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Animal Models for Chronic Lymphocytic Leukemia

Yuri Pekarsky,* Nicola Zanesi, Rami I. Aqeilan, and Carlo M. Croce

Comprehensive Cancer Center, Human Cancer Genetics Program, Department of Molecular Virology, Immunology and Medical Genetics, OSU School of Medicine, Ohio State University, Columbus, Ohio

Abstract B-cell chronic lymphocytic leukemia (B-CLL), the most common leukemia in the Western world, results from an expansion of a rare population of CD5+ mature B-lymphocytes. Although clinical features and genomic abnormalities in B-CLL have been studied in considerable detail, the molecular mechanisms underlying disease development has remained unclear until recently. In the last 4 years, several transgenic mouse models for B-CLL were generated. Investigations of these mouse models revealed that deregulation of three pathways, Tcl1-Akt pathway, TNF-NF-kB pathway, and Bcl2-mediated anti-apoptotic pathway, result in the development of B-CLL. While deregulation of *TCL1* alone caused a B-CLL phenotype in mice, overexpression of Bcl2 required aberrantly activated TNF-NF-kB pathway signaling to yield the disease phenotype. In this article, we present what has been learned from mice with B-CLL phenotype and how these mouse models of B-CLL were used to test therapeutic treatments for this common leukemia. J. Cell. Biochem. 100: 1109–1118, 2007. © 2006 Wiley-Liss, Inc.

Key words: B-cell; mouse model; Tcl1

B-cell chronic lymphocytic leukemia (B-CLL) accounts for approximately 30% of all adult leukemia cases and is the most common leukemia in the Western world [Sgambati et al., 2001]. In a significant proportion of B-CLL cases, patients can survive for a long period of time and show relatively mild symptoms [Sgambati et al., 2001]. B-CLL lymphocytes are usually resting cells showing morphologically mature appearance, that usually do not proliferate spontaneously in vitro [Bullrich and Croce, 2001; Sgambati et al., 2001]. B-CLL cells commonly have low levels of surface immunoglobulins and display CD5 positivity representing an expansion of a rare population of $CD5^+$ B-cells [Sgambati et al., 2001]. Genetic predisposition for B-CLL has been known for many years since B-CLL occurs more commonly in people with at least one first degree relative with CLL [Bullrich and Croce, 2001] although no B-CLL predisposition locus has been identified by linkage studies to date. Several chromosomal aberrations occur frequently in B-CLL cases, including 13q deletions, 11q and 7q deletions, trisomy 12 and 17p deletions [Dohner et al., 2000]. The 13q14 deletion is the most common B-CLL aberration and is seen in approximately half of the cases [Dohner et al., 2000]. This particular deletion is seen in predominantly indolent form of B-CLL and is associated with low levels of ZAP70 expression and a mutated variable region genes of immunoglobulins [Calin et al., 2005]. In contrast, aggressive B-CLL cases most often show unmutated immunoglobulin variable region genes and high ZAP70 expression [Herling et al., 2006]. Analysis of ~30 kb deletion at 13q14.3 and chromosomal breakpoint mapping of a translocation t(2:13)(q32;q14) led to the discovery of two physically linked microRNAs, miR-15a and miR-16-1 as targets of these deletions [Calin et al., 2002]. Subsequent studies demonstrated that both miR-15a and miR-16-1 showed a dramatic reduction in expression in the majority of B-CLL cases and that miR-15a/miR-16-1 negatively regulate Bcl2 expression [Cimmino et al., 2005]. These discoveries suggested that downregulation of miR15/16 and subsequent Bcl2 upregulation contribute to B-CLL pathogenesis and these molecules could be used in mouse models for B-CLL.

*Correspondence to: Yuri Pekarsky, Comprehensive Cancer Center Ohio State University, 410 W. 12th Ave., 435 Wiseman Hall, Columbus, Ohio 43210.
E-mail: Pekarsky.Yuri@osumc.edu

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Another important molecule in the pathogenesis of B-CLL is the *TCL1* oncogene. *TCL1* oncogene was discovered as a target of translocations and inversions of *TCL1* (T cell leukemia/lymphoma 1) locus at 14q32.1, the most common chromosomal aberrations detected in mature T-cell leukemias [Virgilio et al., 1994]. A number of recent studies demonstrated that Tcl1 is also a critical molecule in B-CLL pathogenesis [Pekarsky et al., 2005; Herling et al., 2006]. Interestingly, high Tcl1 expression in human B-CLL correlates with aggressive CLL phenotype showing unmutated immunoglobulin variable region genes and ZAP70 positivity [Herling et al., 2006]. This finding suggests that Tcl1 overexpression is an important factor in B-CLL initiation and/or progression. In fact, two transgenic mice with B-cell overexpression of Tcl1 develop B-CLL.

