HHS Public Access

Author manuscript

Adv Biol Regul. Author manuscript; available in PMC 2020 January 01.

Published in final edited form as:

Adv Biol Regul. 2019 January ; 71: 219–223. doi:10.1016/j.jbior.2018.12.002.

Noncoding RNA genes in cancer pathogenesis

Yuri Pekarsky[1], **Carlo M. Croce**[1,^]

[1]Department of Cancer Biology and Genetics, the Wexner Medical Center, Columbus, Ohio, USA

Abstract

By using chronic lymphocytic leukemia as target for discovery in cancer pathogenesis we discovered that the great majority of CLLs (75-85%) carry a deletion of *miR-15a* and *miR-16-1* at 13q14. We also discovered that miR-15/16 are negative regulators of the *BCL2* oncogene. Thus the loss of the two negative regulators causes *BCL2* overexpression and leukemia. A corollary of this is that CLL is very sensitive to the anti *BCL2* drug venetoclax that can induce complete remission in CLL patients. Since leukemia patients may carry billions of leukemia cells, it is quite likely that some (few) of the leukemic cells are resistant to venetoclax. Thus, since microRNAs have multiple targets, we looked for other proteins that may be overexpressed in CLL because of the low of *miR-15/16*. We discovered that *ROR1* an embryonal antigen expressed on most (~ 90%) CLL, but not on normal B cell, is also regulated by *miR-15/16*. Thus CLL cells are also sensitive to monoclonal antibodies against ROR1. Venetoclax and monoclonal antibodies against ROR1 act synergistically in killing CLL cells.

Keywords

CLL; BCL2; ROR1; miR-15/16; tsRNA; tRNA derived fragments

13q deletions in chronic lymphocytic leukemia, *miR-15/16* and *BCL2*.

Chronic lymphocytic leukemia (CLL) is the most common leukemia in humans. Quite often CLL patients can live normally showing mild symptoms and do not need treatment for a number of years (Sgambati et al., 2001). CLL is a complex disease in which a rare population of CD5 positive B-cells is expanded (Bullrich and Croce, 2001; Sgambati et al., 2001). Most often CLL patients with high expression of ZAP-70 (Zeta-chain-associated protein kinase 70) and unmutated IgH V_H gene show a clinically aggressive disease, while patients expressing low levels of ZAP-70 and mutated IgH V_H do not need immediate treatment (Bullrich and Croce, 2001; Sgambati et al., 2001). The common chromosomal aberrations in CLL cells include deletions at 11q (18%), 17p (8%), 13q (60%), and trisomy 12 (12%−16%) (Dohner et al., 2000; Edelmann et al., 2012).

[^]**Corresponding author**: Carlo M. Croce, MD, The Ohio State University, Comprehensive Cancer Center, Biomedical Research Tower, Room 1082, 460 West 12th Avenue, Columbus, OH 43210, USA, carlo.croce@osumc.edu.

Multiple publications showed that 17p deletions result in inactivation of *TP53* and *miR-3676 - miR-4521* cluster (*miR-3676* targets *TCL1* an import and driver in aggressive CLL) (Balatti et al., 2015; Palamarchuk et al., 2012); trisomy 12 CLLs are associated with *NOTCH1* (Notch homolog 1, translocation-associated (Drosophila)) gain of function mutations (Balatti et al., 2012); while *ATM* (ataxia-telangiectasia mutated) gene may be inactivated by 11q deletions (Bullrich et al., 1999). Interestingly, CLL patients with 11q and 17p deletions usually require immediate treatment while patients with 13q deletions often have indolent disease (Dohner et al., 2000).

The discovery of *miR-15/16* as a target of 13q deletions in CLL and role its role in targeting *BCL2* were reviewed multiple times. Briefly, in 2000-2002 we thought that 13q14 region deleted in most CLLs contains an important tumor suppressor gene. Despite the extensive effort by us and several other laboratories no protein coding gene inactivated in CLL was found (Bullrich et al., 2001; Mertens et al., 2002; Migliazza et al., 2001; Rondeau et al., 2001). At that time we identified several interesting CLL cases, one with a small 30 kb deletion, and another one with a translocation within this deletion. At that time since genomic databases were frequently updated and using one of these and we determined that a cluster of two microRNA genes, *miR-15a* and *miR-16-1*, was located in the 30 kb region (Calin et al., 2002), and *miR-15/16* cluster was the only gene in that region. Subsequent studies revealed that *miR-15/16* was the target of 13q deletions in CLL and that was the first example that a microRNA gene was involved in cancer (Calin et al., 2002).

