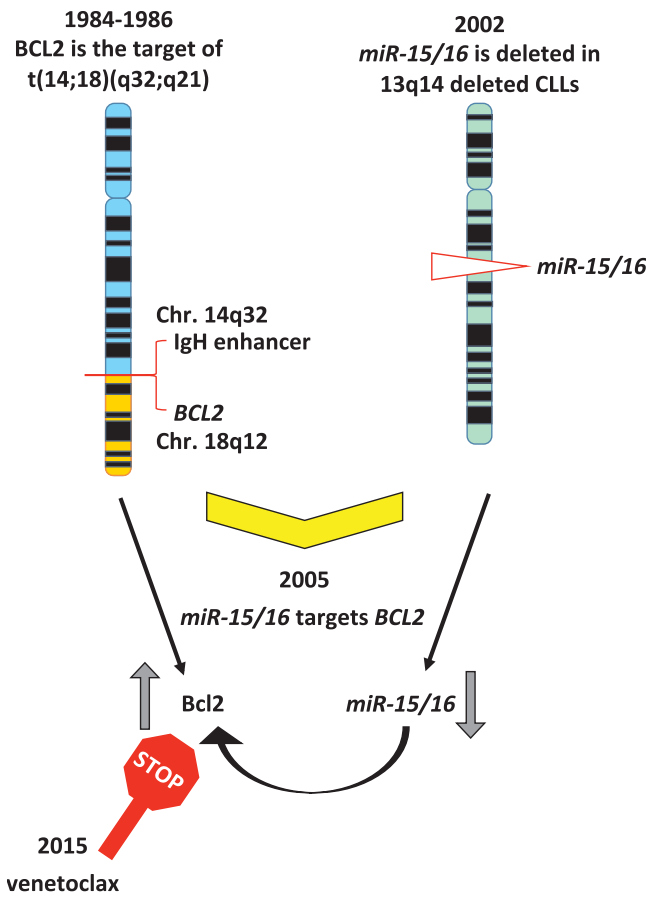
Cell Death and Differentiation (2018) 25, 21–26
 1350-9047/18
www.nature.com/cdd

Review

BCL2 and *miR-15/16*: from gene discovery to treatment

Yuri Pekarsky[1], Veronica Balatti[1] and Carlo M Croce*[,1]

In 1984, we investigated the t(14;18) chromosomal translocations that frequently occur in patients with follicular lymphoma. We first identified a locus on chromosome 18 involved in these translocations with the chromosome 14 containing the immunoglobulin heavy chain locus. Within this region on chromosome 18, we then discovered a gene that we called *BCL2*, which was activated by the translocations. Since that time, many studies determined that *BCL2* is one of the most important oncogenes involved in cancer by inhibiting apoptosis. In 2002, we studied 13q deletions in chronic lymphocytic leukemia (CLL) and found that the microRNA cluster *miR-15a/miR-16-1* (*miR-15/16*) is deleted by 13q deletions. In 2005, we discovered that *miR-15/16* function as tumor suppressors by directly targeting *BCL2*. Thus the loss of two negative regulators of *BCL2* expression results in overexpression of *BCL2*. Very recently, a specific *BCL2* inhibitor ABT-199 (Venetoclax) was developed and approved by FDA for CLL treatment. Thus it took 32 years from fundamental discovery of a critical oncogene to the development of a drug capable to cure CLL. In this review, we discuss the discovery, functions and clinical relevance of *miR-15/16* and *BCL2*.
Cell Death and Differentiation (2018) **25,** 21–26; doi:10.1038/cdd.2017.159; published online 6 October 2017



Graphical Abstract

Facts

- *BCL2* is a key oncogene involved in human cancer by inhibiting apoptosis.
- *miR-15/16* is a tumor-suppressor microRNA cluster deleted in most CLLs.
- The *miR-15/16* cluster targets *BCL2*.
- Venetoclax is a specific *BCL2* inhibitor that can cure CLL.

Open Questions

- Are there other important targets of *miR-15/16* in CLL?
- Can *miR-15/16* itself be of pharmaceutical use in CLL?
- Can Venetoclax be used in combination with inhibitors of other *miR-15/16* targets?

Discovery of *BCL2*

In 1984, we studied t(14;18) chromosomal translocations that almost invariably occurred in patients with follicular lymphoma, a malignant B-cell lymphoma. At first, we identified and cloned a locus on chromosome 18 involved in this translocation.[1] We then cloned recombinant DNA probes spanning the breakpoint on chromosome 18 from cells derived by patients with follicular lymphoma carrying this translocation.[1,2] These probes could detect DNA rearrangements in most of the cases of follicular lymphoma, and almost all chromosome 18 rearrangements were clustered within a short DNA fragment.[1,2] Northern blotting analysis using previously obtained DNA probes detected several transcripts of approximately 6 kb in length in various tissues.[1,2] By screening cDNA libraries using breakpoint DNA probes and sequencing