TCL1 FUNCTION IN LYMPHOID CELLS

Expression analyses of *TCL1* revealed that this gene is primarily expressed in B-cells and to a much lesser extent in T-cells [Virgilio et al., 1994]. Tcl1 is expressed in all stages of B-cell development, except mature B-cells [Narducci et al., 2000; Said et al., 2001]. Accordingly, almost all cell lines derived from B-cell malignancies express Tcl1. The biological function of Tcl1 was not known until several years ago when we and others reported that Tcl1 is an activator in the phosphatidylinositide 3-OH kinase (PI3K)—Akt(PKB) oncogenic pathway [Laine et al., 2000; Pekarsky et al., 2000].

Serine/threonine kinase Akt is encoded by the *AKT* oncogene and plays a critical role in the regulation of numerous signaling pathways involved in cell proliferation, survival, and death [Chan et al., 1999]. Multiple studies showed that Akt is an important factor in regulating the survival and proliferation of a number of cell types including B- and T-cells [Chan et al., 1999]. Figure 1 shows a schematic representation the Akt signaling pathway. Growth and survival factors, such as insulin,

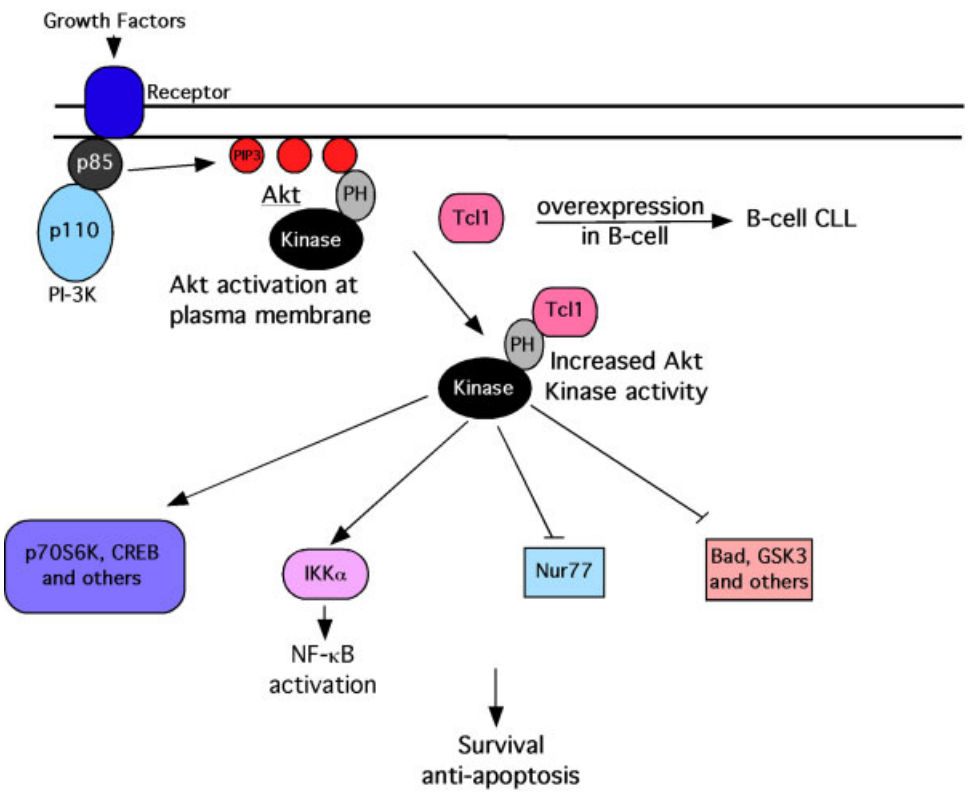

Fig. 1. Tcl1-Akt signaling pathway. Growth and survival factors through their receptors activate PI-3 kinase. PI3K phosphorylates phospholipids located at the plasma membrane. Akt consists of two domains: pleckstrin homology (PH) domain and a kinase domain, Akt binds through its PH domain to phosphorylated phospholipids and translocates to the plasma membrane. At the plasma membrane Akt gets phosphorylated at Thr308 and Ser473 in a catalytic domain and activated. Tcl1 binds Akt, further increases its kinase activity and partially translocates Akt to the nucleus. Tcl1 upregulation increases phosphorylation levels of Akt targets resulting in the resistance to apoptosis and increase in cell survival. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

PDGF and others activate PI3K at the plasma membrane [Chan et al., 1999] leading to the phosphorylation of phospholipids located at the membrane.