Since *miR-15/16* is a target of 13q14 deletions we thought that *miR-15/16* could target important oncogene(s) in B-cells. We searched available databases and identified predicted *miR-15/16* targets. One of the top predicted targets was *BCL2*, a critical oncogene overexpressed in most CLL cases (Cimmino et al., 2005). *BCL2* has a very important role in the pathogenesis of solid cancers as well as lymphoid malignancies. Located mostly in mitochondria, BCL2 protein indices survival and decreases cell death by inhibiting the release of Cytochrome C into the cytoplasm (Grabow et al., 2012; Sanchez-Beato et al., 2003). By analyzing homology between *miR-15/16* and the *BCL2* mRNA we found that both *miR-15* and *miR-16* potentially target bases 3287 to 3279 in the 3'end of the *BCL2* cDNA (Cimmino et al., 2005). By studying the expression levels of *miR-15, miR-16* and *BCL2* in CLL we found that expression of *miR-15/16* was lowest in the samples expressing high levels of *BCL2*; and expression of *miR-15/16* was highest in the samples expressing low levels of *BCL2*. We then confirmed that *miR-15/16* directly targeted *BCL2* by experiments and concluded that *BCL2* overexpression in CLL is due the loss of *miR-15/16* (Cimmino et al., 2005).

MicroRNAs can not be easily delivered into cancer cells, thus, at least at this time *miR-15/16* is not suitable to use as a CLL drug. On the other hand, *BCL2* is highly expressed in almost all CLL cells and thus represents a good target to develop anti cancer drugs. Recently, Abbott was able to develop a drug, an inhibitor of Bcl2 (Souers et al., 2013). Venetoclax (also called ABT-199) targets Bcl2 by inhibiting its protein-protein interactions. Venetoclax was used to treat previously treated relapsed patients showing 17p deletions, and even in these difficult to treat cases venetoclax had of 80% response rate (Croce and Reed, 2016).

ROR1, a new *miR-15/16* target.

Dr. Kipps laboratory has discovered the receptor kinase like orphan receptor 1 (ROR1) and found that is an oncoembryonic antigen found on most CLL B-cells (approximately in 90% of CLL patients) and not on normal B-cells or normal adult tissues, except a small subgroup of B-cell precursors named hematogones (Baskar et al., 2008; Broome et al., 2011). Additionally, antibodies targeting ROR1 can inhibit ROR1 induced cell growth in cells expressing both ROR1 and Tcl1, such as human aggressive CLL (Widhopf et al., 2014). We analyzed the 3' untranslated region (UTR) of the *ROR1* gene with bioinformatic tools and found that *ROR1* is a predicted target of *miR-15/16*. Thus, we carried out luciferase experiments and proved that *miR-15/16* target the 3' UTR of *ROR1* (Rassenti et al., 2017).

Since previous studies have shown that approximately 90% of CLL patients are positive for ROR1 (Fukuda et al., 2008), but approximately 10% have low-to-negligible levels of ROR1, we investigated a cohort of ROR1 low and ROR1 high for the expression of microRNAs (Rassenti et al., 2017). These experiments demonstrated that the expression of ROR1 is inversely correlated with the expression of *miR-15a* and *miR-16-1*. ROR1 high and low cells had a significant difference in expression of *miR-15a* and *miR-16-1* (Rassenti et al., 2017). By Western blotting, we investigated a Burkitt lymphoma cell line that does not express BCL2 (Raji) and a T-cell leukemic cell line that expresses BCL2 (Jurkat), and a panel of CLL cells from patients for the expression of ROR1 and Bcl2. The ROR1 high CLL cells expressed high levels of Bcl2, while ROR1 negative cells expressed very low levels of Bcl2 (Rassenti et al., 2017). At this point we quantitated the differentially expressed microRNA in the ROR1 low and high CLL samples shown a signature of 17 microRNAs can discriminate these two groups of CLL samples (Rassenti et al., 2017). The results of these experiments indicated that the determinants of the expression of ROR1 are *miR-15a and miR-16-1*. The *miR-15/16* expression was low when ROR1 expression was high and *miR-15/16* expression was high when ROR1 expression was low (Rassenti et al., 2017). At this point we wanted to establish the relationship between ROR1 and Bcl2 expressions and percentage of CLL B-cells harboring a 13q14 deletion. We analyzed an additional cohort of 35 cases of CLL for Bcl2 expression and percentage of CLL cells carrying the 13q deletion by FISH analysis. Our results showed that samples with low expression of ROR1 had low expression of intracellular Bcl2 and an average percentage of CLL B-cells carrying in 13q deletion of 22%; while CLL cases with high expression of ROR1 had higher expression of Bcl2 and had an average % of CLL B-cells with a 13q deletion of 55% (Rassenti et al., 2017).

