# The corepressors BCOR and BCORL1: two novel players in acute myeloid leukemia

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Acute myeloid leukemia (AML) is a disease characterized by high molecular heterogeneity. About one-third of AML patients harbor well-defined chromosomal translocations and are included as a distinct entity named "AML with recurrent genetic abnormalities" in the 2008 World Health Organization (WHO) classification of myeloid neoplasms.[1] During the past decade, there have been major efforts to better define the genetic lesions underlying the large group of AML with normal cytogenetics (CN-AML) that accounts for 40-50% of all AML cases. Mutations associated with CN-AML include those affecting *NPM1*, *FLT3*, *MLL*, *CEBPA*, *TET2*, *ASXL1*, *IDH1*, *IDH2*, *DNMT3A*, *RUNX1* and other genes.[2] Because of their distinct biological and clinical features, "AML with mutated *NPM1*" and "AML with mutated *CEBPA*" are already recognized as provisional entities in the 2008 WHO classification.[1,3,4]

The increasing use of next-generation sequencing technologies applied to the study of cancer genomes is making a remarkable contribution to our knowledge of the molecular landscape of CN-AML. Following the identification of *IDH1* and *DNMT3A* mutations,[5-7] this strategy has recently led to the discovery that two homologous genes, i.e. BCOR and BCORL1, are recurrently mutated in AML.[8,9] Their involvement in AML emerged from in-solution whole-exome capture followed by massively parallel sequencing of paired leukemic cells at diagnosis and normal hematopoietic cells at the time of complete remission from patients with AML. However, the strategies leading to the discovery of *BCOR* and *BCORL1* mutations in AML differed. We identified mutated *BCOR* through whole-exome sequencing of a CN-AML patient who was selected for analysis because of lack of any known mutations.[8] Instead, Li *et al.*[9] found *BCORL1* to be mutated by searching for recurrent mutations in a discovery cohort of 8 patients with secondary AML (s-AML) that were subjected to whole-exome sequencing. In both studies, these mutations were subsequently studied in larger series of AML patients.[8,9]

The structures of BCOR and BCORL1 are shown in Figure 1 and their main features are summarized in Table 1. Both *BCOR* and *BCORL1* genes are located on the X-chromosome and encode for large nuclear proteins that are ubiquitously expressed in human tissues.[10,11] The BCOR protein acts as corepressor of BCL6,[10] it can bind to other transcriptional factors[12-14] and appears to play a key role in the regulation of early embryonic development,[15] mesenchymal stem cell function[16] and hematopoiesis.[15] BCORL1 is also a transcriptional corepressor and functional studies have shown that it can bind to class II histone deacetylases (HDAC4, HDAC5, HDAC7), to interact with the CTBP1 corepressor, and to affect the repression of E-cadherin.[11] In spite of their similarities, the BCOR and BCORL1 proteins show a number of different features, including their capability to interact with BCL6, their expression levels in human tissues, and their localization within subnuclear structures (Table 1). These findings strongly suggest that, although *BCOR* and *BCORL1* are homologous genes, they encode for