Akt contains two domains: Pleckstrin homology (PH) domain responsible for protein-protein interactions and binding to phosphoinositides (PIP3), and a kinase domain containing Ser473 and Thr308 residues critical in the regulation of the kinase activity of Akt [Chan et al., 1999]. Upon phosphorylation of phosphoinositides by PI3K, PH domain binds to phosphoinositides and Akt translocates to the plasma membrane. At the plasma membrane Akt is phosphorylated at Thr308 and Ser473 and activated (Fig. 1) [Chan et al., 1999].

This activated Akt phosphorylates and regulates a number of pro- and anti-survival factors including IKKα [Ozes et al., 1999], GSK3 [Cross et al., 1995], CREB [Du and Montminy, 1998], Bad [Mok et al., 1999], Nur77 [Pekarsky et al., 2001], Pdcd4 [Palamarchuk et al., 2005a], Tal1 [Palamarchuk et al., 2005b] and others (Fig. 1). Generally, this regulation increases cell survival and inhibits apoptosis.

In 2000, we and others showed that Tcl1 and Akt act in the same pathway [Laine et al., 2000; Pekarsky et al., 2000]. Coimmunoprecipitation experiments demonstrated that Tcl1 and Akt physically interact and that PH domain of Akt is responsible for this association [Pekarsky et al., 2000]. Using *in vitro* and *in vivo* kinase assays we further showed that Akt physically bound to Tcl1 shows significant increase in the kinase activity [Laine et al., 2000; Pekarsky et al., 2000]. Thus, Tcl1 functions as a co-activator of Akt (Fig. 1). Additional immunofluorescence studies showed that Akt localization was shifted from primarily cytoplasmic to both nuclear and cytoplasmic upon Tcl1 coexpression [Ahmed et al., 1993; Pekarsky et al., 2000]. Thus, Tcl1 mediates the nuclear translocation of Akt [Pekarsky et al., 2000]. A recent study demonstrated that dimerization of Tcl1 and its physical binding to Akt result in the multimeric complex showing increased Akt activity and translocation to the nucleus [Kunstle et al., 2002]. Since a number of Akt phosphorylation targets have been reported, it is important to determine which targets affected by Tcl1 expression are significant in B-CLL pathogenesis. Nur77 is one such candidate. Nur77, also known as NGFI-B or TR3, is a lymphoid-specific transcription factor and an orphan nuclear receptor [Hazel et al., 1988]. Nur77 is induced and required in T-cell apoptosis [Liu et al., 1994; Calnan et al., 1995]. We and others showed that Akt phosphorylates Nur77 in its DNA binding domain and that this phosphorylation inhibits its DNA-binding and transactivation activity of Nur77. Therefore, the ability of Nur77 to transactivate pro-apoptotic genes is decreased [Masuyama et al., 2001; Pekarsky et al., 2001].

Tcl1-DRIVEN MOUSE MODEL OF B-CLL

Previously, we generated transgenic mice in which the expression of *TCL1* was under the control of a V_H promoter-Ig_H-Eμ enhancer that targets expression of the transgene to immature and mature B-cells [Bichi et al., 2002]. Two transgenic founders on a B6C3 background were generated and bred to establish two transgenic lines that expressed Tcl1 in spleen and bone marrow. The immunophenotypic profile of peripheral blood lymphocytes from mice of these two lines between 1 and 9 months of age revealed an expansion of the B220/IgM^+ population, specifically those that were $CD5^+$/B1, whereas such cells are normally infrequent [Kantor and Herzenberg, 1993]. This expansion was detected in 100% of the transgenic mice by 6 months of age, without any sign of disease [Bichi et al., 2002]. Fluorescence-activated cell sorting (FACS) analysis revealed a phenotypically homogeneous $CD5^+IgM^+$ population markedly expanded in the peritoneal cavity of the transgenic mice starting at 2 months of age (44%) that became evident in spleen (9%) by 4 months and bone marrow by 8 months (43%) [Bichi et al., 2002]. At this age, transgenic mice presented a slightly enlarged spleen and a very high cellularity in the peritoneal cavity, ranging between 50- to 100-fold increase. Histopathology of enlarged spleens of Eμ-*TCL1* mice demonstrated a consistent increase in the size of the marginal zone of the white pulp. We also investigated the cell cycle distribution and the rate of cell proliferation in spleen and peritoneal cavity of these transgenic mice and found that IgM^+CD5^+ sorted populations are arrested in the G_0/G_1 phase of the cell cycle and do not actively divide. Eventually, the onset of a frank leukemia in the elderly mice provided evidence of the establishment of a murine model for human B-CLL [Bichi et al., 2002]. All mice around the age 10–20 months became visibly ill

