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Chronic lymphocytic leukemia – genomics lead the way

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As the most common leukemia in the Western world, chronic lymphocytic leukemia (CLL) represents a prime example to demonstrate how modern genomic technologies have rapidly contributed to an improved understanding of cancer in general and how genomics have already started to translate into clinical practice.

In all cancers, characterizing the impact of the genetic aberrations on the phenotype of the disease has led to novel insights into the pathology underlying the disease. CLL is a neoplasm of mature B cells, a tissue in which the immunoglobulin genes are genetically modified to produce a functional B-cell receptor. The specific configuration of the immunoglobulin genes therefore gave further insight into the differentiation process of the malignant clone.[1] While non-malignant B cells use the full repertoire of immunoglobulin gene segments to produce the largest possible number of different B-cell receptors, in CLL cells the usage of immunoglobulin heavy chain variable gene segments (*IGHV*) is drastically restricted and biased towards a limited number of genes.[2] In addition, the process of somatic hypermutation of the variable region, which in non-malignant B cells leads to a change in binding affinity, allows CLL patients to be stratified into two biological and prognostic subgroups according to whether they harbor mutated or non-mutated *IGHV* genes.[1] These genetic findings led to the current model of the pathomechanism of CLL, according to which stimulation by (self-) antigens provides a pro-survival and possibly pro-proliferative advantage for CLL (precursor) cells, most likely leading initially to oligoclonal and subsequently monoclonal selection of malignant cells (Figure 1B). This model was recently improved significantly by findings from a murine xenotransplant model. Here, hematopoietic stem cells

purified from CLL patients and transplanted into immunosuppressed mice matured into mono- or oligo-clonal B cells and produced a CLL-like phenotype.[3] This indicates that the hematopoietic stem cells of CLL patients already have the inherent ability to initiate a CLL precursor state.

The detection of genomic aberrations has been of key importance towards understanding the mechanism of chemotherapy of CLL cells and has enabled the construction of a hierarchical model of genetic lesions that correlate with outcome.[4] Specifically, deletion of the *ATM* and *TP53* tumor suppressor genes, localized at 11q22-q23 and 17p13 respectively, was shown to be associated with poor outcome. Loss of function of these genes likely provides an advantage to the malignant B cells under the selective pressure of chemotherapy, in particular in the case of *TP53* deletion and/or mutation (Figure 1).[5] Over the last years, the prognostic impact of these cytogenetic aberrations has been translated into clinical practice, and while early clinical prognostic parameters such as age, stage and serum markers are still in use,[6] they are nowadays complemented by the information from fluorescence *in-situ* hybridization (FISH) and mutation analysis of the *IGHV* genes.[1]

While interphase FISH has proven valuable, the advent of microarrays that allow genome-wide genomic profiling has further advanced the field. This novel technology increases the resolution of the detection of genomic gains and losses enormously. Resolution is basically limited only by the number and the genomic distribution of arrayed elements on the respective platforms. While the first microarrays used for comparative genomic hybridization had a resolution in the range of several hundred kilobase pairs (kb), later array platforms had a greatly improved resolution of less than 100 kb in selected areas and already allowed the detection of novel CLL-associated genomic aberrations.[7] Recently, novel single nucleotide polymorphism-based microarrays that allow even more detailed insights have been developed. Using these arrays, in a critical region on chromosome 13q14, the most common genetic aberration in CLL, precise mapping has identified the prognostic impact of the size of the deletion, pointing towards a multigeneic tumor suppressor mechanism in that region.[8] Recent studies with high-resolution single nucleotide polymorphism microarrays were also able to identify new risk variants for CLL via genome-wide association studies i.e. linkage analyses. A first study tagging 299,983 single nucleotide polymorphisms in a total of 2,503 CLL cases and 5,789 controls identified four new CLL risk loci at 2q37.3 (rs757978, *FARP2*), 8q24.21 (rs2456449), 15q21.3 (rs7169431), and 16q24.1 (rs305061), and suggested that there were additional risk loci at 15q25.2 (rs783540, *CPEB1*) and 18q21.1 (rs1036935).[9] While these data provide further evidence for genetic susceptibility to CLL (Figure 1), in this issue of Haematologica Wade *et al.* present a linkage analysis based on 346,831 single nucleotide polymorphisms in 356 CLL patients from a clinical phase III trial in order to identify genomic single nucleotide polymorphism variants. They correlated the single nucleotide polymorphism variants with the response to first-line chemotherapy with fludarabine, chlorambucil or a combination of fludarabine plus cyclophosphamide.[10] Interestingly, one of the single nucleotide polymorphisms (rs2255235) that could be associated with progression-free survival is localized at the 5´ end of β_2 microglobulin (*B2M*), a part of the major histocompatibility complex. The serum levels of β_2 microglobulin protein correlate with the tumor burden and β_2 microglobulin is, therefore, a prognostic marker in CLL, with high levels of β_2 microglobulin correlating with a