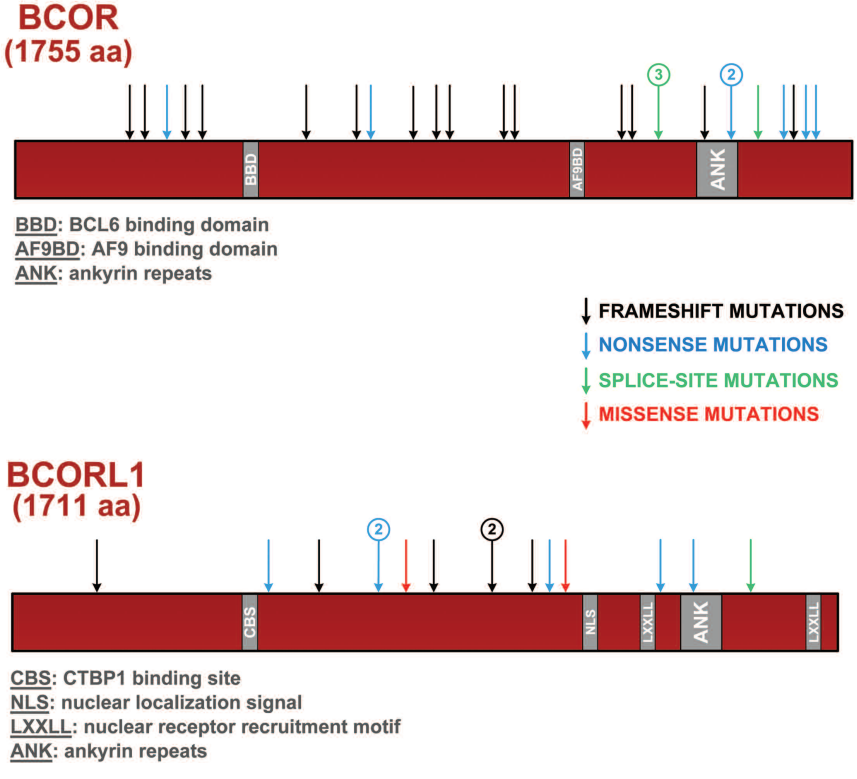


**Figure 1.** Schematic representation of the BCOR (top) and BCORL1 (bottom) transcriptional corepressors with their respective domains and the location and type of mutations occurring in AML patients. The numbers above the arrows indicate the presence, in more than one case, of the same type of mutation occurring at the same exonic location or involving the splicing of the same intron. *BCORL1* mutations also include five mutations that were found in four AML cell lines and in the Jurkat T-lymphoblastic leukemia cell line. The specific type (i.e. frameshift, nonsense, missense or splice-site) of *BCORL1* mutations was inferred from Li *et al.*[9]

proteins that are likely to play distinctly different roles in normal human cells.

In AML, the *BCOR* gene is targeted by both translocations and mutations. One patient with acute promyelocytic leukemia (APL) has been reported to carry the 45,-Y,t(X;17)(p11;q12) karyotype, leading to the formation of a *BCOR-RAR* alpha fusion gene.[17] Compared to typical APL cases with t(15;17), this patient showed distinct morphological features, i.e. rectangular body inclusions in the cytoplasm of leukemic cells and an atypical clinical course characterized by multiple relapses following chemotherapy plus all-transretinoic acid.[17] *BCOR* mutations were detected in about 4% (10 of 262) of an unselected cohort of *de novo* CN-AML. Importantly, they occurred at a higher frequency (about 17%; 14 of 82 cases) in the subset of CN-AML devoid of *NPM1*, *CEBPA*, *FLT3*-ITD, and *MLL*-PTD mutations, and mostly also lacking *IDH1* mutations.[8] Interestingly, the latter genotype mimicked that of the AML index patient initially used for whole-exome sequencing. *BCOR* mutations were demonstrated to be clonal, somatic, disruptive events involving the only functional allele, not only in male but also in female AML patients.[8] Notably, the features of *BCOR* mutations in AML closely resembled those of the germline *BCOR* disruptive mutations causing the oculo-facio-cardio-dental (OFCD) X-linked syndrome[18] that is characterized by microphtalmia, congenital cataracts, dysmorphic appearance, radiculomegaly, and digital and cardiac defects. Finally, *BCOR* mutations in AML were often associated with a decrease in *BCOR* mRNA levels, absence of full-length BCOR protein, and lack or low expression of a truncated BCOR protein.[8] Taken together, the above features conform to those of loss-of-function mutations in a tumor-suppressor gene.

*BCORL1* somatic mutations were found in about 6% of a series of 173 AML patients which included cases with secondary leukemia (showing myelodysplasia-related changes or being therapy-related) and a high incidence (approx. 65%) of abnormal karyotypes.[9] Similarly to mutations of *BCOR*, most of the alterations affecting *BCORL1* were nonsense mutations, out-of-frame insertions/deletions or splice site mutations that, although not resulting in clearly diminished mRNA levels of *BCORL1*, were predicted to encode truncated proteins lacking the last C-terminal LXXLL nuclear receptor recruitment motif.[9] Although targeting of *BCORL1* mutations to the expressed allele in female AML patients was not formally demonstrated by Li *et al.*[9], the above findings again point to *BCORL1* as a tumor-suppressor gene that is inactivated by mutations in a subset of AML.

At present, only limited information is available about the distribution of *BCOR* and *BCORL1* mutations across the main cytogenetic/molecular AML categories. Mutations of *BCOR* mainly clustered with CN-AML, were virtually mutually exclusive with *NPM1* and *FLT3*-ITD mutations and showed an association with *DNMT3A* and *RUNX1* mutations.[8] *BCORL1* mutations mostly occurred in patients with s-AML and/or with abnormal karyotype, and carried germline *TP53*, *CEBPA* and *NPM1* genes.[9] Because a relatively small number of CN-AML cases has been analyzed for *BCORL1* mutations[9] and cases of s-AML have not been investigated for *BCOR* mutations,[8] it is not yet clear whether or not mutations of these two homologous genes may occur in the same clinical-genetic AML backgrounds.