[1]Department of Cancer Biology and Genetics, The Wexner Medical Center, Columbus, OH, USA
*Corresponding author: CM Croce, Comprehensive Cancer Center, The Ohio State University, Biomedical Research Tower, Room 1082, 460 West 12th Avenue, Columbus, OH 43210, USA. Tel: +1 614 292 3063; Fax: +1 614 292 3558; E-mail: carlo.croce@osumc.edu
Received 18.5.17; revised 09.7.17; accepted 03.8.17; Edited by F Pentimalli; published online 06.10.17

obtained clones, we determined the DNA sequence of the *BCL2* gene and the protein sequence of the encoded BCL2 protein.[3] Since that time, many studies contributed to determine that *BCL2* is one of the most important oncogenes involved in cancer by inhibiting apoptosis[4] and causes lymphoma development, particularly in context with c-MYC overexpression.[5] The antiapoptotic function of *BCL2* has been described and reviewed numerous times and newest advances in this field are described in other articles of this issue; thus in this review, we will focus on the regulation of *BCL2* expression by microRNAs.

Chronic Lymphocytic Leukemia, 13q Deletions and *miR-15/16*

Chronic lymphocytic leukemia (CLL) is the most common human leukemia. CLL represents >30% of all lymphoid malignancies diagnosed each year in the United States and represents one-third of all leukemia cases.[6] Interestingly, CLL is more common in men than in women. Very often, newly diagnosed CLL patients can live with mild disease and can survive without treatment for many years.[6] Leukemic CLL cells can survive in tissue culture conditions for several weeks but usually do not proliferate and morphologically look similar to mature B-cells.[6,7] CLL is a heterogeneous disease and mostly defined as an expansion of a rare population of CD5-positive B-cells, as CD5 is commonly expressed on T-cells and not on mature B-cells.[6,7] There are several prognostic markers of CLL. For the most part, CLL patients showing high ZAP-70 (zeta-chain-associated protein kinase 70) expression and unmutated IgH V_H have a clinically aggressive form of CLL and need treatment sooner than patients expressing low levels of ZAP-70 and mutated IgH V_H, though this general rule has many exceptions.[6,7]

CLL is a fascinating disease to study as almost all CLL cases (>80%) show genomic aberrations, and in most cases, these rearrangements occur at several chromosomal locations. The most common chromosomal abnormalities detectable in CLL cells include deletions at 13q (60%), 11q (18%), 17p (8%), and trisomy 12 (12–16%).[8,9] Recent studies demonstrated that trisomy 12 in aggressive CLL is associated with *NOTCH1* (Notch homolog 1, translocation-associated (Drosophila)) activating mutations;[10] 17p deletions often involve inactivation of *TP53* and *miR-3676* (which targets *TCL1*, a critical oncogene in aggressive CLL);[11,12] and 11q deletions may involve the *ATM* (ataxia-telangiectasia mutated) gene.[13] Generally, patients showing 11q and 17p deletions have more aggressive clinical course than patients with 13q deletions.[9]

In 2000–2002, we studied 13q14 deletions in CLL to determine which gene(s) are targets of this most common deletion in CLL. We believed that 13q14 had to contain a very important tumor-suppressor gene involved in the pathogenesis of CLL. Interestingly, in addition to CLL, the same region is often deleted in other malignancies. For example, almost 50% of mantle cell lymphomas (a rare type of B-cell non-Hodkin's lymphoma) have genomic aberrations at 13q14,[14] and the same region is frequently deleted in ~60% of prostate cancers.[15] We and many other research groups extensively studied the 13q14 genomic region using positional cloning and sequencing approaches. We were able to sequence a region of >1 Mb within the 13q14-deleted region in our attempts to identify a specific target of 13q14 deletions.[16,17]

Despite the extensive amount of work including loss of heterozygosity (LOH) studies, sequencing and expression analysis of all protein-coding genes within and around the 13q14-deleted region, the affected gene(s) remained elusive, as no point mutations or loss of expression of any conventional genes were found.[16–19] In 2001–2002, we worked with several interesting CLL cases showing small deletions and translocations at 13q14. We generated several somatic cell hybrids to isolate the relevant chromosomes that included a rearranged 13q34 allele. One of these hybrids (derived from CLL cells of Patient 1) contained a small (~30 kb) deletion located between exons 2 and 5 of the *DLEU2* gene (Figure 1). Another hybrid (derived from CLL cells of Patient 2) contained a translocation breakpoint within the same region.[20,21] Interestingly, *DLEU2* itself was extensively studied previously, and no mutations in the coding region or downregulation of its expression were found.[7,20,21] Thus by investigating these cases with a small deletion and a translocation, we came to the conclusion that the tumor suppressor must have been located within a 30 kb deletion. But all protein-coding genes were excluded. At that

