



The 65th ASH Annual Meeting Abstracts

POSTER ABSTRACTS

641. CHRONIC LYMPHOCYTIC LEUKEMIAS: BASIC AND TRANSLATIONAL

ZMYM3 Mutations Cooperate with NOTCH1 Alterations, Reduce Histone H4 Acetylation and Promote Apoptosis Evasion in Chronic Lymphocytic Leukemia

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Introduction: Recent next-generation sequencing (NGS) studies have identified up to 200 recurrently mutated genes in chronic lymphocytic leukemia (CLL), the vast majority of which appear mutated in <5% of CLL cases. Among the long list of CLL candidate driver genes is ZMYM3, a gene mutated in 2-4% of CLL patients. Since the impact of ZMYM3 mutations is unknown, we aimed to identify their clinical and biological consequences in CLL.

Methods: 487 CLL patients were analyzed by targeted NGS using a customized panel of 54 CLL-related genes to characterize the mutational profile of ZMYM3 mutated patients (ZMYM3^{MUT}) and the clinical impact of these variants. In addition, we introduced ZMYM3 truncating mutations into the CLL-derived HG3 cell line with two genetic backgrounds: wild-type (WT), and NOTCH1 mutated cells (NOTCH1^{MUT}), to generate cells with either single ZMYM3^{MUT}, NOTCH1^{MUT} or combined ZMYM3^{MUT} NOTCH1^{MUT}. In these CLL models, we evaluated the impact of these alterations in gene expression, apoptosis, growth and DNA damage response (DDR).

Results: A total of 32 ZMYM3 variants were identified in 30 CLL patients, most of which were loss-of-function mutations (24/32; 75%). These variants were distributed throughout the protein sequence, with a particular recurrent frameshift mutation occurring in 6 patients (p.P48fs) that was subsequently reproduced in our CLL model. Attending to their mutational profile, we identified that 70% (21/30) of ZMYM3^{MUT} patients harbored mutations in the NOTCH pathway, either in NOTCH1 (60%) or in negative regulators such as MED12, FBXW7 and SPEN, suggesting a cooperation between ZMYM3 dysfunction and NOTCH1 signaling mutations in CLL evolution. Interestingly, ZMYM3^{MUT} patients exhibited significantly shorter time to first treatment (TFT) than ZMYM3^{WT} cases (median: 35 vs 52 months; p=0.010). Furthermore, ZMYM3 mutations shortened TFT of early stage CLL patients, including Binet A (48 vs 108 months, p=0.002) or Rai 0/1 (48 vs 91 months, p=0.016), as well as in low-risk cytogenetics del(13q) patients (median: 45 vs 93 months; p=0.033).

We next addressed the biological implications of ZMYM3 mutations and their co-occurrence with NOTCH1 mutations in HG3 cells. First, we performed RNA-sequencing and analyzed the number of differentially expressed genes (DEGs) (|fold change|>2, adjusted p-value<0.05). Comparing with WT cells, we detected 155 DEGs in ZMYM3^{MUT} and 83 DEGs in NOTCH1^{MUT}, whereas ZMYM3^{MUT} NOTCH1^{MUT} cells showed 690 DEGs, indicating that the combination of both mutations profoundly dysregulate gene expression. Immune regulation, apoptosis and DNA damage response were some of the biological processes dysregulated by ZMYM3 mutations (p<0.05). Furthermore, considering the suggested role of ZMYM3 in chromatin remodeling (Puente et al, Nature. 2015) and that ZMYM3^{MUT} cells showed altered expression of histone acetylation related genes (p<0.05), we identified that ZMYM3 mutations reduced global acetylation levels of histone H4 (p<0.05), which may underlie the ZMYM3-related changes in gene expression.

To further define the functional effects of *ZMYM3* mutations, we assessed *in vitro* their impact in the main dysregulated pathways identified by RNA-seq. First, we identified that *ZMYM3* mutations impair DNA damage response, as reflected by the difficulties to arrest cell cycle in G2/M phase after irradiation. Moreover, we detected more γ H2AX foci ($p < 0.05$) in *ZMYM3*^{MUT} cells, indicating persistent DNA damage; and fewer RAD51 and BRCA1 foci than WT cells ($p < 0.001$) after irradiation, suggesting defective DNA damage response. In parallel, we identified that *ZMYM3* mutations promote apoptosis evasion, especially in the context of *NOTCH1* mutations ($p < 0.001$), which led to enhanced growth of *ZMYM3*^{MUT} *NOTCH1*^{MUT} cells ($p < 0.05$). Of note, *ZMYM3*^{MUT} cells showed down-expression of caspases (-8, -7 and -3) and slightly higher levels of anti-apoptotic proteins (BCL2, MCL1 and BCL-XL), which may explain this apoptosis resistance.

Conclusions: Mutations in *ZMYM3* are mainly loss-of-function, associate with *NOTCH1* signaling mutations and shorten TFT in CLL patients, suggesting that *ZMYM3* mutational status may be a useful marker in the management of early stage CLL patients. Moreover, *ZMYM3* mutations reduce histone H4 acetylation and cooperate with *NOTCH1* mutations to dysregulate gene-expression, leading to impair DNA damage response and apoptosis evasion.

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