Gene expression comparison between B cells expressing high and low level of Zap-70 mRNA reveals distinct profiles, potential therapeutic targets and new prognostic factors for Chronic Lymphocytic Leukemia

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Introduction

Chronic Lymphocytic Leukemia (CLL) is a heterogeneous disease characterized by a highly variable clinical course based on the IgVH mutational status currently considered as one of the powerful prognosis factors. Because of the complexity of this analysis, several surrogate markers of this mutational status have been found in the recent years, among them Zap-70 seems to be a reliable prognostic factor. In a preview study, we compared three predictive markers (Zap-70, lipoprotein lipase and CD38 expression) in terms of treatment-free and overall survival and finally Zap-70 (zeta-associated protein 70) measured by quantitative RT-PCR was chosen for further gene expression analysis because of its strong association with the IgVH status and its high prognostic values (Stamatopoulos B. et al., Clinical Chemistry, 2007, in p r e s s) .

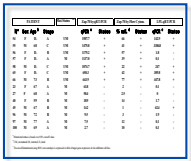

Table 1. Patient characteristics

Methods

We developed a quantitative real time PCR to measure Zap-70 mRNA expression in a cohort of 100 patients and to classify patients with high and low Zap-70 expression. Gene expression profiles of high (Zap-70high, n=7) and low (Zap-70low, n=7) mRNA expression were then compared using Affymetrix U133 plus 2.0 genechips representing more than 47000 transcripts. Patient characteristics are shown in Table 1. Only genes differentially expressed with a FDR ≤ 10% and with fold change of at least 2.0 fold (increase or decrease) were selected. Some of these genes were after confirmed by qPCR in an extended cohort of 90 patients.

Results

Zap-70, the first gene of the list, was followed by the phosphodiesterase 8A (PDE8A, P<0.0001), integrin alpha 4 (ITGA4, P<0.0001) and some genes of Fc receptor like family (FCRL, P<0.0001) (Fig. 1). These genes were confirmed in an extended patient cohort (n=90). Examples of genes differentially expressed are shown in fig. 2.

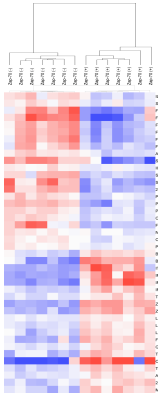

Fig. 1 Microarray profile comparison

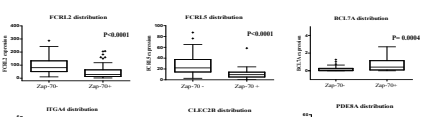

Fig. 2. Distribution of 6 genes between Zap-70 negative and positive patients.

Some of these genes were also able to separate the patients in terms of TFS indicating their relevant clinical predictive power (Fig. 3).

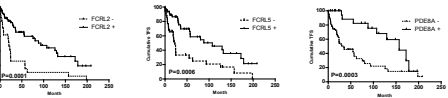

Fig. 3 TFS analysis regarding FCRL2, FCRL5 and PDE8A.

(ROC curve analysis were performed to determine optimal cut-off values that best distinguished between Zap-70 negative and positive cases)

Conclusion

CLL cells expressing high and low level of Zap-70 mRNA are characterized by a distinct gene expression profile that reveals new potential therapeutic target, new prognostic factors and genes implicated in cellular activation, adhesion and migration.