Copy number analysis of the BIRC3 gene by droplet digital PCR in chronic lymphocytic leukemia patients with 11q deletion

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INTRODUCTION

Chronic lymphocytic leukemia (CLL) with 11q deletion (11q- ) has been associated to a poor prognosis, but the clinical course of patients carrying this lesion is variable.

The 11q-, most often monoallelic, is present in 10-17% of newly diagnosed CLL and in 20-30% of patients with progressive or chemotherapy refractory disease. The minimal deleted region (MDR) (2-3 Mbp), located on the 11q22-3-23.1 region, includes ATM. In some cases, 11q- can also include BIRC3 (11q22.2), a gene often deleted or mutated in advanced/chemoresistant CLL. Although BIRC3 disruption has been associated to a poor prognosis, its prognostic implication in addition to ATM deletion is not well defined.

We have previously conducted a study on BIRC3 gene in 55 untreated 11q- CLL patients (discovery cohort) using Cytoscan HD array (Affymetrix) for copy number aberration (CNA) analysis. BIRC3, rarely mutated, was deleted in 82% of cases and the biallelic lesion (D+M) was associated to a marked hyperleukocytosis at diagnosis and immediate need of treatment.

AIMS

The aim of this study was to perform a CNA analysis of BIRC3 gene using the innovative droplet digital PCR (ddPCR) technique, in order to expand our preliminary observations obtained from the discovery cohort analysis.

In particular, we aim at identifying 11q-subgroups with potential prognostic relevance based on the inclusion of BIRC3 gene in the deleted region and on the presence of BIRC3 mutations.

METHODS

Genomic DNA was extracted from peripheral blood samples of 43 untreated 11q- CLL (screening cohort). BIRC3 CNA was performed by QX200™ Droplet Digital™ PCR System (Bio-Rad) (Figure 1) that represents a useful tool to screen known lesions with high accuracy and to provide an absolute quantification of a given target locus relatively to a reference locus. Data were analyzed using QuantaSoft software and CN value was computed by the ratio between the FAM-target (BIRC3) and the HEX-reference (K87RD, centromeric probe on chr 11) molecule concentrations, times the number of copies (2) of reference gene in the genome. By a serial clonion experiment of a CLL case with 95% 11q- by FISH and by preliminary tests on CLL with different types and amounts of FISH lesions, ddPCR proved capable to identify a deletion when present in at least 10% CLL cells and to confirm FISH results in 100% of cases. BIRC3 mutations (exons 6 & 9) were evaluated by Sanger sequencing. Time to first treatment (TFT) was calculated from the date of diagnosis to the date of first therapy or last follow-up.

RESULTS

Given the comparable biological and clinical features of both discovery (n=55) and screening cohorts (n=43), as well as the superimposable TFT (median TFT: 12.3 vs 12 months; p<0.86), the 98 11q- CLL patients were pooled together (Figure 2).

The baseline clinical and biological features of the 98 patients are reported in Table 1. All patients showed 11q- by FISH (median 70.5%, range 10-99.9% of nuclei).

By ddPCR analysis BIRC3 resulted included in the 11q- (Figure 3) in 74/98 cases (75.5%) and the Sanger sequencing analysis revealed the presence of BIRC3 mutations in 7/97 (7.2%), being always deleted on the other allele (D+M). Figure 4 explains the analysis of ddPCR data.

CONCLUSIONS

This study showed that among untreated CLL patients with 11q-:

i) BIRC3 deletion represents a common event (75%), whilst the mutation is rare (7%);
ii) BIRC3 deletion alone does not seem to influence TFT of 11q- CLL;
iii) BIRC3 D+M is strongly associated to a short TFT;
iv) BIRC3 D+M is mostly associated to an immediate need of treatment and to a marked hyperleukocytosis at diagnosis.

Thus, among 11q- CLL the knowledge of the inclusion of BIRC3 gene in the deleted region and of BIRC3 mutation could be useful to identify a novel subgroup of patients with aggressive course. Moreover, the results from this study demonstrate that ddPCR represents a handy tool to screen a known CNA with a good sensitivity and accuracy, resulting in a cost-effective approach.

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