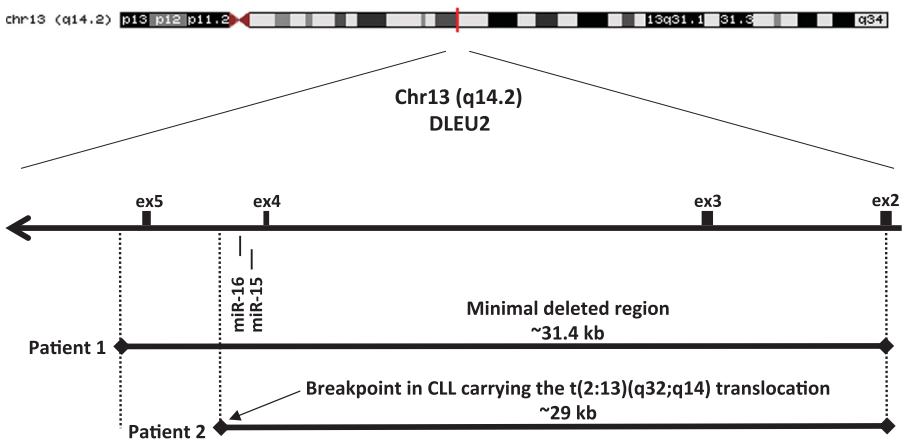


Figure 1 *miR-15/16* in 13q14-deleted region

time, genomic databases were frequently updated, one of these updates contained microRNAs, and we finally found that a cluster of two microRNA genes, *miR-15a* and *miR-16-1*, was located at the translocation breakpoint (Figure 1).[20] As *miR-15/16* was the only gene in the region, we carried out a study to determine its expression in CLL. Northern blotting analysis of 60 13q-deleted specimens derived from patients with CLL revealed that expression of *miR-15/16* was downregulated in approximately 70% of CLL cases compared with normal CD5+ B-cells.[20] As none of the other genes in the region showed any involvement in CLL,[16,17,21] and as the translocation breakpoint and the small deletion involved the *miR-15/16* loss, we concluded that *miR-15/16* is a target of 13q deletions in CLL.[20] This was the first example that an alteration in the non-coding genome could be involved in cancer pathogenesis.[20] MicroRNAs are a large class of non-coding RNAs involved in almost all cellular processes through the regulation of gene expression.[22] MicroRNAs are first produced as long precursor molecules and then processed into hairpin structures of about 70–100 nt in length. These 70–100-nt stem-loop precursors are then processed into microRNAs.[23] For the most part, mature microRNA molecules bind the 3′ UTRs of mRNAs through the conserved seed sequences located at the 5′-ends of the microRNAs by partial complementarity.[23] This binding causes mRNA degradation and/or inhibits mRNA translation, resulting in decreased levels of gene expression.[24]

The *miR-15* and *miR-16* Target BCL2

As we found that the *miR-15/16* is lost in 13q14-deleted CLL cells, we hypothesized that these microRNAs might target important oncogenes responsible for B-cell transformation. Thus we searched available databases to identify oncogenes among the predicted *miR-15/16* targets and focused on genes with possible role on CLL pathogenesis. Remarkably among top predicted targets in this search, we found *BCL2*, the gene we discovered in 1984, and found overexpressed in almost all CLL cases.[25] By analyzing complementarity between *miR-15/16* and the *BCL2* mRNA, we found that the first 9 nucleotides, from the 5′-ends of both *miR-15* and *miR-16*, are complementary to bases 3287–3279 in the 3′-end of the *BCL2* cDNA.[25] As mentioned above, *BCL2* has a critical role in the malignant transformation of solid tissues as well as lymphoid cells. BCL2 protein mostly localizes in mitochondria, and it promotes survival and inhibits apoptosis by preventing the release of Cytochrome *C* from mitochondria into the cytoplasm.[26,27] Several B-cell malignancies (including follicular lymphomas and a fraction of diffuse B-cell lymphomas) carry a t(14;18)(q32;q21) chromosomal translocation. These translocations place immunoglobulin heavy-chain enhancers located at 14q32 near the *BCL2* promoter resulting in upregulated *BCL2* expression.[1,2] Interestingly, however, translocations involving 18q21 (where *BCL2* is located) occur only in a very small minority of CLLs, whereas, on the other hand, overexpression of *BCL2* is a hallmark of CLL.[28,29] Thus it seemed likely that *BCL2* overexpression in CLL might be due to the deletion of *miR-15/16* at 13q14. First, we studied the expression of *miR-15*, *miR-16* and *BCL2* in CLL and found that expression of both *miR-15* and *miR-16* was highest in the CLL

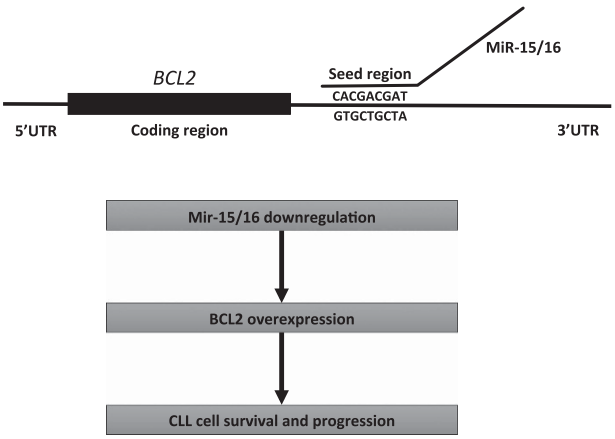


Figure 2 *miR-15/16* target *BCL2* expression

samples with lowest *BCL2* expression and was lowest in the CLL samples showing high *BCL2* expression. We also determined that, in MEG-01 leukemic cells that do not express any *miR-15/16*, the exogenous expression of *miR-15/16* resulted in *BCL2* repression and induction of apoptosis. This was evidenced by cleavage of poly (ADP-ribose) polymerase and of pro-caspase 9.[25] Interestingly, no apoptosis was detected when using mutated *miR-15/16* (this mutation was found in two CLL patients, see below).

