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Supplement Article

IX. Chronic lymphocytic leukaemia: New genetic markers as prognostic factors

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Introduction

In Western countries, chronic lymphocytic leukaemia (CLL) is the most frequent mature B-cell malignancy [1]. The course of CLL ranges from very indolent, with a nearly normal life expectancy, to rapidly progressive leading to early death. Understand the genetic basis of CLL may help in clarifying the molecular determinants of this clinical heterogeneity and improve patients' prognostication.

Recurrent chromosomal aberrations at 13q14, 12q, 11q22-q23 and 17p13 are the first genetic lesions identified as drivers of the disease and have enabled the construction of a hierarchical model of cytogenetic abnormalities that correlates with outcome. Cytogenetic lesions, however, may not entirely explain the genetic basis of CLL clinical heterogeneity, as documented by the contribution of *TP53* mutation assessment in identifying high-risk patients [1]. The recent major improvements in massive parallel sequencing technologies have provided an opportunity to examine the CLL genome, allowing for the identification of genomic alterations underlying the disease and for the discovery of new therapeutic targets and clinically predictive biomarkers such as *NOTCH1*, *SF3B1* and *BIRC3* mutations [2–5].

Molecular aspects of the new CLL genetic markers

The *NOTCH1* gene encodes a heterodimeric transmembrane protein that functions as a ligand-activated transcription factor [2,3]. When the *NOTCH1* receptor interacts with its ligands through the extracellular subunit, two consecutive proteolytic cleavages of the protein are initiated and lead to pathway activation. Upon activation, the cleaved intracellular portion of *NOTCH1* translocates into the nucleus where it modifies the expression of target genes, including upregulation of *MYC* and genes of the NF-κB pathway, and downmodulation of *TP53* and other cell cycle regulators, thus playing an important role in a number of cellular functions, such as proliferation and apoptosis [2,3].

NOTCH1 mutations characterize ~5–10% newly diagnosed CLL, whereas their prevalence increases to 15 – 20% in progressive CLL requiring first treatment and in relapsed cases, and to ~30% in Richter syndrome patients [2,3]. *NOTCH1* mutations are significantly more frequent in CLL with unmutated, rather than mutated, immunoglobulin genes, are significantly enriched in CLL harbouring trisomy 12, and identify a distinct clinico-molecular subgroup of CLL with deregulated cell cycle [2,3]. *NOTCH1* mutations in CLL mainly cluster within a hotspot in exon 34 and are commonly represented by a single 2-bp deletion (c.7544_7545delCT) that accounts for ~80 to 95% of all *NOTCH1* mutations in this leukaemia. The predicted functional consequence of *NOTCH1* mutations in CLL is the disruption of the C-terminal proline-glutamate-serine-threonine (PEST)-rich domain of *NOTCH1*, resulting in impaired degradation and accumulation of the activated *NOTCH1* protein, and sustained deregulation of the signalling. Consistent with this notion, a number of cellular pathways are specifically altered in CLL harbouring *NOTCH1* mutations [2,3].

The *SF3B1* gene encodes a core component of the spliceosome machinery involved in both normal and alternative splicing [4,5]. Whole genome/exome sequencing technologies allowed for the identification of *SF3B1* as a recurrently mutated gene in CLL [4,5]. *SF3B1* mutations recur in ~5 – 10% newly diagnosed CLL, in ~15% progressive CLL requiring first treatment and in ~20 – 25% relapsed and fludarabine-refractory patients. *SF3B1* mutations in CLL cluster in selected HEAT repeats of the *SF3B1* protein, target a number of hotspots (codons 662, 666, 700, 704 and 742), and are generally represented by missense substitutions [4,5]. Among CLL genetic lesions, *SF3B1* lesions have shown a preferential, although not consistent, association with 11q22-q23 deletion and *ATM* mutations [5]. The precise biological consequence of

SF3B1 mutations in CLL is currently unknown. However, the clustering of *SF3B1* mutations within the HEAT domains suggests that they are selected to modify SF3B1 interactions with other proteins of the spliceosome complex, thus resulting in deregulated normal and alternative mRNA splicing.

The *BIRC3* gene encodes a negative regulator of non-canonical NF-κB signalling [6]. *BIRC3* is recurrently disrupted by mutations, deletions or a combination of mutations and deletions in CLL patients. Although occurring at low rate in newly diagnosed CLL (~5% of cases), *BIRC3* lesions are enriched among relapsed and fludarabine-refractory CLL (~25% of cases) [6]. *BIRC3* inactivating mutations and a fraction of *BIRC3* deletions cause a truncation of the C-terminal RING domain of the *BIRC3* protein, essential for ubiquitination and proteasomal degradation of MAP3K14, the downstream activator of non-canonical NF-κB signalling. Consistently, *BIRC3* lesions drive constitutive NF-κB activation in CLL [6]. *BIRC3* is also involved in maintaining wild-type *TP53* levels by preventing NF-κB-mediated transcriptional and post-translational modifications of MDM2 expression and function. Consistently, *BIRC3* knockdown contributes to cancer promotion through downregulation of the *TP53* protein via MDM2.

The *BIRC3* gene maps to 11q22.2, approximately 6 Mb centromeric to the *ATM* locus. The identification of *BIRC3* involvement in CLL might be important for elucidating the molecular genetics of 11q22-q23 deletion, a frequent cytogenetic abnormality predictive of poor outcome. In fact, although *ATM* has been regarded as the relevant gene of this chromosomal abnormality, biallelic inactivation of *ATM* does not exceed ~30% of cases with 11q22-q23 deletion [5]. The presence of an additional tumour suppressor in the 11q22-q23 region has been postulated, and *BIRC3* implicates a suitable candidate.