The discovery that *miR-15/16* target *ROR1*, in addition to *BCL2*, may initiate the development of new combination therapy by targeting the same leukemia cells through different biological pathways. Since normal adult tissues generally lack expression of ROR1 and only leukemia cells are express high levels of ROR1, due of the loss of the *miR-15/16* expression, single agent treatment with anti-ROR1 monoclonal antibodies should not have negative side effects, and should target only the leukemia cells of most CLL patients. However, it is possible that some rare CLL cells may escape the drug effect due to loss or modulation of the expression of ROR1. Thus, a combination therapy with Venetoclax and anti-ROR1 antibodies (cirmtuzumab) should be able to eradicate CLL cells even in case of

selection of a clone mutated for one of the targets that would lead to resistance to one of the two drugs (Rassenti et al., 2017).

Since loss of *miR-15/16* is an initial event in CLL pathogenesis causing overexpression of Bcl2 and ROR1, we hypothesized that combination therapy with molecules targeting each of these proteins would have synergistic effect. To investigate if cirmtuzumab can kill CLL cells, we analyzed viability of CLL cells. We carried out these experimants with and without venetoclax to evaluate for the synergic activity of venetoclax and cirmtuzumab CLL cells expressing high levels of ROR1. After 16 hours of treatment, the combination of venetoclax and cirmtuzumab was significantly more cytotoxic than treatment with venetoclax alone, cirmtuzumab alone or in combination with control human antibody (Rassenti et al., 2017). At 16 hours of treatment cirmtuzumab alone did not significantly affect CLL cells viability. Treatment with venetoclax alone resulted in about 50% of cell death, while addition of cirmtuzumab resulted in about 75% of cell death, suggesting that venetoclax and cirmtuzumab have a synergic effect (Rassenti et al., 2017).

MiR-15/16 deletion causes leukemia in mice.

Tumor suppressor function of any coding or noncoding gene is never fully evidenced until their deletion in mice results in a tumor phenotype. To prove that *miR-15/16* is *bona fide* tumor suppressor Klein at al in 2010 inactivated *miR-15/16* in mice (Klein et al., 2010). They generated two knockout alleles: one of the alleles had small deletion of *miR-15/16* only; another allele was called *MDR* (minimal deleted region) had *miR-15/16* and the neighboring *Dleu2* gene deleted (Klein et al., 2010). Both mouse strains developed CLL like disease at the age of 18 months. Malignant B-cells were CD5, CD19 and IgM positive, and penetrance was ~25% for *miR-15/16* knockouts and ~40% for MDR strain. Interestingly, the knockout of *MDR* allele had more severe phenotype then that of *miR-15/16* only implying that additional regulatory elements in MDR may also contribute to CLL pathogenesis (Klein et al., 2010).

There are two *miR-15/16* clusters in humans, *miR-15a/16-1* and *miR-15b/16-2. MiR-15/16* and *MDR* mice described above were generated by deleting *miR-15a/16-1* cluster. Very recently we generated a mouse expressing no *miR-15/16* by crossing *miR-15a/16-1* and *miR-15b/16-2* knockouts (DKO) (Lovat et al., 2018). Northern Blot analysis confirmed complete absence of *miR-15/16* expression in splenocytes of these mice. DKO mice showed a significant reduction of overall survival (65% at the age of 12 months). Additional examination determined that DKO mice developed ignificant splenomegaly (Lovat et al., 2018). We then carried out histological analysis and to our surprise 77% of DKO mice developed a disease very similar to human AML while 23% developed B-cell malignancies. 20% of DKO mice also developed extra-splenic AML related disease, which involved livers and lungs. Using flow cytometry analysis we were able to confirm AML phenotype. While CD11b/Gr1 double positive cells we 1% in spleens and 40% in bone marrow of control mice, DKO spleens contained 10-15% CD11b/Gr1 double positive cells while DKO bone marrow contained 60-70% such cells (Lovat et al., 2018).

TsRNAs, a new class of small noncoding RNAs involved in CLL.