and presented with spleno- and hepatomegaly associated with high counts of white blood cells. In addition, some mice also developed advanced lumphoadenopathy, another hallmark of human B-CLL [Bichi et al., 2002]. The second Tcl1 transgenic mouse model was designed to express Tcl1 in both, B- and T-cells [Hoyer et al., 2002]. These mice at the average age of 12 months became visibly sick and showed accumulation of $B220^{+}CD5^{+}$ malignant B-cells. The phenotype of B-CLL like disease in these mice was similar to that described above for our Tcl1 transgenics.

Recently, we studied more in detail the spontaneous tumor phenotype of Eμ-*TCL1* transgenic mice [Zanesi et al., 2006]. In addition to B-CLL-like malignancies, an extensive pathologic analysis of these transgenic animals revealed that more than 30% of mice showed the onset of solid malignant tumors, even though the tumors themselves lacked Tcl1 expression. Three of four tumors appeared in relatively young mice (11–12 months) and two of these malignancies were histiocytic sarcomas, a cancer of the macrophagic lineage known to be rare (2%–5%) in 23 month old mice [Frith et al., 1983], whereas 18% of Eμ-*TCL1* mice had these tumors at 11–12 months of age [Zanesi et al., 2006]. Secondary malignancies are also frequent complications in patients with B-CLL [Robak, 2004] and they are the most common cause of death in these patients [Kyasa et al., 2004]. Increased risk of secondary non-hematological neoplasms in patients with B-CLL has several explanations including genetic predisposition, immune deficiency, carcinogen exposure, and the side-effects of therapeutic treatments [Parekh et al., 1999]. In our B-CLL mouse model, 25% of secondary cancers were malignant pilomatrixoma, a type of skin cancer extremely rare in mice. Thus far, the Eμ-*TCL1* model seems to be the only one reported that displays the onset of secondary malignancies in B-CLL-prone mice [Zanesi et al., 2006].

Mouse models of human cancers are useful tools to investigate the effectiveness of therapeutic approaches prior to human clinical trials. The homogeneity of the genetic background of the model is an important factor to evaluate the effects of novel therapies. Consequently, we established B-CLL transplants in syngenic mice that allowed us at first to propagate, and later to therapeutically treat, the same leukemia in different mice with the same genetic background. This system allows for the study of each leukemia and is a necessary step on the road to find drugs that specifically interfere with the pathways involved in the disease [Zanesi et al., 2006]. To determine whether the development of mouse B-CLL is dependent on the Akt pathway, we investigated whether the disruption of Akt signaling through mTOR, by the mTOR inhibitor rapamycin, prevented or cured mouse B-CLL. The treatment prolonged the life of all treated animals in either the prevention or the therapeutic group. mTOR normally regulates translation by regulation key components of the protein synthesis machinery, including ribosomal protein S6 kinase. We found that lymph nodes of untreated mice expressed phosphorylated S6, whereas lymphoid tissues from rapamycin-treated mice did not show any phosphorylation. Hence, rapamycin inhibits mTOR activity in the Tcl1-dependant B-CLL mouse model [Zanesi et al., 2006].

Another recent study further characterized our *TCL1* transgenic mice as a preclinical drug development tool for human B-CLL [Johnson et al., 2006]. This study showed that transformed transgenic murine lymphocytes express relevant therapeutic targets like Bcl-2, Mcl-1, Akt, PDK1, and DNMT1, wild-type p53, and are sensitive to several therapeutic agents active in vitro and/or in vivo in human

B-CLL, including fludarabine and flavopiridol, as well as a novel preclinical agent, OSU03012. Subsequent *in vivo* studies using *TCL1* transgenic mice with established leukemia demonstrated that fludarabine treatment resulted in decreased blood tumor cells and spleen size, and ultimately prolonged survival. Mice with Tcl1-driven B-CLL that initially gained clinical benefit from fludarabine therapy subsequently became resistant and ultimately died from leukemia. This series of studies suggest that the *TCL1* transgenic mouse model is similar to human B-CLL also in its therapeutic response and could be used as a preclinical tool to evaluate new drugs for clinical treatment of B-CLL. The importance of this mouse model to drug development in B-CLL is significant, since until recently no suitable animal models for this purpose have been reported [Johnson et al., 2006].