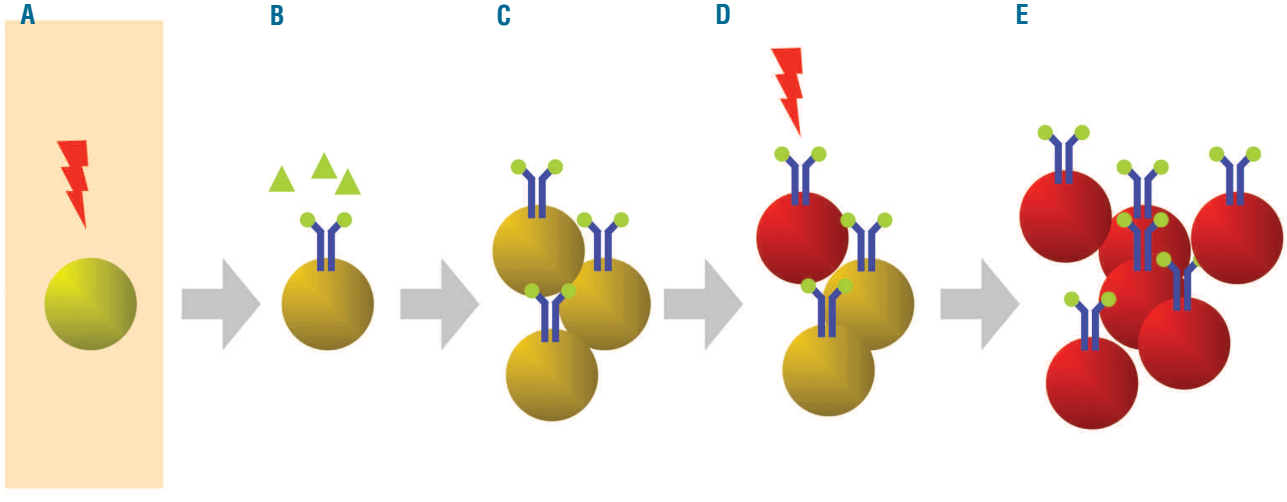

Figure 1. Putative model for the mechanism of leukemogenesis of chronic lymphocytic leukemia. (A) Hematopoietic stem cells sorted from a CLL patient's bone marrow produce a CLL-like disease when transplanted into immunosuppressed mice. It has, therefore, been postulated that hematopoietic stem cells from CLL patients already harbor (pre)leukemia-initiating events. (B) The biased usage of specific immunglobulin heavy chain variable gene segments (*IGHV*) in CLL cells suggests antigenic stimulation as a pro-survival and/or pro-proliferative selective drive. While only shown at a single stage in this figure, antigenic stimulation of CLL cells with non-mutated *IGHV* is likely to occur at all stages of disease development. (C) This results in oligoclonal expansion of pre-malignant cells. (D) Secondary (epi)genomic and/or cytogenetic lesions result in malignant transformation and (E) massive accumulation of CLL cells with further selection e.g. of clones with loss of function of *TP53* (17p) and potentially other genes during the selective pressure of chemotherapy.

worse outcome.[6] In the study by Wade *et al.*, the variation at the single nucleotide polymorphism rs2255235 in the *B2M* gene did not, however, correlate with the levels of serum β_2 microglobulin.[10] The significance of this single nucleotide polymorphism does, therefore, remain to be investigated further, especially with regard to a potential prognostic benefit of β_2 microglobulin serum levels. In addition, the correlation of a single nucleotide polymorphism (rs11158493) in the *PPP2R5E* gene is of interest.[10] Recently, a *PPP2R5E* interacting partner and a member of the same gene family, *PPP2R1B*, were reported to be localized in 11q22-q23, a critical recurrently deleted region in CLL. A second tumor suppressor in this region, besides *ATM*, has been postulated.[11] While no genetic aberrations have been found in *PPP2R1B* at 11q22-q23, its gene product seems to be differently spliced in CLL cells.[11] These findings suggest that single nucleotide polymorphisms might play a significant role in the pathogenicity of this gene family. However, although the findings of Wade *et al.* are very interesting, it must be noted that none of the associations was statistically significant in an independent series of 380 cases of CLL.[10] Thus, all prognostic and predictive effects associated with single nucleotide polymorphisms identified based on linkage analyses should be cautiously interpreted and warrant further validation in independent data sets prior to clinical use.

