Translating genetic and epigenetic knowledge into clinical utility

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Take Home Messages

- While only a handful of ‘driver’ genes are frequently mutated in CLL, a seemingly endless ‘tail’ of low-frequent, recurrently mutated genes have so far been reported.
- Considering the growing list of clinically relevant genetic aberrations identified, it will be necessary to shift from Sanger sequencing to next-generation sequencing-based approaches that also enable sensitive detection of minor subclones.
- Gene mutations linked to development of resistance to B-cell signaling and BCL2 inhibitors have recently been identified.

Introduction

The advent of next-generation sequencing (NGS) technologies revolutionized our understanding of the genomic landscape of CLL. It is now apparent that only a handful of ‘driver’ genes are more frequently mutated (i.e. TP53, ATM, SF3B1 and NOTCH1), while an endless ‘tail’ of low-frequent, recurrently mutated genes is observed affecting key signaling pathways and processes (Figure 1).1-4 This genetic heterogeneity makes it difficult to assess the clinical impact of mutations occurring in only a minor proportion of patients, while also posing a challenge when selecting which gene mutations to study to better understand their functional role in CLL development.5 An additional layer of complexity stems from the clonal architecture evidenced within each tumor with subclones emerging and disappearing throughout the disease course and in response to a particular therapy.6-8

What are the initiating events in CLL?

Despite the discovery of driver mutations in CLL, the precise mechanisms implicated in disease onset remain unknown. In a recent study, whole-genome sequencing (WGS) was performed in individuals with monoclonal B-cell lymphocytosis (MBL), both of the low-count and high-count subtype, as well as in patients with ultra-stable CLL; i.e. >10 years without clinical progression.9 The genomic landscape of these three entities was essentially indistinguishable, with on average 2000-2500 somatic variants detected per case in all settings. In addition to 13q deletions (encompassing miRNA15/16) seen in all three settings, exonic variants in putative CLL driver genes were rare, a finding confirmed by targeted deep-sequencing, and the few driver mutations detected were, per se, not linked to progression.9 Notably, shared somatic variants were detected between MBL/CLL cells and paired polymorphonuclear cells, supporting recent reports describing the presence of CLL mutations in hematopoietic progenitor cells.10

Considering the absence of known putative driver gene mutations in MBL and ultra-stable CLL, this poses the question: what is the driving force in CLL pathogenesis? A plausible answer pertains to the B cell receptor (BcR), an established critical player for both disease ontogeny and patient management.5,11 Indeed, one might envision a scenario where antigen selection of cells expressing particular BcRs may represent a key initiating event, whereas CLL progression may require the acquisition of at least one driver mutation, as evidenced in most cases with clinically aggressive disease.7,8

New players on the scene

Today, >1000 CLL patients have been analyzed by whole-exome sequencing (WES)/WGS and >2000 recurrently mutated genes have been reported, most of which were found in <5% of patients.1,4,7 One such example concerns EGR2 mutations, which were first reported in a study on early hematopoietic progenitor cells in CLL and linked to aggressive disease.10 In a recent, large-scale study, EGR2 mutations were detected in ~4% of cases and independently predicted short time-to-first-treatment and overall survival, similar to patients with TP53 aberrations, hence likely defining a new high-risk group of CLL.12 By applying WES on sequential samples in patients relapsing after chemotherapy, pronounced dynamic changes were observed between baseline and relapse, with increased frequencies of high-risk mutations (e.g. TP53, ATM, NOTCH1, SF3B1, EGR2).7,8 A novel finding of recurrent mutations in RPS15, a gene encoding a component of the 40S ribosomal subunit, was linked to poor outcome and in pilot functional studies, RPS15 mutations appear to negatively impact ribosome fidelity, but may also be involved in dysregulation of p53 through MDM2/MDM2X to which RPS15 binds.7,8 More recently, in studies involving patients relapsing or progressing on the BTK inhibitor, ibrutinib, the majority of patients appear to acquire BTK or PCLG2 mutations.13,14 In contrast, no specific mutation has been identified in patients relapsing on the PI3Kδ inhibitor,
idelalisib, while patients developing venetoclax resistance showed e.g. recurrent BTG1 mutations and homozygous CDKN2A/B deletions.