Since transgenic expression of human *TCL1* produces B-CLL-like tumors in mice, Herling et al. [2006] assessed how Tcl1 is regulated in human B-CLL. They studied the pattern of Tcl1 expression in primary B-CLL samples using

flow cytometry, paraffin-section immunohistochemistry (IHC) and Western blot. A total of 204 B-CLL cases were analyzed by one or more methods. Tcl1 expression levels by all techniques ranged from complete absence to strong uniform expression. Overall, Tcl1 protein was detectable in 90% of B-CLL by IHC. Tcl1 was absent in 10%–14% of the cases analyzed [Herling et al., 2006]. Significant advances in our understanding of B-CLL have come from studying the leukemic B-cell antigen receptor (BCR) [Chiorazzi and Ferrarini, 2003; Chiorazzi et al., 2005]. These observations enabled the categorization of B-CLL patients into subgroups based on the presence or absence of Ig V_H gene mutations [Fais et al., 1998], termed "unmutated B-CLL" (U-CLL) and "mutated B-CLL." Patients with U-CLL follow the more aggressive clinical course with shorter survival [Damle et al., 1999]. Herling et al. correlated Tcl1 levels with different immunophenotypic and molecular B-CLL subsets [Herling et al., 2006]. B-CLL that lacked ZAP70 protein expression in tumor cells by IHC preferentially showed absence or weak expression of Tcl1. On the other hand, strong expression of Tcl1 was less frequent in B-CLL showing V_H gene mutation rates above 2% as compared to U-CLL. An inverse correlation between the level of Tcl1 expression and the V_H mutation rate for any given case was also shown [Herling et al., 2006]. In this report, the authors noted marked intratumoral heterogeneity in Tcl1 expression. Variations in Tcl1 expression suggest that Tcl1 protein levels undergo cyclical changes during tumor cell growth. Tcl1 was expressed at low levels in the actively proliferating tumor components, which were most pronounced in the B-CLL proliferation centers. Other variations in Tcl1 levels appeared related to tumor microenvironment. These changes likely reflect the dynamic effects of tissue-derived cytokines, as Tcl1 expression levels correlated with the density of tumor-associated T-cells. Effective silencing of Tcl1 expression in cultured primary B-CLL cells was predominantly driven by the presence of IL-4, which appeared to provide both proliferative and differentiation signals. Therefore, according to the authors, IL-4 derived from tumor-associated T-cells is a likely candidate for mediating Tcl1 silencing in vivo [Herling et al., 2006].

Recently, in collaboration with Dr. Chiorazzi and colleagues, we carried out a study to determine how the extent of V_HDJ_H and V_LJ_L rearrangements in a series of lethal Tcl1-driven B-CLL resemble those found in patients with B-CLL [Yan et al., 2006]. Our results indicate that the *TCL1* transgenic model replicates the IgV-region rearrangements characteristic of the aggressive, treatment-resistant form of human B-CLL. BCRs comprised of genes differing minimally, if it all, from the germ-line sequence are a hallmark of poor-outcome human B-CLL, and the BCRs in the Tcl1-driven B-CLL consistently displayed this feature. Another important feature of human B-CLL is nonstochastic use and association of individual V region segments with biases differing between U-CLL and mutated B-CLL [Chiorazzi et al., 2005]. Based on the presently known numbers of murine segments, certain V_H, D, J_H, and V_L genes appeared overexpressed in the Tcl1-driven murine B-CLL. This assumption applies particularly for rearrangements involving V_H11, V_H12, and V_H4, because these V_H families contain only one or two genes, a very minor fraction of the entire murine V_H repertoire [Yan et al., 2006].