TCL1 (T-cell leukemia/lymphoma 1) oncogene is a critical gene in the pathogenesis of aggressive CLL. We and others previously showed that transgenic mice overexpressing *TCL1* in B-cells develop the aggressive form of CLL (Allegra et al., 2014; Bichi et al., 2002; Yan et al., 2006). *TCL1* overexpression transforms B-cells by activating Akt and inhibiting AP-1, Dnmt3A and Dnmt3B (Palamarchuk et al., 2012; Pekarsky et al., 2000; Pekarsky et al., 2008). While studying the regulation of *TCL1* expression, we identified the microRNA cluster *miR-4521/3676* and determined that *miR-3676* is a powerful regulator of *TCL1* expression that targets three consecutive 28-bp repeats within the 3' UTR of *TCL1* (Balatti et al., 2015). Subsequently we were studying in detail the *miR-4521/3676* cluster, because of its proximity to the *TP53* gene (this region includes *TP53* and *miR-4521/3676* and is deleted in 7-10% of CLLs). We found that these two microRNAs are associated with tRNA sequences and represent a recently identified class of small noncoding RNAs, tsRNAs (Pekarsky et al., 2016). TsRNAs is a new class of small RNAs derived from tRNAs during tRNA processing (Haussecker et al., 2010). tRNAs are expressed from tRNA genes by RNA polymerase III. TsRNAs are molecules produced from the 3' end of pre-tRNAs by endonuclease RNase Z, they are unique sequences starting at the 3' ends of tRNAs and ending at the stop signal for RNA polymerase III (four consecutive T nucleotides) (Haussecker et al., 2010; Martens-Uzunova et al., 2013).

Since tsRNAs are physically similar to piRNAs (they are both single-stranded short RNAs containing no secondary structures), we investigated if the tsRNAs derived from this region (*ts-3676* and *ts-4521*) can also function as piRNAs (Pekarsky et al., 2016). We carried out an RNA immunoprecipitation experiment and determined that *ts-3676* and *ts-4521* can bind PiwiL2. Indeed PiwiL2 complexes were enriched with *ts-3676* and *ts-4521* but not with control microRNAs. At the same time both, *ts-3676* and *ts-4521* and control microRNAs were present in Ago1 and Ago2 complexes as expected (Pekarsky et al., 2016). These results demonstrate that the *miR-4521/3676* locus expresses two small RNAs, *ts-3676* and *ts-4521*, which may function as microRNAs as well as piRNAs by binding to PiwiL2 (Pekarsky et al., 2016).

We then investigated expression of *ts-3676* and *ts-4521* in CLL and lung cancer. In CLL we found that *ts-3676* and *ts-4521* are down-regulated in all cytogenetic CLL groups (17p deleted, 11q deleted, 13q deleted and normal karyotype) (Pekarsky et al., 2016). To determine the expression of *ts-3676* and *ts-4521* in lung cancer, we used 17 lung cancer samples and matched normal lung tissues. Real-time RT-PCR experiments revealed a drastic down-regulation of *ts-3676* and *ts-4521* expression in lung cancer samples compared to matched normal lung controls. Sequencing analysis of ~500 CLL samples and ~300 lung cancer samples revealed that *ts-3676* and *ts-4521* are mutated in ~1% of CLLs and 2% of lung cancer samples (Pekarsky et al., 2016).

Thus, to our knowledge, we identified first two tsRNAs (produced from 3' end of pre tRNAs) involved in cancer. Our data established that *ts-3676* and *ts-4521* do not originate from the processing that involves microRNAs (Drosha cleavage followed by Dicer cleavage), but derive from a Thr tRNA and a Ser tRNA respectively (Pekarsky et al., 2016).

Similarly to *miR-15/16*, the first two microRNAs found altered in cancer and inactivated in 13q deleted CLL cases, *ts-3676* and *ts-4521* are inactivated in 17p deleted CLL cases (Balatti et al., 2015). While *miR-15/16* target *BCL2* and *ROR1*, critical genes in CLL pathogenesis, *ts-3676* targets *TCL1*, an oncogene critical in aggressive CLL (Balatti et al., 2015).