**Clinical impact of epigenetics?**

Characterization of the methylome has added a new layer of complexity to CLL biology. Recently, DNA methylation profiling of CLL subsets and different B cell subpopulations corresponding to distinct differentiation stages (e.g. naïve or memory cells) revealed that most methylation changes seen in CLL reflect the methylome observed in the cell of origin, while only a minor fraction represents CLL-specific changes. Based on this observation, CLL was grouped into three epigenetic subgroups with distinct outcomes i.e. ‘naïve-like CLL’, ‘memory-like CLL’ and a third intermediate group, potentially originating from different B cell subpopulations. A simplified epigenetic classifier was developed based on only 5 CpG sites, which has been validated by independent groups and accurately predicts the three prognostic subgroups.

**How to transfer recent advances into clinical utility?**

Many attempts have been made to design prognostic indices for CLL that include clinical and/or genetic parameters. In the recently developed CLL-IPI, which includes age, stage, β2-microglobulin levels, IGHV mutation status and TP53 aberrations, patients are grouped into four risk-groups with distinct outcome. However, many poor-risk markers are unevenly distributed between IGHV-mutated versus IGHV-unmutated CLL. Therefore, it is reasonable to consider different prognostic schemes for these two major groups, as recently proposed. The need to follow a more compartmentalized approach was further reinforced by the strikingly different frequencies of genetic lesions in subsets of patients expressing quasi-similar or stereotyped BcR IGs, exemplified by subset #2 which harbors a high frequency (44%) of SF3B1 mutations.

Considering the new clinically relevant genetic aberrations that have been identified, the shift from Sanger sequencing to NGS-based approaches is essential. Recent evidence for the clinical significance of minor clones carrying TP53 mutations indicates

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**Figure 1. Signaling pathways and processes affected in CLL.**

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that it will also be necessary to develop gene panels and methodologies with higher sensitivity (down to 0.1%).29 In order to assess the prognostic impact of mutations within one particular gene in relation to other gene mutations, the European Research Initiative on CLL (ERIC) is currently performing a large-scale study including 10 genes and more than 4000 patients. This effort will hopefully inform us as to which genes to include in future diagnostic set-ups. Finally, in this era of tailored therapy, it will be necessary to appreciate the effect of mutations on drug response and in a future setting potentially screen for such mutations. Furthermore, the impact of subclones containing specific mutations should be addressed by focused efforts where disease evolution will be followed over time, with the ultimate aim of translating this knowledge into clinical routine.