H and L CDR3s (quasi-identical complementarity-determining region 3) of human B-CLL cells can be distinctive in length, amino acid composition and charge, and D and J_H segment pairing [Johnson et al., 1997]. Specifically, U-CLLs frequently exhibit long HCDR3s containing multiple neutral tyrosine and serine residues that may confer CDR3 flexibility and favor polyreactivity. This characteristic is especially true for human leukemic rearrangements using V_H 1-69 and 4-39, two genes virtually always associated with rapid downhill clinical courses [Ghiotto et al., 2004]. The HCDR3s in *TCL1* mice also contain many of these same amino acids. Interestingly, HCDR3s of human B-CLL cells often contain charged amino acids, frequently not coded by germline D and J_H segments. In the Tcl1-driven leukemia, positively and negatively charged residues are seen frequently at or adjacent to the V_H-D and D-J_H junctions, deriving from simple and complex rearrangement events including insertion of nontemplated nucleotides [Yan et al., 2006].

In conclusion, a vast body of information has been gathered in the last 4 years about leukemia developed by the Eμ-*TCL1* transgenic mice, demonstrating its similarity to human B-CLL.

Bcl2-DRIVEN MOUSE MODEL OF B-CLL

As mentioned above, recent discoveries of the role of miR15a/16-1 in B-CLL provided an important link between genomic abnormalities and high expression levels of Bcl2 in the majority of B-CLL samples. This finding suggests that deregulation of Bcl2 may be a major contributor to the pathogenesis of B-CLL, specifically the indolent form of B-CLL often accompanied by 13q14 deletions. A recent report provided an important evidence in favor of this hypothesis [Zapata et al., 2004]. This study took an advantage of two previously developed mouse models, a model over-expressing Bcl2 in the mouse lymphoid system [Katsumata et al., 1992] and a transgenic mouse line over-expressing an isoform of TRAF2 in mouse B- and T-cells [Lee et al., 1997]. TNF receptor associated factor 2 (TRAF2) is a member of a conserved family of adaptor proteins that bind to TNF receptor family members and mediate the activation of NF-kB and JNK by the tumor necrosis factor proteins (Fig. 2) [Chung et al., 2002]. This TNF-mediated signaling leads to the increase in lymphocyte proliferation and survival [Haiat et al., 2006]. These TRAF2 transgenics over-expressed TRAF2 mutant, lacking the N-terminal RING and zinc finger domains located at the N-terminal of the molecule (TRAF2DN), which mimics TRAF1 [Lee et al., 1997]. These mice developed splenomegaly, lymphadenopathy and an increased number of B-cells but failed to develop any frank hematological malignancy. Bcl2 transgenic mice were produced by using a construct which mimics t(14;18) translocation juxtaposing *BCL2* oncogene with the immunoglobulin heavy-chain locus at 14q32 observed in human follicular lymphomas. These mice did not develop any tumor phenotype, but showed polyclonal expansions of B-cells and prolonged B-cell survival *in vitro* [Katsumata et al., 1992]. In contrast to the single transgenics, TRAF2DN/Bcl2 double transgenic mice over time developed severe splenomegaly, and most animals developed B-cell leukemias similar to B-CLL with B-cell blood count as high as 40 times normal [Zapata et al., 2004]. These mice die prematurely at the age of 6–14 months, although Bcl2 or TRAF2DN single transgenics had a normal life span. As in the case of the *TCL1* transgenics, malignant cells were $B220^{+}$ $CD5^{+}$, although a small number of mice had

