Blood Reviews 25 (2011) 131–137

Contents lists available at ScienceDirect

Blood Reviews

journal homepage: www.elsevier.com/locate/blre

REVIEW

Importance of genetics in chronic lymphocytic leukemia

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ARTICLE INFO

Keywords:
CLL
17p13 deletion
11q23 deletion
p53
13q14 deletion
Prognosis

ABSTRACT

Recurrent losses or gains of genomic material as well as mutations of key tumor suppressors (*ATM* and *TP53*) have been identified in chronic lymphocytic leukemia (CLL). These aberrations are important "drivers" of the disease and some of its clinical characteristics. There is a remarkable heterogeneity in the clinical course between patient subgroups with distinct genetic features. While some mutations are associated with poor outcome (particularly 17p− and *TP53* mutation and to a lesser extend 11q−) others are linked to a favorable outcome (13q− as sole aberration; mutated IGHV). Our improved understanding of the clinical course of specific genetic subgroups is beginning to be translated into genotype specific treatment approaches where genetic subgroups (e.g. 17p−) are channeled into separate treatment protocols.
This review will summarize the most important genetic aberrations in CLL and how our improved knowledge of the genetic make-up of leukemic cells may translate into improved treatment results.

1. Introduction

Genetic aberrations are a hallmark of cancer.[1] Recurrent losses or gains of chromosomal material as well as mutations of key tumor suppressors (*ATM* and *TP53*) have been identified in chronic lymphocytic leukemia (CLL).[2] These aberrations are important "drivers" of the disease as well as clinical characteristics. There is a remarkable heterogeneity in the clinical course among patient subgroups with distinct genetic features. Some patients with chronic lymphocytic leukemia survive for many years or decades without need for treatment. Others have a rapidly fatal disease despite therapy. This heterogeneity is in part accounted for by our current prognostic models.[2–4] CLL has turned out to be a disease with multiple facets in its pathogenic mechanisms including genetic aberrations, antigen drive and microenvironmental interactions.

Future approaches aimed at improving treatment strategies should focus on the definition of the clinical use of prognostic factors (Table 1) based on prospective trial data and the identification of predictive factors. This will lead to a genotype-specific approach to the treatment of CLL. The first subgroup of patients to be treated in specific (genotype specific) treatment protocols are patients with 17p deletion, who have repeatedly been shown to have very poor survival once treatment indications arise.[5–8]

This review will summarize the most important genetic aberrations in CLL and how our improved knowledge of the genetic make-up of leukemic cells may translate into improved treatment results.

2. Genomic aberrations in CLL

Our knowledge of genomic aberrations in CLL has initially been based on chromosome banding studies using TPA as a mitogen.[9] These studies have been held back by the low mitotic activity of CLL cells. With this method, clonal chromosomal aberrations were detected in only 40 to 50% of cases, the most common being trisomy 12 and abnormalities of chromosome band 13q14.[9]

More recently, metaphase analysis has had a "revival" because the metaphase yield has been improved by stimulation of CLL cells with alternative methods like CD40 ligand expressing cells and IL-4 or CpG-oligodeoxynucleotides and IL-2.[10] This approach revealed that "translocations" occurred in about one third of CLL patients. After stimulation with CpG-oligodeoxynucleotides and IL-2, the observed aberration rate was comparable to the rate detected by parallel interphase FISH, and the method frequently detected balanced and unbalanced translocations.[11] The studies also demonstrated that additional cases of complex aberrations (more than 3 aberrations) can be detected. In a recent update on the results of metaphase cytogenetics after stimulation with CpG-oligonucleotide DSP30 and IL-2, a total of 500/506 (98.8%) cases were successfully analyzed.[12] Aberrations were detected in 415 of 500 (83.0%) cases by banding and in 392 of 500 (78.4%) cases by FISH due to limitations of the probe set. Future studies will show if the metaphase cytogenetics after stimulation will result in a deeper understanding of the disease and prognostic subgroups.[2]

Fluorescence in situ hybridization (FISH) allows the detection of chromosomal aberrations irrespective of the cell's ability to divide.

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doi:10.1016/j.blre.2011.02.002

Table 1
Summary of current prognostic factors and potential treatment options in patients with CLL and 1st line treatment indication.

	Risk factor	Incidence (1st line treatment)	Treatment approach
Ultra high risk (~10–15%)	17p deletion *TP53* mutation F-refractory CLL	~5–8% ~4–5% ~5%	Clinical trial with investigational agent acting independent of p53, allogeneic stem cell transplantation
High risk (~70%)	Unmutated *IGHV* *V3-21* usage High ß-2M (TK) 11q deletion	~60% <1% ~20%	FCR, maintenance trials, investigational agents + FCR
Low risk (~20%)	Mutated *IGHV* (and none of the above)	~22%	FCR, De-escalation in clinical trials

This approach is also referred to as interphase cytogenetics.[13] Genomic aberrations can be identified in about ~80% of CLL cases by fluorescence in-situ hybridization (FISH) with a disease specific probe set. The most common recurrent chromosomal abnormalities include deletion 13q, trisomy 12, deletion 11q, 17p and 6q.[6] A subdivision based on these aberrations is important, as it is associated with the rate of disease progression and the overall survival time of CLL. Five prognostic categories have been defined in a hierarchical model showing poor survival in patients with 17p deletion and 11q deletion (median survival 32 and 79 months), but better survival for patients with trisomy 12q normal karyotype, and deletion 13q as the sole abnormality (114, 111, and 133 months, respectively).[6]

While certainly the current method of choice, FISH will only "cover" a limited set of aberrations and recent developments of comparative genome hybridization (CGH) and in particular high-resolution SNP arrays have lead to a better understanding of the genetic profile of CLL.

