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POSTER ABSTRACTS

641.CHRONIC LYMPHOCYTIC LEUKEMIAS: BASIC AND TRANSLATIONAL

Normal B Cells in MBL Exhibit Distinct Transcriptomes Compared to Those of Healthy Individuals, Although They Differ in Activation State Based on IGHV Mutation Status

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Background. Immune deficiency is a cardinal feature of CLL and its predecessor MBL. To understand this issue, we studied individuals with MBL since a higher proportion of normal B cells (NBC) are present in this condition as compared to CLL and none of the individuals have been exposed to therapies that might alter immune function.
Objectives. Weevaluated the transcriptomes of NBC from people with MBL (NBC-MBL) and compared these to NBC of healthy controls (NBC-HC) and to MBL clonal B cells (Clonal-MBL). Also, we compared the transcriptomic profiles of $CD5^-$ NBC-M-MBL, NBC-U-MBL, and NBC-HC.
Methods. PBMCs from 13 people with MBL (9 M-MBL, 4 U-MBL) and from 13 HC were FACS-isolated to obtain $CD5^+$ clonal B cells ($CD19^+CD20^{Dim}CD5^+Ig\kappa^+$ or $Ig\lambda^+$) for MBL, and up to 4 B-cell fractions of NBC ($CD19^+CD20^{Bright}CD5^-$ or $CD5^+/Ig\kappa^+$ or $Ig\lambda^+$) for MBL and HC. Each cell fraction was >99% pure. In all, 76 cell fractions were collected, 34 from MBL and 42 from HC. RNA was sequenced using SMART-Seq v4 Ultra Low Input and HiSeq platform. DESeq2 was used to analyze RNA-seq data. PCA clustered samples based on transcriptomic variance. Differentially expressed genes (DEG) were obtained with adjusted $P<0.05$ and $|FC|\geq 3$. IPA identified relevant biological pathways. DEG protein products were validated by flow cytometry (FC) in 6 MBL (4 M-MBL and 2 U-MBL) and 8 HC.
Results. PCAof all B-cell fractions showed that NBC-HC, NBC-MBL, and Clonal-MBL fell into 3 distinct groups, indicating each group has distinct transcriptomes. Focusing on NBC from HC and from MBL based on IGHV-mutation status, 3 groups with a similar gradient were identified: NBC-HC > NBC-M-MBL > NBC-U-MBL (**Fig 1**), showing that NBC-HC differ from NBC-MBL and NBC differ within MBL based on IGHV-mutation types. Next, IPA was used to determine up- and down-regulated pathways (**Fig 2**). For $CD5^-$ NBC-MBL from all MBL people vs. NBC-HC (C1; 1,704 DEG), IPA revealed activation of inhibitory (e.g., FcγRIIB, the most significant pathway, which can inhibit B-cell function) and stimulatory pathways (e.g., [1] S100 signaling, the second most significant pathway, which can trigger NF-κB activation; [2] phospholipase C signaling, the third most significant pathway, involved in cell survival and proliferation; and [3] IL-4, essential for B-cell survival and maturation). To discriminate contributions of NBC from M-MBL and U-MBL, IPA was first performed for $CD5^-$ NBC-M-MBL vs. NBC-HC (C2; 1,086 DEG), showing 76% (13/17) of pathways significantly inhibited (e.g., HMGB1, TNFR1, IL17, and IL6). Notably, of the only 4 upregulated pathways, 2 were inhibitory, IL-10 signaling (the most significant pathway) and NFKBIE signaling. These findings suggest downregulation/anergy of NBC in M-MBL. Strikingly, the $CD5^-$ NBC-U-MBL vs. NBC-HC comparison (C3; 10,959 DEG) showed that 100% (11/11) of IPA pathways were activated and stimulatory (e.g., S100 signaling), consistent with increased NBC-U-MBL proliferation and migration. FC analyses detected significant ($P<0.05$) protein overexpression of CD24, CD27, CD300a, CD32 and IL10RA in $CD5^-$ NBC from all MBL vs. NBC-HC; CD27, CD300a and IL10RA overexpression in $CD5^-$ NBC-M-MBL vs. NBC-HC; and CD27 and TLR10 overexpression in $CD5^-$ NBC-U-MBL vs. NBC-HC, validating gene expression results.
Conclusions. PCA showed clear distinctions between NBC-MBL compared to NBC-HC, for both U-MBL and M-MBL. NBC-M-MBL appear to be functionally suppressed presumably due to IL-10 and NFKBIE signaling. In contrast, NBC-U-MBL appear to be stimulated through the S100 pathway. This activation of NBC-U-MBL seems paradoxical since in CLL there is greater immune suppression in U-CLL than M-CLL. Protein validation strengthens these findings.
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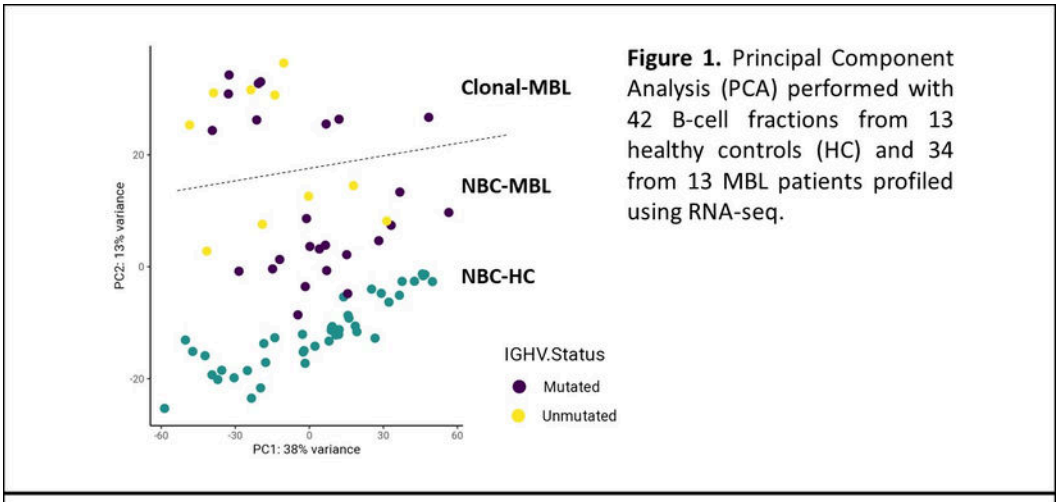