References

1. Puente XS, Pinyol M, Quesada V, et al. Whole-genome sequencing identifies recurrent mutations in chronic lymphocytic leukaemia. Nature 2011;475:101-5.
2. Quesada V, Conde L, Villamor N, et al. Exome sequencing identifies recurrent mutations of the splicing factor SF3B1 gene in chronic lymphocytic leukaemia. Nat Genet 2011;44:47-52.
3. Wang L, Lawrence MS, Wan Y, et al. SF3B1 and other novel cancer genes in chronic lymphocytic leukaemia. N Engl J Med 2011;365:2497-506.
4. Puente XS, Bea S, Valdes-Mas R, et al. Non-coding recurrent mutations in chronic lymphocytic leukaemia. Nature 2015;526:519-24.
5. Sutton LA, Rosenquist R. The complex interplay between cell-intrinsic and cell-extrinsic factors driving the evolution of chronic lymphocytic leukaemia. Semin Cancer Biol 2015;34:22-35.
6. Landau DA, Carter SL, Stojanov P, et al. Evolution and impact of subclonal mutations in chronic lymphocytic leukaemia. Cell 2013;152:714-26.
7. Ljungstrom V, Cortese D, Young E, et al. Whole-exome sequencing in relapsing chronic lymphocytic leukaemia: clinical impact of recurrent TPS15 mutations. Blood 2016;127:1007-16.
8. Landau DA, Tausch E, Taylor-Weiner AN, et al. Mutations driving CLL and their evolution in progression and relapse. Nature 2015;526:525-30.
9. Agathangelidis A, Scarfò L, Fazi C, et al. Highly similar genomic landscapes in monoclonal B-cell lymphocytosis and ultra-stable chronic lymphocytic leukaemia with low frequency of driver mutations. Haematologica 2018; doi:10.3324/haematol.2017.177212
10. The genomic landscapes in low-count and high-count monoclonal B-cell lymphocytosis and ultra-stable CLL are indistinguishable.
11. Damm F, Mylonas E, Coisson A, et al. Acquired initiating mutations in early hematopoietic cells of CLL patients. Cancer Discov 2014;4:1085-101.
12. Young E, Noerenberg D, Mansouri L, et al. EGR2 mutations define a new clinically aggressive subgroup of chronic lymphocytic leukaemia. Leukemia 2017;31:282-91.
13. Landau DA, Sun C, Rosebrock D, et al. The evolutionary landscape of chronic lymphocytic leukaemia treated with ibrutinib targeted therapy. Nat Commun 2017;8:2185.
14. Ahn IE, Underbayev C, Albitar A, et al. Clonal evolution leading to ibrutinib resistance in chronic lymphocytic leukaemia. Blood 2017;129:1469-79.
15. Ghia P, Tausch E, Agathangelidis A, et al. Whole-exome sequencing revealed no recurrent mutations within the PI3K pathway in relapsed chronic lymphocytic leukaemia patients progressing under ibrutinib treatment. Blood 2017;128:2770.
16. Herling CD, Abedpour N, Weiss J, et al. Clonal dynamics towards the development of venetoclax resistance in chronic lymphocytic leukaemia. Nat Commun 2018;9:727.
17. Mansouri L, Wierzbinska JA, Plass C, Rosenquist R. Epigenetic deregulation in chronic lymphocytic leukaemia: clinical and biological impact. Semin Cancer Biol 2018; pii: S1044-579X(17)30048-2.
18. Kulis M, Heath S, Bibikova M, et al. Epigenomic analysis detects widespread gene-body DNA hypomethylation in chronic lymphocytic leukaemia. Nat Genet 2012;44:1236-42.
19. Cahill N, Bergh AC, Kanduri M, et al. 450K-array analysis of chronic lymphocytic leukaemia cells reveals global DNA methylation to be relatively stable over time and similar in resting and proliferative compartments. Leukemia 2013;27:150-8.
20. Kulis M, Merkel A, Heath S, et al. Whole-genome fingerprint of the DNA methylome during human B cell differentiation. Nat Genet 2015;47:746-56.
21. Most of the DNA methylation changes observed in prognostic subgroups of CLL reflect the methylome observed in the cell of origin.
22. Queiros AC, Villamor N, Clot G, et al. A B-cell epigenetic signature defines three biologic subgroups of chronic lymphocytic leukaemia with clinical impact. Leukemia 2015;29:598-605.
23. Bhoi S, Ljungstrom V, Baliakas P, et al. Prognostic impact of epigenetic classification in chronic lymphocytic leukaemia: The case of subset #2. Epigenetics 2016;11:449-55.
24. Oakes CC, Claus R, Gu L, et al. Evolution of DNA methylation is linked to genetic aberrations in chronic lymphocytic leukaemia. Cancer Discov 2014;4:348-61.
25. Baliakas P, Mattsson M, Stamatopoulos K, Rosenquist R. Prognostic indices in chronic lymphocytic leukaemia: where do we stand how do we proceed? J Intern Med 2016;279:347-57.
26. International CLLIPwg. An international prognostic index for patients with chronic lymphocytic leukaemia (CLL-IPI): a meta-analysis of individual patient data. Lancet Oncol 2016;17:779-90.
27. Baliakas P, Moyssiadis T, Hadzidimitriou A, et al. Tailored approaches for refined prognostication in chronic lymphocytic leukaemia patients with mutated versus unmuted immunoglobulin receptors. Blood 2016;128:3199.
28. Sutton LA, Young E, Baliakas P, et al. Different spectra of recurrent gene mutations in subsets of chronic lymphocytic leukaemia harboring stereotyped B-cell receptors. Haematologica 2016;101:959-67.
29. Sutton LA, Ljungstrom V, Mansouri L, et al. Targeted next-generation sequencing in chronic lymphocytic leukaemia: a high-throughput yet tailored approach will facilitate implementation in a clinical setting. Haematologica 2015;100:270-6.
30. Rossi D, Khiabanian H, Spina V, et al. Clinical impact of small TP53 mutated subclones in chronic lymphocytic leukaemia. Blood 2014;123:2139-47.