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ORAL ABSTRACTS

641.CHRONIC LYMPHOCYTIC LEUKEMIAS: BASIC AND TRANSLATIONAL

A Comprehensive DNA Methylome Analysis of Stereotyped and Non-Stereotyped CLL Reveals an Epigenetic Signature with Strong Clinical Impact Encompassing IGHV Status, Stereotypes and IGLV3-21^{R110}

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Abstract Although up to 41% of chronic lymphocytic leukemia (CLL) patients belong to subsets with stereotyped or quasi-identical B cell receptors, a thorough epigenomic characterization of CLL stereotypy has been limited by the low frequency (<2.5%) of each individual subset. To address this challenge, we assembled a series of 995 cases profiled by 450k and EPIC DNA methylation arrays. The dataset was enriched with the 8 most frequent subsets (#1-8, n=180) and other less common and satellite stereotypes (n=18). The remaining cases were not recognized as any known subset (n=451) or were not classified (n=346), and were used for control and validation purposes.

Unsupervised analyses showed subset and non-subset cases overlapping, suggesting that stereotypy per se is not a major source of DNA methylation variability. Instead, and reinforcing previous reports, we found that the major principal components were related to the 3 cell-of-origin groups or epitypes (naïve (n)-CLL, intermediate (i)-CLL and memory (m)-CLL), and to the CLL proliferative history measured by the epiCMIT mitotic clock. Nonetheless, focused analyses of each epitype revealed specific clustering and differential patterns for all subsets, with subsets #2, #5, #8 demonstrating the most prominent DNA methylation signatures.

Focusing on #2, the most frequent subset almost exclusively composed of i-CLL, we found mainly a loss of DNA methylation compared to non-subset i-CLL cases (217 differentially methylated CpGs). Part of this methylation loss was also observed in post-germinal center B cells and mainly reflected the higher epiCMIT of subset #2 (P=0.002). The majority of the remaining CpGs were de novo hypomethylated in subset #2 compared to non-subset and normal B cell samples, and targeted regulatory elements, which may represent a hypomethylation linked to subset #2-specific regulatory programs. Noticeably, a group of non-subset cases displayed molecular features similar to subset #2, including the hypomethylation signature, epiCMIT levels, IGHV gene mutational status, and enrichment for the IGLV3-21^{R110} mutation. As all but one subset #2 patient carried the IGLV3-21^{R110}, we then grouped patients having either of the two features and found a ~50% increase of differentially methylated CpGs (n=315), strongly supporting that the driver of the differential methylation patterns in subset #2 was indeed the presence of the IGLV3-21^{R110}.

A subsequent comparison of the identified signature with n-CLLs and m-CLLs showed that i-CLLs with IGLV3-21^{R110} shared methylation patterns with n-CLLs, whereas i-CLLs without such mutation resembled m-CLLs. In fact, a consensus clustering approach of the 315 CpGs with 631 cases profiled with the 450k array resulted in 2 robust clusters, which could also be identified with high accuracy with few CpGs (~98% cross-validated accuracy, ~5 CpGs). The first cluster was composed globally, but not entirely, by unmutated IGHV (U-CLL), n-CLL, subsets #1-3 and #5-8, and cases with the IGLV3-21^{R110} from the mutated IGHV (M-CLL), m-CLL and i-CLL subgroups (n=313), while the second cluster encompassed M-CLL, m-CLL and i-CLL lacking the IGLV3-21^{R110} and subset #4 (n=318). As expected, these two clusters showed a dramatically different clinical outcome, with a difference in median time to first treatment from diagnosis of ~25 years (P=3x10^{-77}). Remarkably, this high prognostic power was also observed for overall survival, both from diagnosis and from date of sampling, and was independent from IGHV mutational status, subsets #1-8, IGLV3-21^{R110}, epitypes, and epiCMIT, as indicated by multivariate Cox models. In fact, few U-CLL cases classified as cluster 2 showed the same favorable outcome than M-CLL. Conversely, M-CLL, m-CLL and i-CLL cases classified as cluster 1 displayed the same adverse outcome similar to U-CLL or n-CLL, even those few cases without the IGLV3-21^{R110}. Finally, the molecular (n=364) and clinical (n=257) features of these 2 groups were collectively validated through 3 independent cohorts containing both 450k and EPIC data.

Altogether, this study reveals novel insights into the epigenome of CLL stereotypy and into the yet poorly characterized i-CLL epitype. Our analyses unveil an epigenetic signature that dichotomizes CLLs into two clusters with markedly different clinical outcome, which mainly, but not exclusively, encompass IGHV mutational status, epitypes, stereotypes and IGLV3-21^{R110}.

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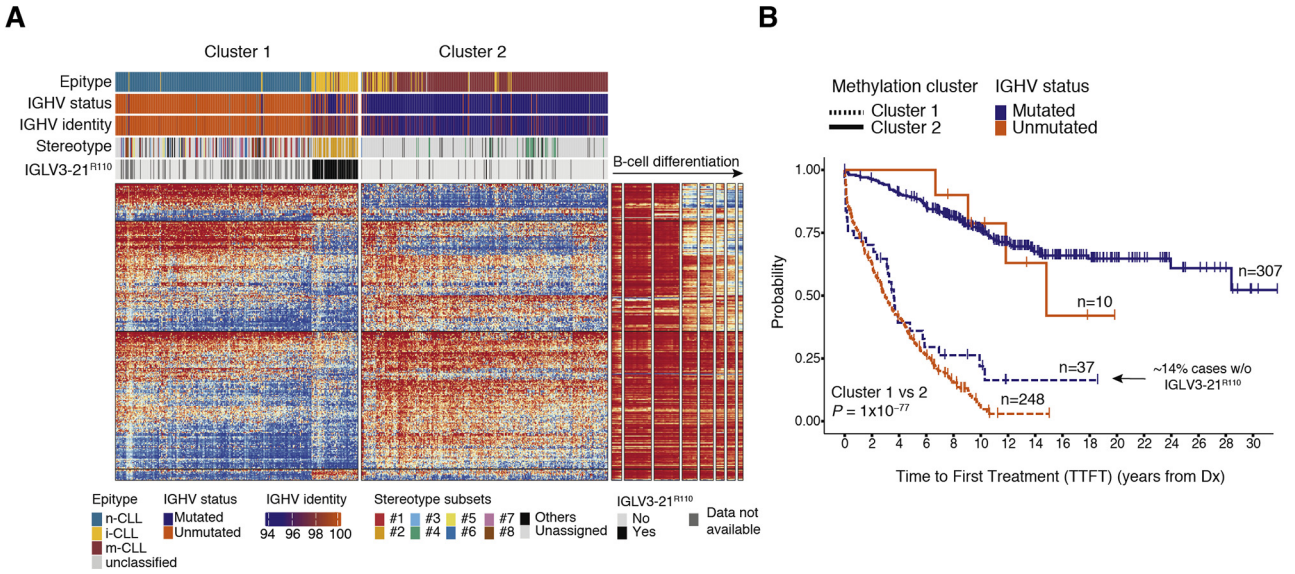

A, A novel DNA methylation signature dichotomizes CLLs in 2 clusters, which mainly, but not exclusively, encompass IGHV mutational status, epitypes, stereotypes and IGLV3-21^{R110}. **B**, TTFT from diagnosis of the 2 clusters from A. Cluster membership and IGHV gene mutational status are indicated.

Figure 1.

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