To demonstrate that *miR-15/16* directly targeted *BCL2*, we carried out a series of luciferase assay experiments. We cloned a small fragment of the 3′ UTR of *BCL2*, which was predicted to interact with *miR-15/16*, into the 3′ UTR of the luciferase gene. Expression of *miR-15/16* significantly decreased luciferase activity when the WT construct was used, whereas no difference was observed when a construct containing a mutated microRNA–mRNA interaction site was used.[25] This showed that both microRNAs directly interact with and inhibit *BCL2* expression (Figure 2).[25] Overall, these results indicate that the loss of *miR-15/16* is the main cause of *BCL2* overexpression in CLL (Figure 2).

miR-15/16: Mechanisms of Deregulation Beyond deletion

As our initial observation that *miR-15/16* is deleted or downregulated in most of CLLs, additional studies indicated that *miR-15/16* could also be downregulated by additional mechanisms. Allegra *et al.*[30] showed that some CLLs have defective DROSHA processing that contributes to the loss of *miR-15/16* expression. Sampath *et al.*[31] and Allegra *et al.*[30] demonstrated that histone deacetylases are overexpressed in CLL leading to the aberrant epigenetic silencing of *miR-15/16* expression. Thus *miR-15/16* can also be silenced by an epigenetic mechanism. As tumor suppressors are frequently mutated, and most of these mutations are loss-of-function mutations, it is very important to identify and characterize such mutations. Thus it is possible that mutations can also contribute to the loss of *miR-15/16* in CLL. To identify such mutations, we carried out an additional study analyzing 75 CLL cases and 160 normal controls for mutations of several microRNAs, including *miR-15/16*.[32] We found one germline mutation in the pre-*miR-15/16* sequence in CLL samples

derived from two CLL patients, and no mutations were found in the controls. This C-to-T mutation is located only seven base pairs after the end of the *miR-16-1* precursor.[32] We also analyzed this genomic region in all primates and determined that this region is strongly conserved, indicating that this mutation can have an important role in the processing of *miR-16-1*. This was confirmed by studying *miR-16-1* expression levels in both patients by microchip analysis and Northern blotting. Both methods showed a significant decrease of expression of *miR-16-1* in both patients.[32] In addition, both patients had a monoallelic deletion at 13q14 while in their normal DNA the mutation was heterozygous. Thus in CLL cells from these patients one allele of *miR-16-1* was deleted and the other one was mutated. Interestingly, the mother of one of these patients also had CLL and the sister of the same patient had breast cancer, suggesting an important role for this mutation or rare polymorphism.[32]

We also functionally tested this mutation and showed that it is a loss-of-function mutation, resulting in the decrease of *miR-15/16* expression.[32] All the data discussed above clearly showed that *miR-15/16* is a target of 13q14 deletions and can also be mutated and downregulated by other mechanisms.

miR-15/16 Upstream and downstream Mechanisms

After these results, we investigated whether *miR-15/16* functions as a tumor suppressor *in vivo*. We transiently transfected MEG-01 cells, which lack *miR-15/16* expression, with *miR-15/16* or empty vector and tested their tumorigenic activity upon inoculation in immunosuppressed mice. After 4 weeks, no tumors originated from cells transfected with *miR-15/16*, whereas, in sharp contrast, large tumors originated from cells transfected with the empty vector.[33] Thus we concluded that *miR-15* and *miR-16* function as a tumor suppressor in MEG-01 leukemia cells. We also investigated the transcriptional and translational effects of *miR-15/16* exogenous expression in MEG-01 cells. We found that *miR-15/16* expression resulted in the induction of transcription of 265 genes and suppression of 3300 genes. Interestingly, among the repressed genes we found 85 predicted *miR-15/16* targets. We also identified 27 proteins repressed by *miR-15/16* using proteomics analysis (including BCL2). Most of these proteins were found to be involved in tumorigenesis, apoptosis or cell growth and eight of these proteins were predicted *miR-15/16* targets.[33]

By analyzing the genomic region surrounding *miR-15/16*, we found several *TP53*-binding sites upstream of the cluster.[34] To prove that p53 was bound to these sites, we carried out chromatin immunoprecipitation experiments. These analyses revealed that indeed p53 binds upstream of *miR-15/16* cluster. Further experiments revealed that p53 transactivates the *miR-15/16* cluster.[34] Interestingly, the 3′ UTR of *TP53* also contains binding sites for *miR-15* and *miR-16*. We carried out a series of luciferase experiments and found that indeed these sites are targeted by *miR-15/16*. These results showed the interplay between *miR-15/16* and p53: activation of p53 results in *miR-15/16* overexpression, resulting in increased targeting of *TP53* by *miR-15/16*, and decrease of p53 expression.[34]