Since *ts-3676* and *ts-4521* are down-regulated in CLL and lung cancer, we decided to determine if other tsRNAs can be differentially expressed in cancer. We retrieved all tRNA sequences from genomic databases, generated a list of all tsRNAs by isolating unique sequences starting from the 3' end of tRNAs and ending with the four-T RNA Polymerase III stop signal. Our list contained ~120 unique tsRNA sequences (Pekarsky et al., 2016). Using this list we designed a microarray chip with a goal of studying expression patterns of tsRNAs in a variety of tumor tissues and normal controls. First we studied tsRNA expression signatures in CLL. We selected 11 indolent CLL patient samples, 12 aggressive CLL patient samples and 8 normal controls and carried out a microarray experiment using a tsRNA chip we designed. We found that 15 tsRNAs were significantly up- or down-regulated in indolent CLL versus aggressive CLL comparison. In addition, nine tsRNAs were significantly differentially expressed in aggressive CLL compared normal controls; and that 10 tsRNAs were dysregulated in indolent CLL versus normal controls (Pekarsky et al., 2016). To investigate tsRNA are signatures in lung cancer, we carried out a similar experiment using seven lung cancer samples and five normal paired lung tissue samples. This resulted in the identification of six tsRNAs dysregulated in lung cancer samples versus normal lung tissues (Pekarsky et al., 2016). Interestingly, *ts-46* and *ts-47* were significantly down-regulated in lung cancer samples versus normal lung tissues, and in aggressive CLL when compared to normal controls. This indicates that *ts-46* and *ts-47* could potentially have tumor suppressor functions (Pekarsky et al., 2016). To confirm these observations in another study we showed that both, *ts-46* and *ts-47* suppress colonies formation is lung cancer cell lines (Balatti et al., 2017). In a subsequent study we investigated 14 paired samples (tumor and normal surrounding tissue) from 7 colon adenoma patients and 16 paired samples (tumor and normal surrounding tissue) from 8 patients with colon adenocarcinoma. This resulted in a signature of 8 tsRNAs for colon adenoma and another signature of 7 tsRNAs for colon adenocarcinoma and a signature (Balatti et al., 2017). Interestingly *ts-3676* and *ts-4521* were inactivated in adenomas but not in adenocarcinomas. Both comparisons showed up-regulation of *Ts-40* in colon cancer indicating possible oncogenic function for this tsRNA (Balatti et al., 2017). In another experiment we studied tsRNA signature in human lymphocytes with and without activation of *c-MYC*. Among 15 differentially expressed tsRNAs, *ts-47* was the most down-regulated by *c-MYC* overexpression, indicating that *c-MYC* may be responsible for don-regulation of *ts-47* in CLL and lung cancer (Balatti et al., 2017).

References.

Allegra D, Bilan V, Garding A, Dohner H, Stilgenbauer S, Kuchenbauer F, Mertens D, 2014 Defective DROSHA processing contributes to downregulation of MiR-15/−16 in chronic lymphocytic leukemia. Leukemia 28(1), 98–107. [PubMed: 23974981]

Balatti V, Bottoni A, Palamarchuk A, Alder H, Rassenti LZ, Kipps TJ, Pekarsky Y, Croce CM, 2012 NOTCH1 mutations in CLL associated with trisomy 12. Blood 119(2), 329–331. [PubMed: 22086416]

Balatti V, Nigita G, Veneziano D, Drusco A, Stein GS, Messier TL, Farina NH, Lian JB, Tomasello L, Liu CG, Palamarchuk A, Hart JR, Bell C, Carosi M, Pescarmona E, Perracchio L, Diodoro M, Russo A, Antenucci A, Visca P, Ciardi A, Harris CC, Vogt PK, Pekarsky Y, Croce CM, 2017 tsRNA signatures in cancer. Proceedings of the National Academy of Sciences of the United States of America 114(30), 8071–8076. [PubMed: 28696308]

Balatti V, Rizzotto L, Miller C, Palamarchuk A, Fadda P, Pandolfo R, Rassenti LZ, Hertlein E, Ruppert AS, Lozanski A, Lozanski G, Kipps TJ, Byrd JC, Croce CM, Pekarsky Y, 2015 TCL1 targeting miR-3676 is codeleted with tumor protein p53 in chronic lymphocytic leukemia. Proceedings of the National Academy of Sciences of the United States of America 112(7), 2169–2174. [PubMed: 25646413]

Baskar S, Kwong KY, Hofer T, Levy JM, Kennedy MG, Lee E, Staudt LM, Wilson WH, Wiestner A, Rader C, 2008 Unique cell surface expression of receptor tyrosine kinase ROR1 in human B-cell chronic lymphocytic leukemia. Clinical cancer research : an official journal of the American Association for Cancer Research 14(2), 396–404.

Bichi R, Shinton SA, Martin ES, Koval A, Calin GA, Cesari R, Russo G, Hardy RR, Croce CM, 2002 Human chronic lymphocytic leukemia modeled in mouse by targeted TCL1 expression. Proc Natl Acad Sci U S A 99(10), 6955–6960. [PubMed: 12011454]

Broome HE, Rassenti LZ, Wang HY, Meyer LM, Kipps TJ, 2011 ROR1 is expressed on hematogones (non-neoplastic human B-lymphocyte precursors) and a minority of precursor-B acute lymphoblastic leukemia. Leukemia research 35(10), 1390–1394. [PubMed: 21813176]

Bullrich F, Croce C, 2001 Molecular Biology of Chronic Lymphocytic Leukemia. Chronic Lymphocytic Leukemias, Second Edition, Revised and Expanded, Bruce Cheson , Ed. Dekker Marcel, Inc. New York, 9–32.