In a series of 106 CLL cases a study of array-based CGH uncovered previously unrecognized recurrent genomic imbalances including a copy number gain of chromosome 19 and the *MYCN* oncogene on 2p.[14]

In an early study on high resolution SNP-array,[15] 10k and 50k SNP arrays were evaluated as a diagnostic tool and revealed chromosomal imbalances in 65.6% and 81.5% of 70 CLL cases, respectively. The aberrations previously shown to have prognostic importance were identified at the expected frequency. In addition, 24 large (>10 Mb) copy number neutral regions with LOH were identified in 14 cases. These abnormalities are not detectable by most other methods and the identification may lead to the discovery of imprinted genes or loss-of-function alleles which may be important for the pathogenesis of CLL.

More recent studies using SNP-arrays have focused on the architecture of recurrent aberrations, their clinical impact and an association between a higher number of aberrations and outcome.[16–18]

2.1. 13q14 deletion

Loss of 13q14.3 is the most common chromosome aberration in CLL, which is present in the majority of CLL cases. In contrast to other recurrent aberrations, 13q14 deletions may be hetero- or (less commonly) homozygous. Deletions at 13q14 are not confined to CLL but also occur at high frequencies in other lymphomas and solid tumors, such as mantle cell lymphoma, multiple myeloma, and prostate and lung cancer. In addition to deletions, copy number neutral loss of heterozygosity has been demonstrated for the 13q14 region.

Studies of serial samples suggest that heterozygous deletion of 13q14 is an early event, whereas deletion of the second copy of this region occurs at a later stage.[19,20]

The discovery of the genes targeted by the deletion has been one of the fascinating stories of the molecular genetics of leukemia. Despite extensive efforts, mutation analysis of protein-coding genes in this region revealed no inactivation of candidate genes. A complex epigenetic regulatory mechanism that would control the expression of the entire region (and may account also for cases without 13q14 deletion) has been proposed.[21,22]

In a crucial study, Calin and co-workers showed that 13q14 deletion in CLL is associated with down-regulation of miR-15a and miR-16-1, whose genes cluster in the minimally deleted region (MDR) within 13q14.[23] Mutations in the miR locus appear to be very rare.[23,24] In mice, a study identified a point mutation in the 3′-DNA neighboring the miR-16-1 region that has been associated with reduced miR-16-1 expression in the New Zealand black mouse, a model for indolent, late-onset CLL.[25]

Definite evidence for the pathogenic significance of the 13q14 MDR and miR-15a/miR-16-1 in B-cell-malignancies has been provided recently by generating a mouse model deleting the miR-15a/16-1 cluster (Fig. 4).[26] Deletion in mice of the 13q14-minimal deleted region (MDR) causes development of indolent B cell-autonomous, clonal lymphoproliferative disorders, recapitulating the spectrum of CLL-associated phenotypes observed in humans. Mice presenting deletions of the MDR or with sole miR-15a/16-1 deletion developed clonal B cell lymphoproliferation at the age of 15 to 18 months, including CD5+ MBL, CLL/SLL, and NHL in 42% of MDR−/− and 26% of miR-15a/16-1−/− cases. While these data suggest that the deletion of the miR-15a/16-1 is sufficient for lymphomagenesis, it also suggests that additional components in 13q14 are contributing to the pathomechanism in CLL because the phenotype in these mice was more pronounced and there was a greater penetrance of lymphoma/CLL. Therefore additional tumor suppressive functions within the MDR on 13q14 are likely to contribute to the disease.[26]

In the work of Ulf Klein and colleagues, the miR15a/16-1 cluster has been shown to influence growth, cell-cycle control and/or apoptosis although the exact regulatory function by which miR15a/16-1 exert their pathogenic effects in CLL remains unclear.[26] The group found limited evidence of the miR-15a/miR-16-1 to regulate BCL-2.[23,27,28]

Patients with a monoallelic del 13q14 show slower lymphocyte growth kinetics than patients with biallelic deletions,[15] implicating mechanisms which regulate the gene-dosage of these miRs. Nonetheless, questions remain as for example the question if and how miR-15a/16-1 levels could be deregulated in cases without 13q deletion.

Patients with 13q14 as the only aberration based on FISH data (13q− sole) show a favorable prognosis. In the initial study of the hierarchical model of Döhner patients with this aberration had the most favorable outcome with a median survival of 133 months. In more recent analysis of prospective trial cohorts, the outcome of patients with 13q− as the sole aberration is similar to patients with no aberrations on FISH analysis or patients with trisomy 12 (Fig. 1).[29]

In addition, recent analysis of the 13q14 deletion architecture by SNP arrays suggests that the extent of the deletion contributes to disease characteristics.[16,30]

While it is possible that there are subtle differences among the good risk groups, which will just need longer follow-up to be uncovered, our better understanding of 13q14 deletion and its influence on outcome has not yet translated into its use as a predictive factor. Given the favorable outcome, patients with this aberration (as sole abnormality) may be considered for trials studying de-escalation strategies.

2.2. Trisomy 12

Trisomy 12 is among the most frequent aberrations in CLL (10 to 20%). Often the aberration is subclonal and potential genes involved in the pathogenesis of CLL with trisomy 12 are unknown. Trisomy 12 has been associated with "atypical" CLL with more frequent atypical