Since our discovery that *miR-15/16* targets BCL2, several reports described other important targets of *miR-15/16*. For example, *miR-15/16* was found to inhibit *Cyclin D1* in several malignancies, including bladder cancer and osteosarcoma.[35,36] *MCL1* oncogene in CLL and the *BMI1* oncogene in mantle call lymphoma were also validated as *miR-15/16* targets.[33,37]

Interestingly, *miR-15/16* has tumor-suppressor functions not only in lymphoid malignancies. Reid *et al*.[38] showed that *miR-15/16* expression is inactivated in malignant pleural mesothelioma. Transfection of synthetic oligos mimicking *miR-15/16* resulted in growth inhibition in mesothelioma cell lines and in reduction of tumor sizes in xenograft models.[38]

Mouse Models for *miR-15/16*

Supporting evidence for the tumor-suppressor activity of any gene is a tumor phenotype resulting upon its knockout in mouse models. To verify the function of *miR-15/16*, Klein *et al*.[39] generated *miR-15/16* knockout mouse models. Two knockout alleles were generated. One allele had the minimal deleted region (*MDR*) containing *miR-15/16* and the *Dleu2* gene; another allele had only *miR-15/16* deleted.[39] Both mouse models developed CD5+ B-cell proliferation and/or malignancies. At the age of 1 year, *MDR* mice had 50% of CD5 positive B-cells in their spleens similarly to *miR-15/16* mice, which had a slightly lower number.[39] At the age of 18 months, 26% of *miR-15/16* mice and 42% of *MDR* mice developed B-cell proliferation or malignancies, while no WT controls developed any lymphoid malignancies.[39] Generally, the *MDR* knockout phenotype was more severe than that of *miR-15/16*, suggesting that other sequences in the *MDR* locus may additionally contribute to the tumor-suppressor function of *miR-15/16*.[39] Another interesting mouse model of CLL is the New Zealand Black (NZB) mouse strain.[40] NZB is a naturally occurring mouse strain that late in life develops a CD5+ B-cell proliferation similar to CLL.[40] Dr. Raveche's laboratory carried out a genome-wide linkage study attempting to identify genomic loci responsible for the development of such CLL-like disease in NZB mice.[41] They identified three genomic regions linked to the disease, which were located on chromosomes 14, 18, and 19. Intriguingly, the region on NZB chromosome 14 is homologous to the human 13q14 and contains mouse *Dleu2* and *miR-15/16*.[41] Sequencing of the mouse *miR-15/16* region revealed a point mutation resembling a mutation previously found in *miR-15/16* in human CLL samples.[32,41] Further investigation showed that this NZB mutation caused reduced the expression of *miR-16* in these mice.[41] These data confirmed the importance of *miR-15/16* loss in CLL pathogenesis.

The tumor-suppressor function of *miR-15a/16-1* cluster (referred above as *miR-15/16*) is well established as it is deleted, mutated and downregulated by other mechanisms in CLL and other cancers. The second member of *miR-15/16* family (*miR-15b/16-2* cluster) is located at 3q25.[42] As *miR-15b/16-2* is almost identical to *miR-15a16-1*, to determine whether *miR-15b/16-2* could have any role in cancer, we generated *miR-15b/16-2* knockout mice and studied their phenotype.[42] Pathological examination of these knockout mice revealed enlarged spleens, twofolds to fourfolds compared with wild-type mice. The *miR-15b/16-2* knockout mice developed CD5+ B-cell proliferation and lymphomas at the age of 15–18 months with a penetrance of 60%. FACS