Bullrich F, Fujii H, Calin G, Mabuchi H, Negrini M, Pekarsky Y, Rassenti L, Alder H, Reed JC, Keating MJ, Kipps TJ, Croce CM, 2001 Characterization of the 13q14 tumor suppressor locus in CLL: identification of ALT1, an alternative splice variant of the LEU2 gene. Cancer research 61(18), 6640–6648. [PubMed: 11559527]

Bullrich F, Rasio D, Kitada S, Starostik P, Kipps T, Keating M, Albitar M, Reed JC, Croce CM, 1999 ATM mutations in B-cell chronic lymphocytic leukemia. Cancer Res 59(1), 24–27. [PubMed: 9892178]

Calin GA, Dumitru CD, Shimizu M, Bichi R, Zupo S, Noch E, Aldler H, Rattan S, Keating M, Rai K, Rassenti L, Kipps T, Negrini M, Bullrich F, Croce CM, 2002 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 99(24), 15524–15529. [PubMed: 12434020]

Cimmino A, Calin GA, Fabbri M, Iorio MV, Ferracin M, Shimizu M, Wojcik SE, Aqeilan RI, Zupo S, Dono M, Rassenti L, Alder H, Volinia S, Liu CG, Kipps TJ, Negrini M, Croce CM, 2005 miR-15 and miR-16 induce apoptosis by targeting BCL2. Proc Natl Acad Sci U S A 102(39), 13944–13949. [PubMed: 16166262]

Croce CM, Reed JC, 2016 Finally, An Apoptosis-Targeting Therapeutic for Cancer. Cancer research 76(20), 5914–5920. [PubMed: 27694602]

Dohner H, Stilgenbauer S, Benner A, Leupolt E, Krober A, Bullinger L, Dohner K, Bentz M, Lichter P, 2000 Genomic aberrations and survival in chronic lymphocytic leukemia. N Engl J Med 343(26), 1910–1916. [PubMed: 11136261]

Edelmann J, Holzmann K, Miller F, Winkler D, Buhler A, Zenz T, Bullinger L, Kuhn MW, Gerhardinger A, Bloehdorn J, Radtke I, Su X, Ma J, Pounds S, Hallek M, Lichter P, Korbel J, Busch R, Mertens D, Downing JR, Stilgenbauer S, Dohner H, 2012 High-resolution genomic profiling of chronic lymphocytic leukemia reveals new recurrent genomic alterations. Blood 120(24), 4783–4794. [PubMed: 23047824]

Fukuda T, Chen L, Endo T, Tang L, Lu D, Castro JE, Widhopf GF 2nd, Rassenti LZ, Cantwell MJ, Prussak CE, Carson DA, Kipps TJ, 2008 Antisera induced by infusions of autologous Ad-CD154-leukemia B cells identify ROR1 as an oncofetal antigen and receptor for Wnt5a. Proceedings of

the National Academy of Sciences of the United States of America 105(8), 3047–3052. [PubMed: 18287027]

Grabow S, Waring P, Happo L, Cook M, Mason KD, Kelly PN, Strasser A, 2012 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 19(4), 623–632. [PubMed: 21997189]

Haussecker D, Huang Y, Lau A, Parameswaran P, Fire AZ, Kay MA, 2010 Human tRNA-derived small RNAs in the global regulation of RNA silencing. Rna 16(4), 673–695. [PubMed: 20181738]

Klein U, Lia M, Crespo M, Siegel R, Shen Q, Mo T, Ambesi-Impiombato A, Califano A, Migliazza A, Bhagat G, Dalla-Favera R, 2010 The DLEU2/miR- 15a/16–1 cluster controls B cell proliferation and its deletion leads to chronic lymphocytic leukemia. Cancer Cell 17(1), 28–40. [PubMed: 20060366]

Lovat F, Fassan M, Sacchi D, Ranganathan P, Palamarchuk A, Bill M, Karunasiri M, Gasparini P, Nigita G, Distefano R, Veneziano D, Dorrance AM, Garzon R, Croce CM, 2018 Knockout of both miR-15/16 loci induces acute myeloid leukemia. Proceedings of the National Academy of Sciences of the United States of America.

