Late Breaking Chromosomes

Don't Mess with *RUNX1*

Disturbed regulation of genes controlling leukomogenesis frequently underlays hematologic malignancies. Some 50 years ago, Nowell and Hungerford [1961] discovered in leukemic cells of patients with chronic myelogenous leukemia that a G-group chromosome had been replaced by a small and characteristic acrocentric chromosome, named after the location of the authors, the Philadelphia chromosome (Ph¹). This discovery suggested that an acquired chromosomal rearrangement may lead to a disease involving clonal expansion of a genetically altered cell, which no longer adequately responded to normal controls of cell proliferation and differentiation. By Q-banding, Rowley [1973] showed that the Ph¹ chromosome was due to a translocation of the long arms of chromosomes 9 and 22. Almost a decade later, disruption of the *ABL1* proto-oncogene resulting from the t(9; 22)(q34, q11) was demonstrated [de Klein et al., 1983]. This result provided the first molecular clue toward a basic mechanism(s) leading to hematological malignancies and to some types of solid cancers. Up to 2007, more than 50,000 cases of clonal, somatic chromosomal rearrangements, involving 337 distinct genes, have been described in human malignant disease [Mitelman et al., 2007]. This tremendous rise in our knowledge has been made possible by leaps in genetic technology from classical karyotyping and Q-banding to Southern blotting.

Meanwhile, next-generation sequencing has further expanded our ability to investigate genomes. Application of 2 of these novel techniques, whole-exome and mate-pair sequencing, has provided new insights into how another gene, *RUNX1*, may be involved in acute lymphoblastoid leukemia (ALL) and in familial platelet disorder with myeloid malignancy (FPD/AML). *RUNX1*, the runt-related transcription factor 1, being located in chromo-

somal region 21q22.12, encodes a heterodimeric transcription factor that binds to many enhancers and promoters. This gene was initially identified in acute myelogenous leukemia (AML) as the fusion partner in the t(8;21)(q22;q22) [Miyoshi et al., 1991]. Later on, the t(12;21)(p13;q22) in B-lineage-ALL and the t(4;21)(q28;q22) in T-lineage-ALL were also shown to affect *RUNX1* [Golub et al., 1995; Mikhail et al., 2004]. These findings emphasize the importance of *RUNX1* in governing proper hematopoiesis and indicate that this gene may be involved in several hematological disorders, depending on the mechanism of its disruption or perturbation. While fusion of 2 unrelated genes, resulting from structural genome variations in somatic cells, is still considered a major genetic cause of hematologic malignancies, 2 recent papers point toward other, equally important mechanisms [Buijs et al., 2012; Lilljebjörn et al., 2012].

Performing whole-exome sequencing of bone marrow and corresponding normal tissue-derived DNA samples from 2 patients with pediatric ALL, carrying the *ETV6/RUNX1* fusion gene, Lilljebjörn et al. [2012] found 14 somatic mutations. Of those, 11 had already been described in various solid tumors and one, of *FLT3*, in hematologic malignancies. Interestingly, no somatic single nucleotide variants (SNVs) were shared between the 2 ALL-derived DNA samples, and none were found in an extended series of 29 *ETV6/RUNX1*-positive ALL cases. Predictions of pathogenicity with PolyPhen2 and SIFT [Ng and Henikoff, 2003; Adzhubei et al., 2010] gave divergent outcomes, ranging from benign to damaging for each SNV. The authors conclude that the functional importance of these SNVs needs to be determined by sequencing larger series of *ETV6/RUNX1*-positive ALL cases and by functional evaluation.

Buijs et al. [2012] studied 2 patients with familial platelet disorder with an associated propensity to develop my-

eloid leukemia (FPD/AML). In the first family, they found a c.508G>A mutation in exon 4 in all available patients, leading to p.Gly107Arg, which has previously been reported in sporadic cases of AML with myelodysplastic syndrome. In the second family, sequencing of all 4 affected members revealed a c.784C>T substitution in exon 6 leading to a premature stop (p.Gln262X). This mutation would produce a truncated RUNX1 protein lacking the TA domain.

In an isolated case with storage pool deficiency, thrombocytopenia and AML, Buijs et al. [2012] found a t(16;21) (p13;q22), which they mapped to the *RUNX1* locus (in 21q22) and upstream of *ATF7IP2* (in 16p13.2) by fluorescence in situ hybridization. No mutations within the *RUNX1* gene were detected, nor were any copy number variations (CNVs) found by oligonucleotide and SNP array analysis. Therefore, the authors resorted to mate-pair sequencing to identify the translocation breakpoints at the nucleotide level [Kloosterman et al., 2011]. The 16p13.2 breakpoint was located at ~97 kb distal to *ATF7IP2* and the 21q22 breakpoint localized within intron 1 of *RUNX1* at 39.4 kb distal to exon 2. *RUNX1* transcription is regulated by 2 non-redundant promoters, P1 and P2. This particular translocation separates the RUNT from the TA domain encoding exons, which would abrogate expression of *RUNX1* isoform c. In addition, the enhancer at position +23 of exon 1 will be disconnected from promoter P2, which would possibly perturb expression of

RUNX1 isoform b. Since no mRNA from this patient was available, the authors were not able to test these hypotheses in terms of *RUNX1* expression. Yet, these findings lead the authors to suggest investigation of as yet unresolved cases of FPD/AML.

Both papers underscore the resolving power of the recently developed next-generation sequencing technology, in particular in cases in which no CNVs could be detected [Buijs et al., 2012]. Although the latter has increasingly become the standard of genetic testing [Hochstenbach et al., 2011], absence of CNVs can no longer be taken as the final result [Poot et al., 2011]. Novel SNVs detected by exome sequencing need, as a first step, analysis of potential pathogenicity by bioinformatic prediction algorithms. In most cases, these yield divergent predictions, such that functional testing will become inevitable. In the clinical work-up of hematological disorders, mRNA isolation from a bone marrow sample may be mandatory to perform mRNA expression analysis as a first step of functional investigation of a given SNV. The latter is of particular importance if allogenic bone marrow transplantation from a clinically unaffected sibling is considered. Since the first, heroic, attempts to identify chromosomal rearrangements, science followed a circuitous path of ever deeper analysis of our genome while making unexpected and challenging discoveries, which at each step allowed to raise the standard of care for patients with hematological malignancies.

M. Poot

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