Mutational profile of *ZMYM3*^{MUT} patients. Each column represents a *ZMYM3*^{MUT} patient; and each row a CLL-related gene (blue = mutated). Bar graphs indicate the number of mutations per patient (above) and the frequency of each mutation (right). **C.** Clinical impact of *ZMYM3* mutations in terms of time to first treatment in the entire cohort of CLL patients (left) and in Binet stage A cases (right).

2 *ZMYM3* mutations reduce histone acetylation and their co-occurrence with *NOTCH1* mutations impacts CLL transcriptome.

Analysis of differentially expressed genes (DEGs) identified by RNA-sequencing determined that co-occurrence of *ZMYM3* and *NOTCH1* mutations profoundly dysregulates transcriptome, as *ZMYM3*^{MUT} *NOTCH1*^{MUT} cells showed 690 DEGs compared with WT, most of which downregulated (458 *vs* 232) (Figure 2.A). In addition, enrichment analysis revealed that DNA damage response, apoptosis and histone deacetylation were some of the processes dysregulated by *ZMYM3* mutations (Figure 2.B). In line with this notion, *ZMYM3*^{MUT} cells showed reduced levels of histone H4 acetylation (Figure 2.C), which may explain the changes in gene expression associated with *ZMYM3* mutations.

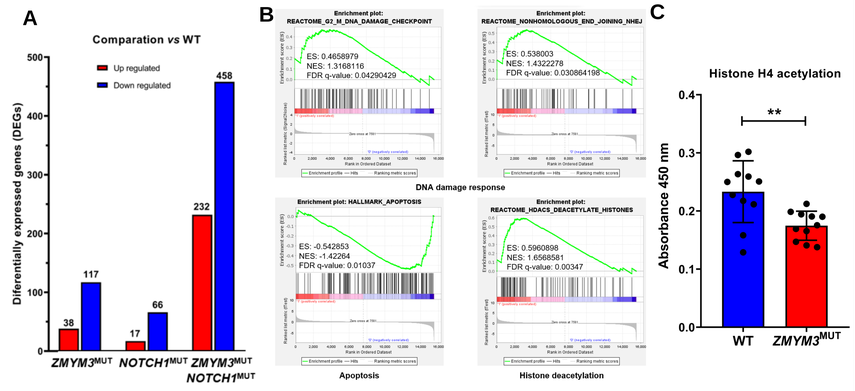

Figure 2. A. Number of diferentially expressed genes (DEGs) (|fold change|>2; False Discovery Rate, FDR<0.05) compared with WT cells. Red: up-regulated; Blue: down-regulated. **B.** Gene Set Enrichment Analysis (GSEA) for apoptosis, DNA damage response and histone deacetylation in *ZMYM3*^{MUT} *vs* WT cells. **C.** Determination of total histone H4 acetylation levels using an Abcam fluorometric kit.** = p<0.01

3 *ZMYM3* mutations impair DNA damage response.

To determine the impact of *ZMYM3* mutations in DNA damage response, we evaluated cell cycle profile after induction of DNA double strand breaks by irradiation (Figure 3.A). Consistent with transcriptomic data, *ZMYM3*^{MUT} cells exhibited difficulties to arrest cell cycle in G2/M. Furthermore, these cells displayed persistent γH2AX *foci* and fewer BRCA1 *foci* than WT cells in DNA lesions (Figure 3.B), which suggest a key role of *ZMYM3* in DNA damage response.

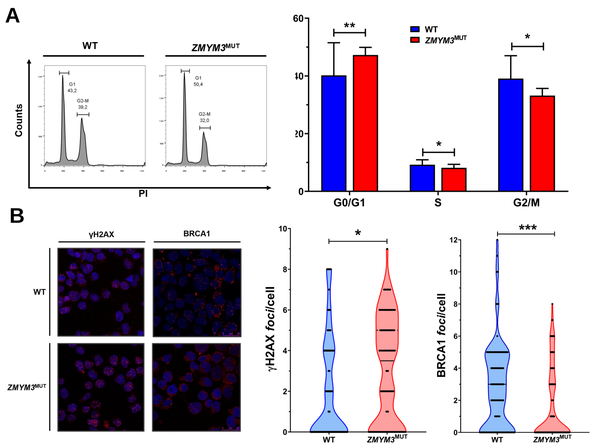

Figure 3. A. Cell cycle profile determined by propidium iodide (PI) staining 24 hours after irradiation (2 Gy). **B.** Quantification by immunofluorescence of γH2AX and BRCA1 *foci* after irradiation (2 Gy). *:p<0.05; ***: p<0.001

4 Co-occurrence of *ZMYM3* and *NOTCH1* mutations promotes apoptosis evasion and enhances growth capacity of CLL cells.

Focusing on apoptosis, we found that *ZMYM3*^{MUT} cells showed reduced expression of caspases -8, -7 and -3; as well as a slightly increase in the anti-apoptotic proteins BCL2, BCL-XL and MCL1 (Figure 4. A). In agreement with this expression and consistently with RNA-seq, *ZMYM3*^{MUT} cells exhibited higher apoptosis evasion, which was potentiated in *ZMYM3*^{MUT} *NOTCH1*^{MUT} cells (Figure 4. B) and led to enhanced growth capacity of these cells(Figure 4. C).

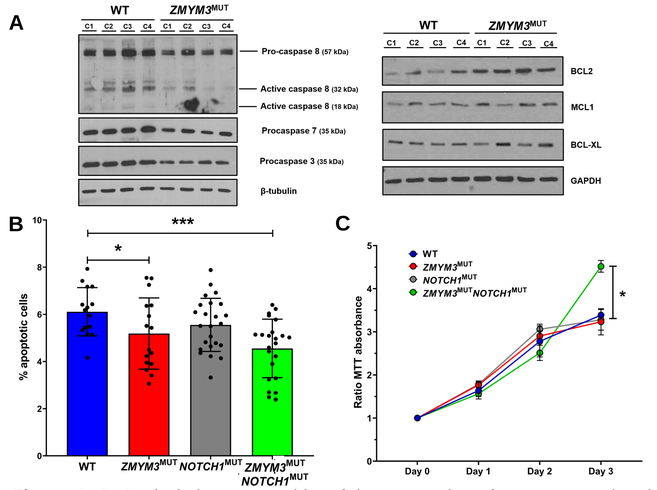

Figure 4. A. Analysis by western blot of the expression of caspases and anti-apoptotic proteins. **B.** Study of apoptotic cells by annexin V/PI staining. **C.** Proliferation curves determined by MTT absorbance. *:p<0.05; ***: p<0.001

5. CONCLUSIONS

1. *ZMYM3* mutations are mainly loss-of-function, associate with mutations in *NOTCH1* and a shorter time to first treatment, suggesting that *ZMYM3* mutational status may be considered in the management of early stage CLL patients.
2. *ZMYM3* mutations reduce histone H4 acetylation and impair DNA damage response, leading to DNA damage accumulation and genomic instability.
3. Co-occurrence of *ZMYM3* and *NOTCH1* mutations leads to changes in gene expression, promotes apoptosis evasion and enhances growth capacity of CLL cells.