Martens-Uzunova ES, Olvedy M, Jenster G, 2013 Beyond microRNA--novel RNAs derived from small non-coding RNA and their implication in cancer. Cancer letters 340(2), 201–211. [PubMed: 23376637]

Mertens D, Wolf S, Schroeter P, Schaffner C, Dohner H, Stilgenbauer S, Lichter P, 2002 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 99(11), 4116–4121. [PubMed: 12010815]

Migliazza A, Bosch F, Komatsu H, Cayanis E, Martinotti S, Toniato E, Guccione E, Qu X, Chien M, Murty VV, Gaidano G, Inghirami G, Zhang P, Fischer S, Kalachikov SM, Russo J, Edelman I, Efstratiadis A, Dalla-Favera R, 2001 Nucleotide sequence, transcription map, and mutation analysis of the 13q14 chromosomal region deleted in B-cell chronic lymphocytic leukemia. Blood 97(7), 2098–2104. [PubMed: 11264177]

Palamarchuk A, Yan PS, Zanesi N, Wang L, Rodrigues B, Murphy M, Balatti V, Bottoni A, Nazaryan N, Alder H, Rassenti L, Kipps TJ, Freitas M, Croce CM, Pekarsky Y, 2012 Tcl1 protein functions as an inhibitor of de novo DNA methylation in B-cell chronic lymphocytic leukemia (CLL). Proceedings of the National Academy of Sciences of the United States of America 109(7), 2555–2560. [PubMed: 22308499]

Pekarsky Y, Balatti V, Palamarchuk A, Rizzotto L, Veneziano D, Nigita G, Rassenti LZ, Pass HI, Kipps TJ, Liu CG, Croce CM, 2016 Dysregulation of a family of short noncoding RNAs, tsRNAs, in human cancer. Proceedings of the National Academy of Sciences of the United States of America 113(18), 5071–5076. [PubMed: 27071132]

Pekarsky Y, Koval A, Hallas C, Bichi R, Tresini M, Malstrom S, Russo G, Tsichlis P, Croce CM, 2000 Tcl1 enhances Akt kinase activity and mediates its nuclear translocation. Proceedings of the National Academy of Sciences of the United States of America 97(7), 3028–3033. [PubMed: 10716693]

Pekarsky Y, Palamarchuk A, Maximov V, Efanov A, Nazaryan N, Santanam U, Rassenti L, Kipps T, Croce CM, 2008 Tcl1 functions as a transcriptional regulator and is directly involved in the pathogenesis of CLL. Proceedings of the National Academy of Sciences of the United States of America 105(50), 19643–19648. [PubMed: 19064921]

Rassenti L, Balatti V, Ghia EM, Palamarchuk A, Tomasello L, Fadda P, Pekarsky Y, Widhopf Ii GF, Kipps T, Croce CM, 2017 MicroRNA dysregulation to identify therapeutic target combinations for Chronic Lymphocytic Leukemia. Proc Natl Acad Sci U S A 114(40), 10731–10736 [PubMed: 28923920]

Rondeau G, Moreau I, Bezieau S, Petit JL, Heilig R, Fernandez S, Pennarun E, Myers JS, Batzer MA, Moisan JP, Devilder MC, 2001 Comprehensive analysis of a large genomic sequence at the putative B-cell chronic lymphocytic leukaemia (B- CLL) tumour suppresser gene locus. Mutat Res 458(3–4), 55–70. [PubMed: 11691637]

Sanchez-Beato M, Sanchez-Aguilera A, Piris MA, 2003 Cell cycle deregulation in B-cell lymphomas. Blood 101(4), 1220–1235. [PubMed: 12393483]

Sgambati M, Linet M, Devesa S, 2001 Chronic Lymphocytic Leukemia, Epidemiological, Familial, and Genetic Aspects. Chronic Lymphocytic Leukemias, Second Edition, Revised and Expanded, Bruce Cheson , Ed. Dekker Marcel, Inc. New York, 33–62.

Souers AJ, Leverson JD, Boghaert ER, Ackler SL, Catron ND, Chen J, Dayton BD, Ding H, Enschede SH, Fairbrother WJ, Huang DC, Hymowitz SG, Jin S, Khaw SL, Kovar PJ, Lam LT, Lee J, Maecker HL, Marsh KC, Mason KD, Mitten MJ, Nimmer PM, Oleksijew A, Park CH, Park CM, Phillips DC, Roberts AW, Sampath D, Seymour JF, Smith ML, Sullivan GM, Tahir SK, Tse C, Wendt MD, Xiao Y, Xue JC, Zhang H, Humerickhouse RA, Rosenberg SH, Elmore SW, 2013 ABT-199, a potent and selective BCL-2 inhibitor, achieves antitumor activity while sparing platelets. Nature medicine 19(2), 202–208.