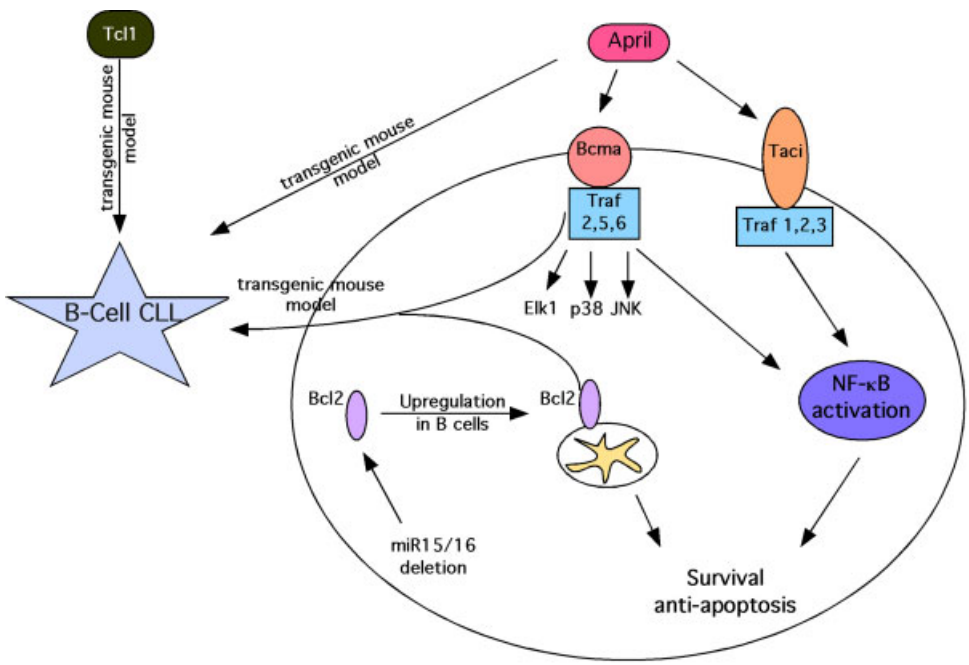

Fig. 2. A schematic representation of a role of TNF pathway in B-CLL phenotype in mice. APRIL binds to its receptors, Bcma and Taci. Activated Bcma and Taci interact with Trafs and activate NF-kB pathway. 13q14 deletions result in downregulation of miR15/16, upregulation of Bcl2 and resistance to apoptosis. Thansgenic expression of April, or co-expression of an isoform of Traf 2 and Bcl2 in mouse lymphoid cells result in B-CLL phenotype. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

B-cell expansions of a different subtype. These cells showed markedly increased survival and were resistant to apoptosis induced by chemotherapeutic agents and glucocorticoids [Zapata et al., 2004]. The authors speculated that TRAF2DN and TRAF2DN/Bcl2 transgenic lymphocytes may accumulate in spleen and lymph nodes due to upregulation of cell adhesion proteins. For example, they found an increased expression of CD29 and CD54 adhesion molecules in malignant mouse B-CLL cells. After leaving nodal compartment these TRAF2DN-driven B-cells die unless apoptosis is blocked by Bcl2. This hypothesis needs additional verification since TRAF2DN-driven expanding B-cells are $B220^{+}CD5^{-}$ [Lee et al., 1997] but TRAF2DN/Bcl2 B-CLL cells are $B220^{+}CD5^{+}$. This difference in subtype cannot be explained by increased survival alone of expanding TRAF2DN transgenic B-cells. Since many human B-CLLs overexpress TRAF1 and Bcl2, these findings suggested a cooperation between the Bcl2 and TNF-NF-kB pathways in the development of B-CLL. It is still unclear if this TRAF2DN/Bcl2-driven disease resembles indolent or aggressive form of B-CLL. Additional studies will be necessary to determine whether this mouse B-CLLs are transplantable and therefore could be used in drug trials, like Tcl1-driven mouse B-CLLs.

APRIL-DRIVEN MOUSE MODEL OF B-CLL

Since NF-kB is a downstream target of TRAFs, it is possible that NF-kB activation plays an important role in the pathogenesis of B-CLL. A recent report described another mouse model for B-CLL suggesting an importance of NF-kB activation. This study described a transgenic mouse line specifically expressing APRIL (a proliferation-inducing ligand) under a control of T-cell specific lck promoter [Planelles et al., 2004]. Since APRIL is a secreted protein, its over-expression in T-cells caused elevated APRIL serum levels. Therefore APRIL works systemically in these transgenics affecting B-cell proliferation and survival [Planelles et al., 2004]. APRIL and its close relative BAFF are two recent members of TNF superfamily, expressing almost exclusively in hematopoietic cells [Haiat et al., 2006]. APRIL can act as a stimulator of B- and T-cells in vitro, its in vivo application caused splenomagaly due to the expansion of B-cells [Haiat et al., 2006]. Both BAFF and APRIL show elevated expression levels in patients with various B-cell malignancies including diffuse large cell lymphoma, mantle cell lymphoma and CLL [Haiat et al., 2006]. APRIL binds with high affinity to two receptors, BCMA (B-cell maturation antigen) and TACI (Fig. 2) [Haiat et al., 2006]. BCMA is detected in mature B- and T-cells, while TACI is mostly expressed in activated T-cells and subpopulations of B-cells [Haiat et al., 2006]. BCMA and TACI are members of TNF receptor superfamily [Haiat et al., 2006]. They interact with various TRAFs and stimulate NF-kB pathway (Fig. 2). APRIL transgenic mice did not show significant alterations in their lymphoid organs (except slight increase of $B220^{+}$ cells) up to the age of 9 months [Planelles et al., 2004]. Closer assessment of 9–12 months old transgenics by flow cytometric analysis revealed significant expansions of $B220^{+}CD5^{+}$ cells in mesenteric lymph nodes and Peyers patches [Planelles et al., 2004]. The incidence and severity of these alterations increased over time suggesting progressive expansion of mature $CD5^{+}$ B-cells. These expanded mature B-cells displayed significant increase in survival in vitro when compared to normal B-lymphocytes [Planelles et al., 2004]. The expansion of $B220^{+}CD5^{+}$ cells were observed in ~40% of transgenic animals in contrast with two described above B-CLL mouse models where the penetrance was about 100%. Generally, APRIL transgenic mice showed much milder phenotype than *TCL1* or TRAF2DN/Bcl2 transgenics. APRIL transgenics showed only mild expansion of spleenic B-cells, they did not develop high blood white cells count and did not die prematurely from leukemia or lymphoma (at least these occurrences were not yet reported). Therefore it is likely that APRIL predisposes CD5+ B-cells to malignant transformation, but this event also requires a second hit, such as Bcl2 or Tcl1 overexpression. Clearly, crosses of APRIL transgenics with mice overexpressing known oncogenes in B-cells, showing mild phenotypes, like c-Myc or Bcl2, would clarify this issue.