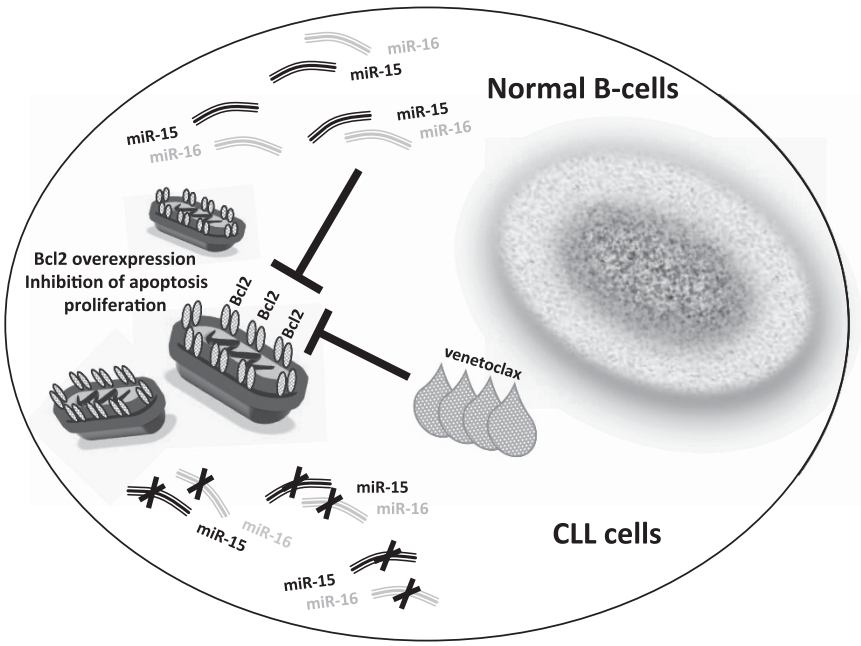


Figure 3 Therapeutic implications of *miR-15/16* targeting *BCL2*

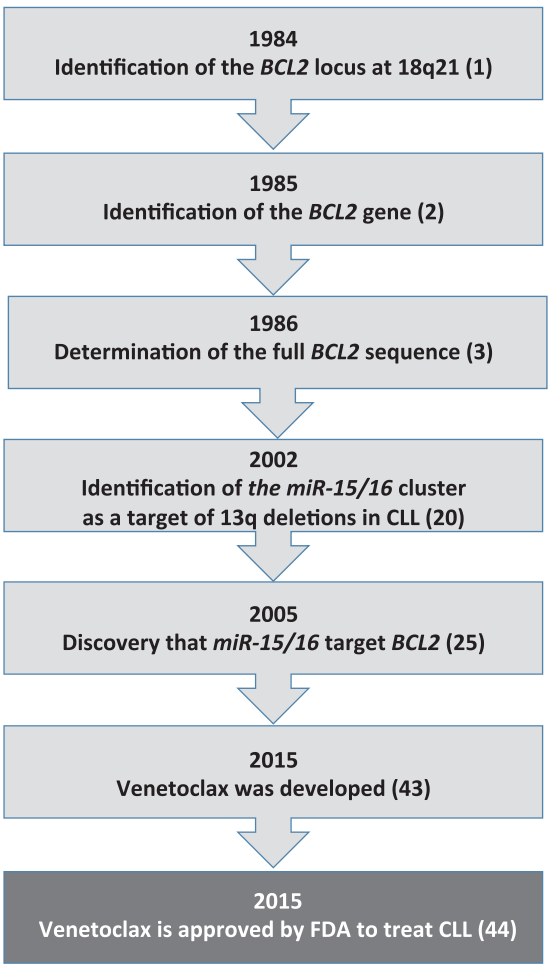


Figure 4 Major steps in the discovery of *BCL2* and *miR-15/16*

analysis revealed expanded CD5+ B-cell proliferation in the spleens of these mice. Interestingly, we observed only mild upregulation of *BCL2* expression in these knockout mice, on the other hand we found more robust upregulation of several other predicted *miR-15/16* targets (*Ccnd1*, *Ccnd2*, and *Igf1r1*) in spleen lymphocytes from these mice.[42]

Venetoclax, a BCL2 Inhibitor in CLL

Generally, microRNAs are not used directly as drugs, mostly because they cannot be easily delivered in 100% of malignant cells. Thus, at present, *miR-15/16* cannot be used as an anti-CLL drug directly. As we discussed above, *BCL2* is over-expressed in almost all CLLs and could therefore be used as a target for development of drugs against CLL. There were several attempts in the past to develop BCL2 inhibitors. Most of them inhibited not only BCL2 but also other members of the BCL2 family, including BCLXL, making them toxic. More recently, Abbott was able to develop a drug targeting exclusively BCL2.[43] The drug named ABT-199 (venetoclax) targets protein–protein interactions of BCL2. Venetoclax can induce complete remission in CLL patients, even in previously treated relapsed patients with 17p deletions, which are normally the most aggressive and difficult to treat.[44] But even in these cases, venetoclax has a response rate of 80% and was recently approved by the FDA to treat these CLL cases (Figure 3).[44] Venetoclax also showed promising results in treating different B-cell malignancies, including large B-cell lymphoma and follicular lymphoma.[44]

Conclusions

Since we first identified a locus on chromosome 18 involved in translocations in patients with follicular lymphoma and

discovered a gene we named *BCL2* that is activated by these translocations, it took >30 years to develop a drug inhibiting BCL2 and capable to cure CLL. Meanwhile, many studies showed the importance of *BCL2* in many cancers, including other hematological malignancies. During this time, we identified the major regulators of *BCL2* expression, *miR-15 and miR-16*, which are the first example of tumor-suppressor microRNAs, deleted or downregulated in >70% of CLLs. Thus, in the past 30 years, this research completed a full circle from the discovery of *BCL2* and *miR-15/16* to cancer treatment through a precision medicine approach (Figure 4).

Conflict of Interest

The authors declare no conflict of interest.

