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Increased aldehyde dehydrogenase activity in high-risk chronic lymphocytic leukemia

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Chronic lymphocytic leukemia (CLL), the most common type of leukemia in the Western world, has a clinically variable course, with differences in the pace of malignant B-lymphocyte accumulation and in the time until cytoreductive treatment is needed. In various solid tumors, the expression of stem cell associated markers, such as aldehyde dehydrogenase (ALDH), has been associated with aggressiveness and a poor prognosis. However, the level of ALDH has not been assessed in CLL B cells.

Despite their mature appearance, the B cells from patients with CLL possess immature characteristics both functionally and biochemically. CLL B cells often display biochemical markers of cells early in the blood lineage, including ROR1 [1], Wnt16 [2] and LEF1. In addition, CLL B cells have higher levels of reactive oxygen species (ROS) than normal B cells [3]. The ALDH enzyme family is thought to protect cells from accumulated aldehydes formed during lipid peroxidation and poorly regulated oxidative metabolism in a low oxygen environment [4]. Cells with a high level of ALDH activity are considered to have improved self-renewal capacity [5].

The goal of this study was to ascertain whether increased ALDH activity is associated with more aggressive disease in CLL. To this end, we compared ALDH activity with two CLL biomarkers, immunoglobulin heavy chain variable gene (IgHV) mutation status and ZAP-70 expression. These biomarkers are regularly used to classify patients with CLL as high-risk: IgHV unmutated (> 98% homology to IgHV germline gene) or ZAP-70 positive (> 20% of CD19 CLL B cells expressing ZAP-70) and low-risk: IgHV mutated and ZAP-70 negative [6].

Blood samples had been previously collected and viably stored from 40 consenting subjects diagnosed with CLL and followed at the University of California San Diego (UCSD) Moores Cancer Center, with the approval of the UCSD Institutional Review Board. Twenty cases were selected with unmutated IgHV or that were ZAP-70 positive (high risk), and an additional 18 cases were selected which had both mutated IgHV and were ZAP-70 negative (low risk) [6]. Two samples were excluded because of insufficient clinical data. All blood samples were collected from patients prior to treatment. In addition, peripheral blood mononuclear cells (PBMCs) were prepared from samples collected from healthy volunteers registered at the San Diego Blood Bank, as previously described [6].

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Cells (2.5×10^6) were stained for ALDH activity with an Aldefluor assay kit (StemCell Technologies, Vancouver, Canada) according to the manufacturer's instructions. The cells were then stained for the B cell surface marker CD19, and analyzed using a FACSCalibur instrument (BD Biosciences, San Diego, CA). A total of 50,000 events were acquired. Data analysis was performed using Flowjo software (Ashland, OR) and Prism (GraphPad Software, La Jolla, CA). The cells were gated for viable CD19⁺ cells and mean fluorescent activity was determined.

Receiver operating characteristic (ROC) curves were computed in R v2.13.0 [7] using the package ROCR, with standard errors and corresponding 95% confidence intervals (CIs) computed based on the standard errors of Somers' Dxy rank correlation using the function `rcorr.cens` from the Hmisc package [8]. Significance was attained at the 5% level if the CI for the AUC was entirely above 0.50, demonstrating greater predictive ability than by chance alone.

We assayed mean ALDH levels in CD19⁺ B cells in 18 previously characterized low-risk (ZAP-70 negative and IgHV mutated) and 20 high-risk (IgHV unmutated or ZAP-70 positive) patients with CLL, as well as PBMCs [6] from six healthy individuals. The characteristics of the patients are summarized in Table I.

The mean ALDH activity was elevated in the high-risk CLL samples, compared to the low-risk CLL group ($p = 0.002$, one-sided Wilcoxon test) [Figure 1(A); combined high-risk groups, $n = 20$, vs. low risk group, $n = 18$]. Normal CD19⁺ B cells exhibited low levels of ALDH activity. The area under the curve (AUC) for classifying high-risk versus low-risk samples using the ALDH level was 0.77 (95% CI: 0.62, 0.92) [Figure 1(B)], indicating that ALDH activity had a moderate but statistically significant ability to discriminate between these two groups. Considering each biomarker separately, ALDH had a similar ability to classify IgHV mutational status alone [AUC of 0.77; 95% CI: 0.62, 0.92; Figure 1(C)] and ZAP-70 status alone [AUC of 0.76; 95% CI: 0.59, 0.93; Figure 1(D)]. Moreover, for the 16 samples with unmutated IgHV and an ALDH value above 400, ZAP-70 expression correlated with ALDH activity ($r = 0.90$ with $p < 10^{-5}$) [Figure 1(E); open triangles > 400 ALDH]. These data collectively show: (i) that ALDH activity is increased in CLL B cells, (ii) that ALDH has a similar moderate ability to classify patients with CLL according to IgHV mutational status and ZAP-70 expression status alone, and jointly, and (iii) that increased ALDH activity is associated with high-risk CLL.

The observation that ALDH activity is increased in CLL samples obtained from patients with high-risk characteristics has implications related to disease pathogenesis and treatment. Recently, elevated ALDH activity in CD34⁺ CD38⁺ stem-like cells from acute myelogenous leukemia has been correlated with subsequent clinical relapse [9]. Increased ALDH activity in CLL cells might similarly reflect an immature, aggressive phenotype. ALDH enzymes enable cells to remove toxic products formed in a hypoxic environment as byproducts of oxidative metabolism. Together with other factors, the elevated ALDH in high-risk CLL may promote cell survival in stressful tissue compartments. ALDH may also aid in the detoxification of oxidation products resulting from overexpression of c-Myc and activated phosphatidylinositol-3 kinase (PI3K), as commonly observed in CLL [10,11].

In summary, ALDH activity, which can be measured relatively easily, warrants further investigation for use as a screening biomarker to classify patients with CLL according to a high-risk versus low-risk disease course. Future studies are needed to determine the precise role of ALDH in the pathogenesis of CLL.

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Figure 1.

ALDH activity in chronic lymphocytic leukemia. (A) Mean ALDH activities were determined in the gated CD19⁺ population. Mean ALDH activities of different groups of CLL and CD19⁺ B cells from healthy volunteers are shown. Bars indicate mean values. (B–D) The binary classification ability of ALDH for different groups is shown as area under the curve (AUC) in receiver operating characteristic (ROC) curves. AUC values in ROC curves represent the probability of classifying two test groups correctly. While an AUC value of 0.5 indicates no classification value, an AUC value of 1 represents perfect classification power. Classifications of (B) disease risk, (C) IgHV mutation and (D) ZAP-70 expression status (positive + and negative –) by ALDH are shown, along with 95% confidence intervals (CIs) for AUC values. (E) ZAP-70 expression values were plotted against mean ALDH activities. Samples with unmutated and mutated IgHV are shown as open and filled triangle symbols, respectively.

Table I

Characteristics of patients with CLL.

	<i>n</i> (%)
Gender	
Male	22 (58%)
Female	16 (42%)
Age at diagnosis, median (range)	59 (42–80)
Rai stage	
0	16 (45%)
0–1	3 (8%)
1	9 (25%)
1–2	1 (3%)
2	7 (19%)
Unknown	2
High risk	
IgHV unmutated, ZAP-70 positive	14 (70%)
IgHV unmutated, ZAP-70 negative	6 (30%)
Low risk	
IgHV mutated, ZAP-70 negative	18 (100%)

CLL, chronic lymphocytic leukemia; IgHV, immunoglobulin heavy chain variable gene.