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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 γH2AX *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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