1. Tsujimoto Y, Finger LR, Yunis J, Nowell PC, Croce CM. Cloning of the chromosome breakpoint of neoplastic B cells with the t(14;18) chromosome translocation. *Science* 1984; **226**: 1097–1099.
2. Tsujimoto Y, Cossman J, Jaffe E, Croce CM. Involvement of the bcl-2 gene in human follicular lymphoma. *Science* 1985; **228**: 1440–1443.
3. Tsujimoto Y, Croce CM. Analysis of the structure, transcripts, and protein products of bcl-2, the gene involved in human follicular lymphoma. *Proc Natl Acad Sci USA* 1986; **83**: 5214–5218.
4. Vaux DL, Cory S, Adams JM. Bcl-2 gene promotes haemopoietic cell survival and cooperates with c-myc to immortalize pre-B cells. *Nature* 1988; **335**: 440–442.
5. Strasser A, Harris AW, Bath ML, Cory S. Novel primitive lymphoid tumours induced in transgenic mice by cooperation between myc and bcl-2. *Nature* 1990; **348**: 331–333.
6. Sgambati M, Linet M, Devesa S. Chronic lymphocytic leukemia, epidemiological, familial, and genetic aspects. In: Cheson BD (ed). *Chronic Lymphocytic Leukemias, Second Edition, Revised and Expanded*,, Marcel Dekker, Inc: New York, USA, pp 33–622001.
7. Bullrich F, Croce C. Molecular biology of chronic lymphocytic leukemia. In: Cheson BD (ed). *Chronic Lymphocytic Leukemias, Second Edition, Revised and Expanded*. Marcel Dekker, Inc: New York, USA, pp 9–322001.
8. Edelmann J, Holzmann K, Miller F, Winkler D, Buhler A, Zenz T *et al.* High-resolution genomic profiling of chronic lymphocytic leukemia reveals new recurrent genomic alterations. *Blood* 2012; **120**: 4783–4794.
9. Dohner H, Stilgenbauer S, Benner A, Leupolt E, Krober A, Bullinger L *et al.* Genomic aberrations and survival in chronic lymphocytic leukemia. *N Engl J Med* 2000; **343**: 1910–1916.
10. Balatti V, Bottoni A, Palamarchuk A, Alder H, Rassenti LZ, Kipps TJ *et al.* NOTCH1 mutations in CLL associated with trisomy 12. *Blood* 2012; **119**: 329–331.
11. Palamarchuk A, Yan PS, Zanesi N, Wang L, Rodrigues B, Murphy M *et al.* Tcl1 protein functions as an inhibitor of de novo DNA methylation in B-cell chronic lymphocytic leukemia (CLL). *Proc Natl Acad Sci USA* 2012; **109**: 2555–2560.
12. Balatti V, Rizzotto L, Miller C, Palamarchuk A, Fadda P, Pandolfo R *et al.* TCL1 targeting miR-3676 is codeleted with tumor protein p53 in chronic lymphocytic leukemia. *Proc Natl Acad Sci USA* 2015; **112**: 2169–2174.
13. Bullrich F, Rasio D, Kitada S, Starostik P, Kipps T, Keating M *et al.* ATM mutations in B-cell chronic lymphocytic leukemia. *Cancer Res* 1999; **59**: 24–27.
14. Stilgenbauer S, Nickolenko J, Wilhelm J, Wolf S, Weitz S, Dohner K *et al.* Expressed sequences as candidates for a novel tumor suppressor gene at band 13q14 in B-cell chronic lymphocytic leukemia and mantle cell lymphoma. *Oncogene* 1998; **16**: 1891–1897.
15. Dong JT, Boyd JC, Frierson HF Jr. Loss of heterozygosity at 13q14 and 13q21 in high grade, high stage prostate cancer. *Prostate* 2001; **49**: 166–171.
16. Bullrich F, Fujii H, Calin G, Mabuchi H, Negrini M, Pekarsky Y *et al.* Characterization of the 13q14 tumor suppressor locus in CLL: identification of ALT1, an alternative splice variant of the LEU2 gene. *Cancer Res* 2001; **61**: 6640–6648.
17. Migliazza A, Bosch F, Komatsu H, Cayanis E, Martinotti S, Toniato E *et al.* Nucleotide sequence, transcription map, and mutation analysis of the 13q14 chromosomal region deleted in B-cell chronic lymphocytic leukemia. *Blood* 2001; **97**: 2098–2104.
18. Rondeau G, Moreau I, Bezieau S, Petit JL, Heilig R, Fernandez S *et al.* Comprehensive analysis of a large genomic sequence at the putative B-cell chronic lymphocytic leukaemia (B-CLL) tumour suppresser gene locus. *Mutat Res* 2001; **458**: 55–70.
19. Mertens D, Wolf S, Schroeter P, Schaffner C, Dohner H, Stilgenbauer S *et al.* Down-regulation of candidate tumor suppressor genes within chromosome band 13q14.3 is independent of the DNA methylation pattern in B-cell chronic lymphocytic leukemia. *Blood* 2002; **99**: 4116–4121.
20. 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 USA* 2002; **99**: 15524–15529.
21. Pekarsky Y, Calin GA, Aqeilan R. Chronic lymphocytic leukemia: molecular genetics and animal models. *Curr Top Microbiol Immunol* 2005; **294**: 51–70.
