

## Downregulation of miR-15a and miR-16-1 at 13q14 in Chronic Lymphocytic Leukemia

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**Featured Article:** Calin GA, Dumitru CD, Shimizu M, Bichi R, Zupo S, Noch E, et al. Frequent deletions and down-regulation of micro-RNA genes miR15 and miR16 at 13q14 in chronic lymphocytic leukemia. *Proc Natl Acad Sci U S A* 2002;99:15524–9.<sup>2</sup>

MicroRNAs (miRNAs)<sup>3</sup> are involved in many biological processes due to their posttranscriptional gene regulation function. Consequently, their crucial role in many diseases, including cancer, is now well known. Nevertheless, the connection between miRNA deregulation and tumorigenesis was unclear for quite some time. In the 2002 article discussed here (1), Dr. Croce's group reported the very first direct association between miRNAs and cancer. In the time preceding this important discovery, Dr. Croce devoted himself to the study of the most common human leukemia: chronic lymphocytic leukemia (CLL). CLL is a malignancy of CD5-positive B cells occurring, for the most part, in individuals over the age of 60 years. At presentation the disease is usually indolent, although it often progresses to an aggressive form. An aggressive form at presentation occurs in 30% of patients. Consistent chromosomal alterations also occur in CLL, with the most common being a deletion of chromosome 13q14, which is observed by cytogenetics in approximately 50% of CLL patients (2). Dr. Croce's group focused its efforts on this region and used a genetic approach called loss of heterozygosity (LOH) to narrow the region of loss and identify the altered gene(s) involved in CLL. After narrowing to approximately 700 kb, the Croce group sequenced this region including the epicenter of loss in the middle. Unfortunately, 7 years of searching amounted to no results. Finally, Dr. Croce decided to consider translocations at 13q14 occurring in CLL, asking colleagues at the CLL Research Consortium for cases of CLLs with such translocations. An interesting opportunity came when Michael Keating of MD Ander-

son provided Dr. Croce's laboratory with samples of a CLL patient with a t(2;13) chromosome translocation within breakpoint at 13q14 (3). After obtaining somatic cell hybrids with mouse cells to immortalize the CLL genome, a brilliant postdoc of Dr. Croce's lab, George Calin, precisely mapped the translocation breakpoint as a single simple cut in the region at the epicenter of loss identified by our loss of heterozygosity study (2). Nonetheless, a gene was not found. The breakthrough finally arrived while studying another case of CLL in a patient with retinoblastoma, provided by Dr. Kanti Rai. After hybrids were again made, Dr. Croce's group successfully segregated the 2 chromosome 13s of the CLL cells. Intriguingly, one chromosome displayed a small deletion, approximately 30 kb as determined by Calin, which occurred precisely in the same region as in the patient with the t(2;13) chromosome translocation, but no CLL gene could be found (2).

The lack of a coding gene directed Dr. Croce's attention elsewhere, specifically to the noncoding component of the genome. Particular interest started to rise toward the class of small noncoding RNAs we today know as miRNAs. The first, Lin-4, was discovered by Victor Ambros in 1993 in the worm *Caenorhabditis elegans* (3). Mutations in this gene were found to affect the development of *C. elegans*, although this gene did not encode a protein, but instead encoded a short RNA. This discovery did not trigger any interest in miRNA. This situation changed in 1998, due to their similarity to siRNAs (small interfering RNAs), which were discovered that year. By 2001 the genomes of *Drosophila*, mice, rats, and humans were also found to contain miRNA genes. In light of this, Dr. Croce decided to scan region 13q14 for miRNA genes and found that it indeed contained 2: miR-15a and miR-16-1. Clearly, the loss of these 2 miRNAs was responsible for CLL. Thus Dr. Croce's group analyzed many cases of CLL and found that in approximately 70% of them miR-15a/16-1 were lost (2, 4). This was an extraordinary discovery because it showed that alterations in noncoding genes could cause disease, specifically cancer. Indeed, several miRNA genes known at the time mapped precisely to regions of loss or amplification or rearrangement in a variety of human cancers (5).

Concurrently, Dr. Croce found that by binding through partial complementarity mainly to the 3' untranslated region of mRNAs, miRNAs inhibit translation and/or cause degradation of their targets (4).

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<sup>2</sup> This article has been cited more than 2200 times since publication.

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<sup>3</sup> Nonstandard abbreviations: miRNA, microRNA; CLL, chronic lymphocytic leukemia; LOH, loss of heterozygosity.

As reasonably expected, BCL2 was at the top of the predicted targets for miR-15/-16-1. Two major indolent B-cell malignancies occur in humans: follicular lymphoma, where a t(14-18) chromosome translocation dysregulates BCL2, and CLL. Dr. Croce's group was able to prove that miR-15/-16-1 were negative regulators of BCL2, their loss leading to BCL2 overexpression in CLL (6).

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