As for microarray studies of genomic aberrations, microarray-based gene expression profiles have started to be developed into clinical diagnostic tools in lymphoma and leukemia. However, in the future, recent advances in sequencing technologies are very likely to initiate the next diagnostic revolution. Here again, CLL can be viewed as a model disease entity since next-generation sequencing was successfully applied for whole-genome sequencing of four cases of CLL.[12] Forty-six somatic mutations that potentially affect gene function were identified. The most frequently recurrent aberrations were gain-of-function mutations of the outer-membrane receptor and transcriptional activator *NOTCH1*. Found in 12% of CLL patients, most *NOTCH1* mutated cases seem to carry unmutated *IGHV* genes. In addition, the authors demonstrated that the mutations mostly affected the functional PEST domain of *NOTCH1*, thereby leading to enhanced stability of the protein and to differential gene expression patterns of target genes in CLL patients.[12] Interestingly, an elevated rate of *NOTCH1* mutation was also recently observed during disease progression towards Richter's transformation as well as in chemotherapy-refractory CLL,[13] underscoring the importance of this gene in the pathomechanism of the disease. Furthermore, recent developments of sequencing technology, including so-called paired-end next-generation sequencing, offer improved detection of chromosomal rearrangements. The methodology led to the discovery of cataclysmic shattering of a single chromosome in cancer.[14] Initially observed in CLL, this unprecedented genomic aberration, termed chromothripsis, may be present in 2-3% of all cancers.[14]

In addition to genetic aberrations, epigenetic modifications seem to play a major role in the pathomechanism of CLL. First genome-wide analyses revealed that up to 8% of all CpG units are aberrantly methylated in CLL cells.[15] An even more striking finding in CLL was the aberrant epigenetic silencing of the death-associated protein kinase 1 (DAPK1) in virtually all CLL samples analyzed (67/69).[16] In addition, epigenetic lesions seemed to precede cytogenetic aberrations in a CLL mouse model.[17] Similarly, in 13q14, the region that is most commonly genetically inactivated, we have identified a complex epigenetic tumor suppressor mechanism.[18] It is, therefore, possible that a similar sequence of events at 13q14 leads to the development of CLL: first a gradual epigenetic silencing of a tumor suppressor mechanism which is then superseded by full genetic inactivation by deletion (Figure 1).[19]

An initial epigenetic aberration predisposing to CLL could also explain the surprising recent finding that hematopoietic stem cells isolated from CLL patients are capable of forming a transplantable CLL precursor-like disease in mice, suggesting a common aberration already present in the hematopoietic stem cells (Figure 1A).[3] While this hematopoietic stem cell defect could also be a genetic lesion, the cytogenetic aberration(s) present in the mature CLL cells were not detected in the hematopoietic stem cells from CLL patients, and no common genetic mutation was found in the coding regions of four CLL whole genomes sequenced which could thus be causative.[12] It remains to be seen whether mutations in non-coding RNA genes or sequence variants in intergenic regions could be the underlying cause of the development of CLL: for example, in the most commonly deleted region in 13q14, a rare sequence variation was found in the non-coding RNA genes miR15a and miR16-1 leading to a defect in the maturation process of these non-coding genes.[20] Thus, genomics will lead the way to improved characterization of the pathomechanisms underlying CLL and further advance prognostication and risk-adapted management for patients.

Daniel Mertens studied biochemistry at the University of Tübingen and took his PhD under Prof. Lichter at the German Cancer Research Center (DKFZ) in Heidelberg. He received a Junior Group at the DKFZ and at the same time a Max-Eder Research Group at the Department of Internal Medicine III of the University of Ulm (Prof. Döhner). These groups are combined in the Cooperation Unit "Mechanisms of Leukemogenesis" which allows the translational application of basic research at the DKFZ to preclinical research at the University of Ulm. The focus of his research is on the role of epigenetics and the microenvironment in leukemogenesis.

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