Figure 1. Principal Component Analysis (PCA) performed with 42 B-cell fractions from 13 healthy controls (HC) and 34 from 13 MBL patients profiled using RNA-seq.

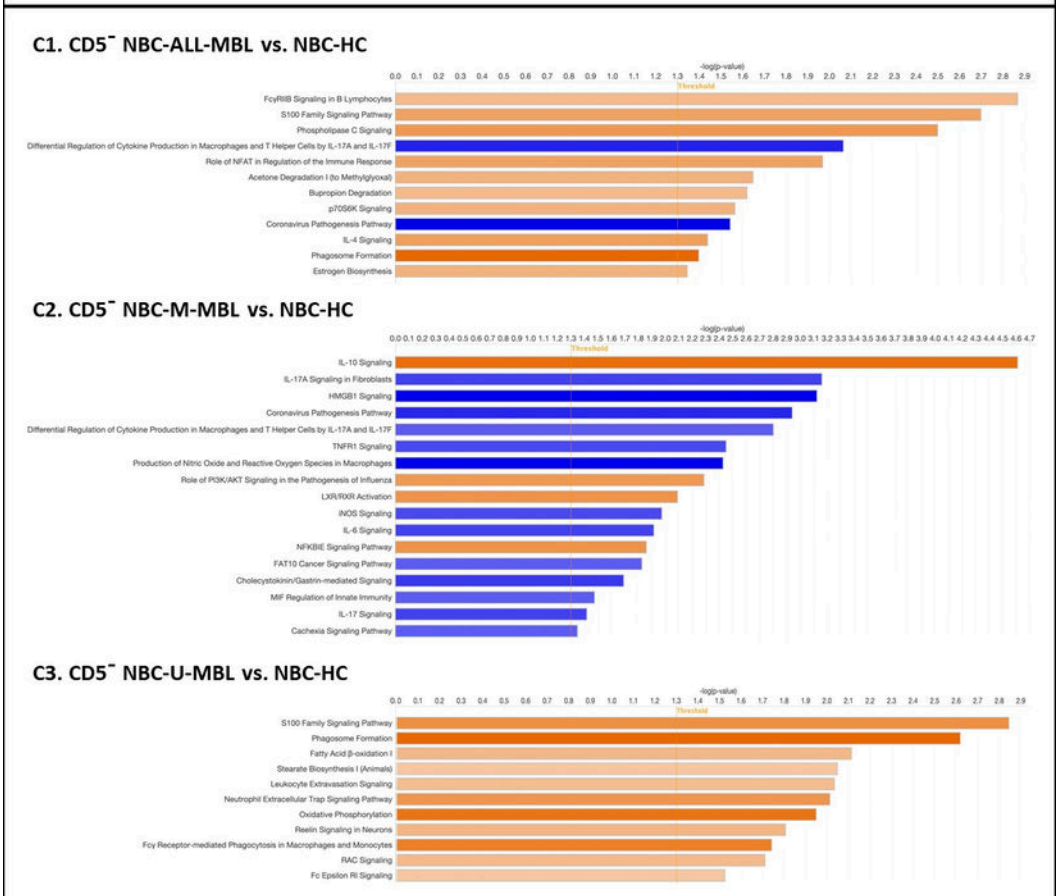

Figure 2. Pathways highlighted by IPA for each of the 3 comparisons. Only those pathways with a P-value < 0.05, Z-score ≥ 2 (orange, predicting activation) or Z-score ≤ -2 (blue, predicting inhibition) are detailed. A cell type filter (lymphocyte) was applied. NBC: Normal B cells; HC: Healthy controls; U-MBL: MBL patients with unmutated IGHV ; M-MBL: MBL with mutated IGHV.

Figure 1

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