CONCLUSIONS

Although clinical features and chromosomal rearrangements in B-CLL have been studied in detail, this information have recently started to provide clues of what molecular mechanisms

underlie the origin of this common leukemia. The only definitive proof that deregulation of certain gene(s) or pathway(s) is a causal event in the pathogenesis of any malignancy is the tumor phenotype in transgenic or knockout animal model(s) for this gene. Human B-CLL presents in two major forms: indolent form, associated with mutated variable region genes of immunoglobulins and low levels of expression of ZAP70 and an aggressive form showing unmutated immunoglobulin variable region genes and high ZAP70 expression [Herling et al., 2006]. Development of B-CLL in Eμ-*TCL1* mice suggests that deregulation of *TCL1* is a critical event in the pathogenesis of this disease. The fact that high Tcl1 expression in human B-CLL correlates with aggressive B-CLL phenotype [Herling et al., 2006] and that mouse B-CLL tumors from Eμ-*TCL1* mice resemble aggressive form of human B-CLL [Yan et al., 2006] provides further evidence of the importance of *TCL1* deregulation in the origin of this disease. Given that B-CLL cell lines do not exist, Eμ-*TCL1* transplantable mouse B-CLLs are extremely important, since it provides the only model system available to experiment with potential therapeutic agents for B-CLL. In fact, the first two such studies were recently published [Johnson et al., 2006; Zanesi et al., 2006]. Although the involvement of Tcl1 in B-CLL is clear, molecular mechanisms linking *TCL1* deregulation and B-CLL phenotype remain poorly understood. Studies demonstrating that Tcl1 is a co-activator of Akt shed some light into this question; however, it is not clear whether Tcl1 is strictly involved in the Akt pathway or other molecular partners of Tcl1 exist that are significant for B-CLL.

Loss of miR-15a/16-1 by 13q14 deletions provided a novel molecular mechanism of Bcl2 overexpression in B-CLL [Cimmino et al., 2005]. On the other hand, deregulation of Bcl2 expression alone in mouse B-cells is not sufficient to cause B-CLL like disease [Katsumata et al., 1992]. Studies of transgenic mouse models demonstrated that cooperation of Bcl2 deregulation and activation of the NF-kB pathway (by TRAF2DN overexpression) is necessary to generate B-CLL phenotype [Zapata et al., 2004]. The importance of NF-kB pathway in the pathogenesis of B-CLL is confirmed by the phenotype of APRIL transgenics. Although activation of NF-kB pathway alone (by overexpression of TRAF2DN or APRIL) causes B-cell expansion and does not progress to malignant disease [Planelles et al., 2004]. It would be interesting to determine if APRIL would cooperate with *BCL2* (as TRAF2DN does) to generate B-CLL phenotype. Since 13q14 deletions are associated with indolent form of human B-CLL, an intriging question is whether Bcl2 driven mouse B-CLL is similar to indolent B-CLL.

In conclusion, mouse models of B-CLL clearly demonstrated that three molecular pathways: a pathway downstream of Tcl1, Bcl2 anti-apoptotic pathway, and NF-kB pathway are critical in the origination of this common leukemia.

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