Clinical relevance of the new CLL genetic markers

Retrospective studies have consistently shown the impact of *NOTCH1* and *SF3B1* mutations on newly diagnosed CLL outcome. *NOTCH1* mutated patients have a more rapidly progressive disease and a significantly shorter overall survival (OS) probability (21–45% at 10 years) compared with *NOTCH1* wild-type cases (56–66% at 10 years) [2,3,7]. The poor prognosis associated with *NOTCH1* mutations in CLL may be explained, at least in part, by a substantial risk (~40–50%) of developing Richter syndrome [8]. *SF3B1* mutated patients are characterized by a significantly shorter time to treatment requirement and a significantly shorter OS (10–48% at 10 years) compared with wild-type cases (60–77% at 10 years) [4,5]. In a retrospective analysis of newly diagnosed CLL, *BIRC3* disruption identifies patients with a poor survival (median OS of 3 years) similar to that associated with *TP53* abnormalities [6].

The integration of these new CLL genetic markers into the backbone of the fluorescence *in situ* hybridization (FISH) hierarchical model has allowed a better understanding of the genetic basis of CLL heterogeneity and a significant improvement in patients' prognostication [9]. According to this integrated mutational and cytogenetic model, four risk groups of patients are hierarchically classified [9]: (i) high-risk patients, harbouring *TP53* and/or *BIRC3* abnormalities independent of co-occurring genetic lesions, that account for ~15–20% newly diagnosed CLL and show a 10-year survival of 29%; (ii) intermediate-risk patients, harbouring *NOTCH1* and/or *SF3B1* mutations and/or del11q22-q23 in the absence of *BIRC3* and *TP53* abnormalities, that account for ~15–20% newly diagnosed CLL and show a 10-year survival of 37%; (iii) low-risk patients, harbouring +12 or a normal genetics, that account for ~40% of newly diagnosed CLL and showed a 10-year survival of 57%; and (iv) very low-risk patients, harbouring del13q14 only in the absence of any additional abnormality, that account for ~20–25% newly diagnosed CLL, and showed a nearly normal life expectancy with a 10-year survival (69%) that did not significantly differ from a matched general population.

Data from prospective studies and clinical trials validate the clinical importance of *NOTCH1* and *SF3B1* mutations in CLL. In a prospectively collected population-based cohort of newly diagnosed CLL patients, the presence of *NOTCH1* or *SF3B1* mutations was strongly associated with poor outcome, both in terms of shorter time to treatment (4.8 months in patients harbouring *NOTCH1* mutations and of 2.4 months in patients harbouring *SF3B1* mutations) and decreased OS (66 months in *NOTCH1* mutated patients and 63 months in *SF3B1* mutated patients) [10].

In the UK LRF CLL4 trial, that enrolled progressive and previously untreated patients, CLL harbouring *NOTCH1* and *SF3B1* mutations have an OS (55 and 54 months, respectively) that was significantly shorter compared with wild-type patients (83 months) and longer than that of patients carrying *TP53* abnormalities (26 months) [11]. These data document that, also at the time of treatment requirement, patients with *NOTCH1* and *SF3B1* mutations display an outcome that is intermediate between the one marked by *TP53* abnormalities and the one characterizing wild-type cases [11].

The German CLL Study Group (GCLLSG) is currently exploring the impact of monoclonal antibodies and allogeneic hematopoietic stem cell transplantation in overcoming the prognostic impact of *NOTCH1* and *SF3B1* alterations. Preliminary data from the GCLLSG CLL8 trial indicate that both *SF3B1* and *NOTCH1* mutations represent independent predictors of short progression-free survival after treatment with fludarabine, cyclophosphamide and rituximab (FCR) [12]. In particular, in this trial, *NOTCH1* mutations appear to identify a subset of

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CLL patients that may not benefit from the addition of rituximab to fludarabine and cyclophosphamide. Conversely, on the basis of a preliminary analysis of the GCLLSG CLL2H trial, patients harbouring *NOTCH1* mutations may have a superior progression-free survival after alemtuzumab treatment compared with *NOTCH1* wild-type cases, at least in the relapsed/refractory setting [13]. Data from the GCLLSG CLLX3 trial suggest that reduced-intensity allogeneic hematopoietic stem cell transplantation can provide long-term disease control in patients with poor-risk CLL independent of the presence of *TP53*, *SF3B1* and *NOTCH1* mutations [14].

Although information on the impact of *BIRC3* lesions on response to treatment is currently lacking, their enrichment among fludarabine-refractory CLL might suggest an association of these molecular defects with chemorefractory progression [6].

Conclusions

Given the growing number of new targeted agents, the management of CLL will conceivably be revised. In this changing scenario, there is increasing interest in the use of prognostic markers that may guide management of patients since the early phases of the disease. To advance the field, it will be crucial to build stronger genetic models of CLL subgroups and to discover and implement genetic predictors of treatment response and genotype-specific approaches. Future challenges are also to design rapid and affordable molecular assays and to prospectively define if specific treatments may overcome the poor prognosis conferred by higher risk lesions.

Conflicts of interest

The author has no conflict of interest to disclose.

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