Widhopf GF 2nd, Cui B, Ghia EM, Chen L, Messer K, Shen Z, Briggs SP, Croce CM, Kipps TJ, 2014 ROR1 can interact with TCL1 and enhance leukemogenesis in Emu-TCL1 transgenic mice. Proceedings of the National Academy of Sciences of the United States of America 111(2), 793–798. [PubMed: 24379361]

Yan XJ, Albesiano E, Zanesi N, Yancopoulos S, Sawyer A, Romano E, Petlickovski A, Efremov DG, Croce CM, Chiorazzi N, 2006 B cell receptors in TCL1 transgenic mice resemble those of aggressive, treatment-resistant human chronic lymphocytic leukemia. Proc Natl Acad Sci U S A 103(31), 11713–11718. [PubMed: 16864779]

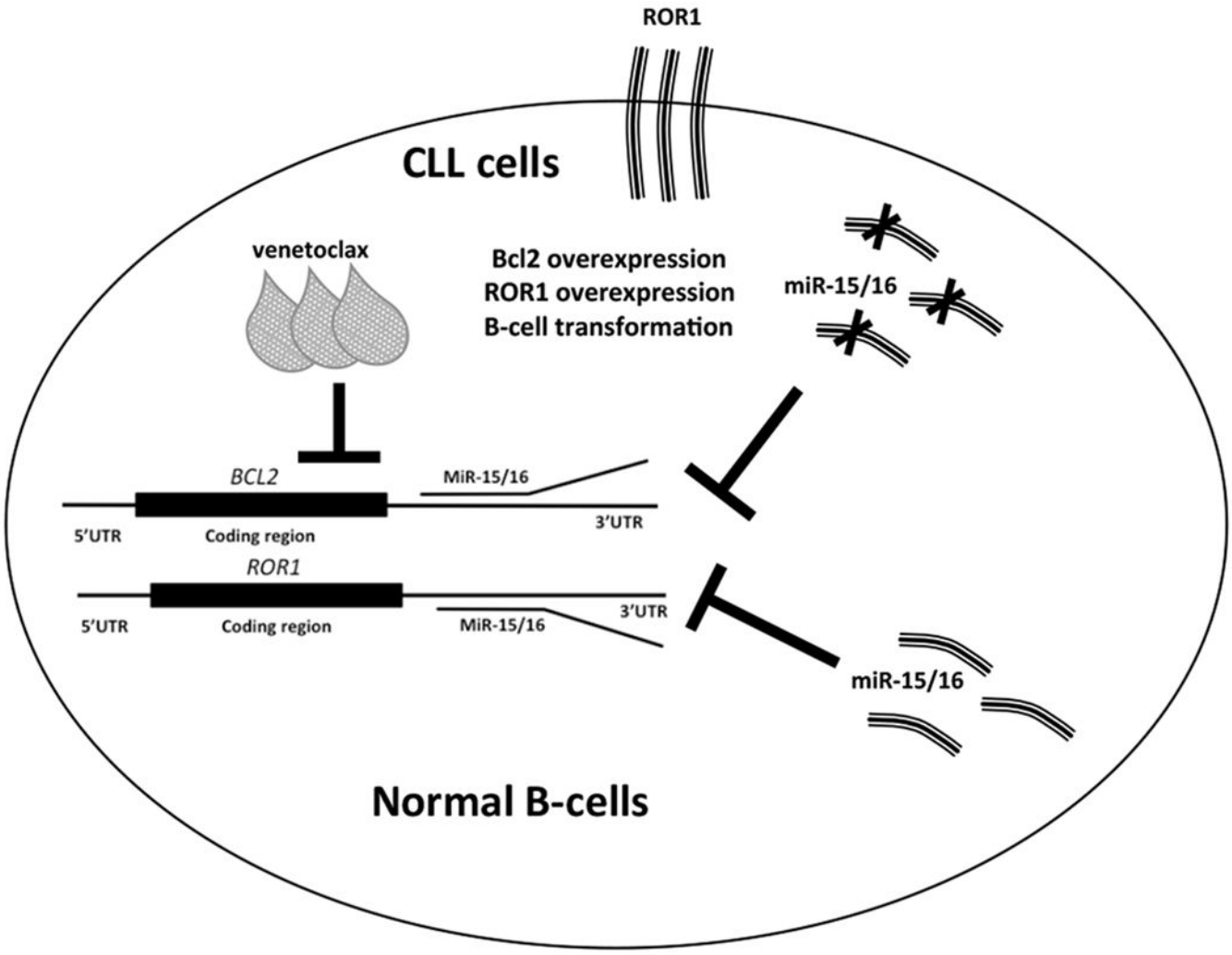

Figure 1. *MiR-15/16, BCL2* and *ROR1* in CLL.

In normal B-cells *miR-15/16* inhibit *ROR1* and *BCL2*. In CLL cells *miR-15/16* is deleted, *ROR1* and *BCL2* are overexpressed causing B-cell transformation.