22. Ambros V. The functions of animal microRNAs. *Nature* 2004; **431**: 350–355.
23. Ambros V. MicroRNA pathways in flies and worms: growth, death, fat, stress, and timing. *Cell* 2003; **113**: 673–676.
24. Bartel DP. MicroRNAs: genomics, biogenesis, mechanism, and function. *Cell* 2004; **116**: 281–297.
25. Cimmino A, Calin GA, Fabbri M, Iorio MV, Ferracin M, Shimizu M *et al.* miR-15 and miR-16 induce apoptosis by targeting BCL2. *Proc Natl Acad Sci USA* 2005; **102**: 13944–13949.
26. Sanchez-Beato M, Sanchez-Aguilera A, Piris MA. Cell cycle deregulation in B-cell lymphomas. *Blood* 2003; **101**: 1220–1235.
27. Grabow S, Waring P, Happo L, Cook M, Mason KD, Kelly PN *et al.* Pharmacological blockade of Bcl-2, Bcl-x(L) and Bcl-w by the BH3 mimetic ABT-737 has only minor impact on tumour development in p53-deficient mice. *Cell Death Differ* 2012; **19**: 623–632.
28. Kitada S, Andersen J, Akar S, Zapata JM, Takayama S, Krajewski S *et al.* Expression of apoptosis-regulating proteins in chronic lymphocytic leukemia: correlations with in vitro and in vivo chemoresponses. *Blood* 1998; **91**: 3379–3389.
29. Adachi M, Tefferi A, Greipp PR, Kipps TJ, Tsujimoto Y. Preferential linkage of Bcl-2 to immunoglobulin light chain gene in chronic lymphocytic leukemia. *J Exp Med* 1990; **171**: 559–564.
30. Allegra D, Bilan V, Garding A, Dohner H, Stilgenbauer S, Kuchenbauer F *et al.* Defective DROSHA processing contributes to downregulation of MiR-15/-16 in chronic lymphocytic leukemia. *Leukemia* 2014; **28**: 98–107.
31. Sampath D, Liu C, Vasan K, Sulda M, Puduvalli VK, Wierda WG *et al.* Histone deacetylases mediate the silencing of miR-15a, miR-16, and miR-29b in chronic lymphocytic leukemia. *Blood* 2012; **119**: 1162–1172.
32. Calin GA, Ferracin M, Cimmino A, Di Leva G, Shimizu M, Wojcik SE *et al.* A microRNA signature associated with prognosis and progression in chronic lymphocytic leukemia. *N Engl J Med* 2005; **353**: 1793–1801.
33. Calin GA, Cimmino A, Fabbri M, Ferracin M, Wojcik SE, Shimizu M *et al.* MiR-15a and miR-16-1 cluster functions in human leukemia. *Proc Natl Acad Sci USA* 2008; **105**: 5166–5171.
34. Fabbri M, Bottoni A, Shimizu M, Spizzo R, Nicoloso MS, Rossi S *et al.* Association of a microRNA/TP53 feedback circuitry with pathogenesis and outcome of B-cell chronic lymphocytic leukemia*JAMA* 2011; **305**: 59–67.
35. Cai CK, Zhao GY, Tian LY, Liu L, Yan K, Ma YL *et al.* miR-15a and miR-16-1 downregulate CCND1 and induce apoptosis and cell cycle arrest in osteosarcoma. *Oncol Rep* 2012; **28**: 1764–1770.
36. Jiang QQ, Liu B, Yuan T. MicroRNA-16 inhibits bladder cancer proliferation by targeting Cyclin D1. *Asian Pac J Cancer Prev* 2013; **14**: 4127–4130.
37. Teshima K, Nara M, Watanabe A, Ito M, Ikeda S, Hatano Y *et al.* Dysregulation of BMI1 and microRNA-16 collaborate to enhance an anti-apoptotic potential in the side population of refractory mantle cell lymphoma. *Oncogene* 2014; **33**: 2191–2203.
38. Reid G, Pel ME, Kirschner MB, Cheng YY, Mugridge N, Weiss J *et al.* Restoring expression of miR-16: a novel approach to therapy for malignant pleural mesothelioma. *Ann Oncol* 2013; **24**: 3128–3135.
39. Klein U, Lia M, Crespo M, Siegel R, Shen Q, Mo T *et al.* The DLEU2/miR-15a/16-1 cluster controls B cell proliferation and its deletion leads to chronic lymphocytic leukemia. *Cancer Cell* 2010; **17**: 28–40.
40. Raveche ES. Possible immunoregulatory role for CD5+B cells. *Clin Immunol Immunopathol* 1990; **56**: 135–150.
41. Raveche ES, Salerno E, Scaglione BJ, Manohar V, Abbasi F, Lin YC *et al.* Abnormal microRNA-16 locus with synteny to human 13q14 linked to CLL in NZB mice. *Blood* 2007; **109**: 5079–5086.
42. Lovat F, Fassan M, Gasparini P, Rizzotto L, Cascione L, Pizzi M *et al.* miR-15b/16-2 deletion promotes B-cell malignancies. *Proc Natl Acad Sci USA* 2015; **112**: 11636–11641.
43. Souers AJ, Leverson JD, Boghaert ER, Ackler SL, Catron ND, Chen J *et al.* ABT-199, a potent and selective BCL-2 inhibitor, achieves antitumor activity while sparing platelets. *Nat Med* 2013; **19**: 202–208.
44. Croce CM, Reed JC. Finally, an apoptosis-targeting therapeutic for cancer. *Cancer Res* 2016; **76**: 5914–5920.