**Table 1. Features of the BCOR and BCORL1 transcriptional corepressors.**

| Feature | BCOR | BCORL1 |
|---|---|---|
| **Gene** | | |
| Location | Chromosome X (band Xp11.4) | Chromosome X (band Xq26.1) |
| Number of exons | 15 | 13* |
| Association with a genetic syndrome | Oculo-facio-cardio-dental (OFCD) syndrome | Not reported |
| **Protein** | | |
| Length | 1755 amino acids | 1711 amino acids* |
| Subcellular location | Nucleosol and nuclear dots of various size# | Speckle-like nuclear dots of consistent size# |
| Tissue expression | High levels in many tissues | Low levels in many tissues. High levels in testis and prostate |
| Major domains and motifs | BCL6-binding domain, MLLT3-binding domain^, tandem ankyrin repeats | CTBP1-binding site, nuclear localization signal (NLS), tandem ankyrin repeats, two LXXLL motifs |
| Function | Transcriptional corepressor | Transcriptional corepressor |
| Interactors° | BCL6, class I and II HDACs, MLLT3, FBXL10/JHDM1B, MLLT1/ENL, ZBTB5, SP1, ZBTB2, ZBTB7A/Pokemon | Class II HDACs, CTBP1 |

**BCORL1a (an alternatively spliced form of BCORL1) contains additional 74 amino acids (for a total of 1785) encoded by a further exon (exon 9). #*BCOR *and* BCORL1 *localize in different subnuclear structures. ^Shown in the Bcor murine ortholog; MLLT3 is also known as AF9 and is a fusion partner of MLL in acute leukemias. °Direct or indirect.*

All these findings point to mutational targeting of corepressors as a new mechanism of leukemogenesis. However, the way in which *BCOR* and *BCORL1* mutations contribute to AML development is still not clear. The observation that about half the *BCOR*-mutated cases also carry mutations of the *DNMT3A* gene suggests that these two mutations may act synergistically to induce AML, possibly interfering with epigenetic mechanisms. Indeed, *DNMT3A* encodes a methyltransferase enzyme catalyzing the addition of methyl groups to CpG dinucleotides.[6] Moreover, BCOR increases the repression of transcriptional activity by interacting with class I and II HDACs, the polycomb group protein PCGF1/NSPC1 and the histone demethylase FBXL10,[13,14,16] which implies BCOR may suppress gene transcription by epigenetic mechanisms.[16,19] Because BCOR (and BCORL1) are ubiquitously expressed, it will be crucial to define the key targets of their corepressive transcriptional activity specifically in AML cells, in order to gain more precise insights into mechanistic role of *BCOR* and *BCORL1* mutations in leukemogenesis.

Do *BCOR* and *BCORL1* mutations have any clinical impact in AML? Analysis of a large series of CN-AML patients suggests that *BCOR* mutations may confer a poorer prognosis.[8] However, due to the low frequency of the mutation, additional studies are necessary to confirm these findings. For the moment, no prognostic information is available concerning *BCORL1* mutations[9] and this needs to be clarified.

In conclusion, *BCOR* and *BCORL1* add to the growing list

of genes recurrently mutated in AML. These findings also highlight the cost and the huge amount of time involved to investigate the functional and clinical aspects of genetic lesions that occur at a relatively low frequency in AML. Researchers should be prepared to take on this difficult task, since in the future, novel low frequency mutations in other genes are likely to emerge from the sequencing of additional AML genomes.

*Acknowledgments: this work was supported by the Italian Association for Cancer Research (AIRC).*

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*Financial and other disclosures provided by the author using the ICMJE (www.icmje.org) Uniform Format for Disclosure of Competing Interests are available with the full text of this paper at www.haematologica.org.*

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# *ATM* and chronic lymphocytic leukemia: mutations, and not only deletions, matter

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Chronic lymphocytic leukemia (CLL) is the most common leukemia in adults. Though modern treatments are highly effective in most CLL, a challenging subgroup of patients shows poor response to standard regimens and a survival of less than two years.[1-3] Identifying chemorefractory patients early, ideally before treatment, and designing therapeutic strategies tailored to overcoming chemorefractoriness remain key issues toward an opti-