miR16–1. Transfection of MEG-01 cells with a vector encoding these microRNAs repressed expression of *BCL2* and rendered these cells highly susceptible to apoptosis and less tumorigenic, raising the prospect that such microRNAs might have potential for use in cancer therapy.

In this issue of *Blood*, Raveche and colleagues report on another important facet of this developing story. These investigators examined genes that contribute to the known propensity of New Zealand Black (NZB) mice to develop a CD5 B-cell lymphoproliferative disease that has many features in common with CLL.[7] They backcrossed NZB mice with a control strain of mice and found that the development of lymphoproliferative disease was linked to 3 loci, one of which was syntenic to human 13q14. At this locus, they identified a point mutation in the 3′ region flanking the mouse homolog to human *miR16–1*. This mutation defined a genetic polymorphism in *miR16–1* that was peculiar to the NZB mouse and that also resulted in reduced expression of *miR16–1* in the lymphoid tissues of these animals. Similar to studies with the MEG-01 human B-cell tumor line, transfection of neoplastic CD5 B cells derived from NZB mice with exogenous *miR16–1* rendered the mouse tumor cells more susceptible to apoptosis. Collectively, these studies indicate that abnormal, low-level expression of *miR15a/miR16–1* can lead to lymphoproliferative disease of CD5 B cells in both mice and humans. In addition to providing evidence that altered expression of *miR15a/miR16–1* is, in fact, the molecular lesion for most cases of CLL, this study also suggests that the NZB mouse might provide a useful model with which to evaluate strategies for correcting abnormal expression of microRNA for the prevention and/or treatment of neoplastic disease.

Conflict-of-interest disclosure: The author declares no competing financial interests. ■

REFERENCES

1. Ambros V. The functions of animal microRNAs. Nature. 2004;431:350-355.
2. Bartel DP. MicroRNAs: genomics, biogenesis, mechanism, and function. Cell. 2004;116:281-297.
3. Calin GA, Dumitru CD, Shimizu M, et al. Frequent deletions and down-regulation of micro- RNA genes miR15 and miR16 at 13q14 in chronic lymphocytic leukemia. Proc Natl Acad Sci U S A 2002;99:15524-15529.
4. Calin GA, Liu CG, Sevignani C, et al. MicroRNA profiling reveals distinct signatures in B cell chronic lymphocytic leukemias. Proc Natl Acad Sci U S A. 2004;101:11755-11760.
5. Calin GA, Ferracin M, Cimmino A, et al. A MicroRNA signature associated with prognosis and progression in chronic lymphocytic leukemia. N Engl J Med. 2005;353:1793-1801.
6. Cimmino A, Calin GA, Fabbri M, et al. miR-15 and miR-16 induce apoptosis by targeting BCL2. Proc Natl Acad Sci U S A. 2005;102:13944-13949.
7. Marti GE, Metcalf RA, Raveche E. The natural history of a lymphoproliferative disorder in aged NZB mice. Curr Top Microbiol Immunol. 1995;194:117-126.

IMMUNOBIOLOGY

Comment on Bixel et al, page 5327

To transmigrate or not to transmigrate

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Neutrophil transmigration through the endothelial lining of blood vessels is regulated by signaling and adhesion molecules. Now, CD99L2 joins the club of molecules implicated in this process.

In a remarkable study published in this issue of *Blood*, Bixel and colleagues describe the important role of CD99L2, a molecule with a modest 32% homology to CD99 and no other close relatives, in neutrophil transendothelial migration. This increases the number of molecules known to be involved in neutrophil transmigration by one fifth, expanding on PECAM-1, JAM-A, JAM-C, ESAM and CD99, which have previously been implicated in this process.[1] In addition, ICAM-1 and ICAM-2 are involved in transendothelial migration, but also mediate neutrophil adhesion. Based on electron microscopy, CD99L2 seems to inhibit a distal step in transmigration, because in the presence of an antibody to CD99L2 neutrophils are found trapped between the endothelium and the basement membrane. A similar phenotype was previously seen in mice treated with an antibody to PECAM-1 and in PECAM-1 knockout mice.[2,3]

In an in vitro transmigration experiment through bEnd.5 endothelioma cells, transmigration of bone marrow neutrophils was inhibited by 40% by an intact antibody to CD99L2 and by 30% by $F(ab')_2$ fragments. The effect of the full-length antibody was similar whether only endothelial cells were preincubated or the antibody remained present during the transmigration assay. Although no effect of exposing bone marrow neutrophils to CD99L2 antibody was seen, this remains to be confirmed with blood neutrophils, which express 100-fold more CD99L2. Incidentally, this regulation pattern makes CD99L2 one of the very few markers that distinguish mature (blood) from immature (marrow) neutrophils.

Although CD99L2 has a significant extracellular domain, it does not support homophilic adhesion. The calcium and magnesium sensitivity of the CHO-cell aggregation assay might suggest a possible integrin ligand for CD99L2, but this is pure speculation and awaits further study. Since all antibody reagents used were bivalent, ligation of CD99L2 may trigger a signaling event in endothelial cells, but the nature of this signaling and the domains in CD99L2 responsible remain unknown. Neutrophil transmigration requires an exquisitely regulated dance between the leukocyte and the vessel wall. When endothelial CD99L2 is ligated by antibody, the partners still find each other, since rolling and adhesion are unaffected, but their ability to dance is reduced. Future work will have to determine what the relevant ligands for CD99L2 might be and whether they are expressed on transmigrating neutrophils, adjacent endothelial cells, or both. The phenotype of CD99L2-deficient endothelial cells and mice remains to be explored. Based on the data presented by Bixel and colleagues, it seems unlikely that CD99L2 deficiency will produce a neutrophil transmigration defect.

Why are so many molecules involved in neutrophil transmigration? Possibly they form a chain of adhesion and signaling events, one handing off to the next. CD99L2 and CD99 are in the same general pathway, because blocking both has no additive effect on transmigration (as pointed out by Bixel and colleagues), but we don't know the details of their relationship. PECAM-1 has been described as functioning upstream (luminal) from CD99 in neutrophil transmigration[4] and may engage in homophilic adhesion, which is clearly different from

CD99L2. While many aspects of CD99L2 biology remain to be explored, the present paper takes the all-important first step in linking this molecule to transmigration.

Conflict-of-interest disclosure: The author declares no competing financial interests. ■

REFERENCES

1. Nourshargh S, Krombach F, Dejana E. The role of JAM-A and PECAM-1 in modulating leukocyte infiltration in inflamed and ischemic tissues. J Leukoc Biol. 2006;80:714-718.
2. Wakelin MW, Sanz M-J, Dewar A, et al. An anti-Platelet-Endothelial Cell Adhesion Molecule-1 antibody inhibits leukocyte extravasation from mesenteric microvessels in vivo by blocking the passage through the basement membrane. J Exp Med. 1996;184:229-239.
3. Thompson RD, Noble KE, Larbi KY, et al. Platelet-endothelial cell adhesion molecule-1 (PECAM-1)-deficient mice demonstrate a transient and cytokine-specific role for PECAM-1 in leukocyte migration through the perivascular basement membrane. Blood. 2001;97:1854-1860.
4. Lou O, Alcaide P, Luscinskas FW, Muller WA. CD99 is a key mediator of the transendothelial migration of neutrophils. J Immunol. 2007;178:1136-1143.

NEOPLASIA

Comment on Guo et al, page 5463

Uncontrolled Wnt signaling causes leukemia

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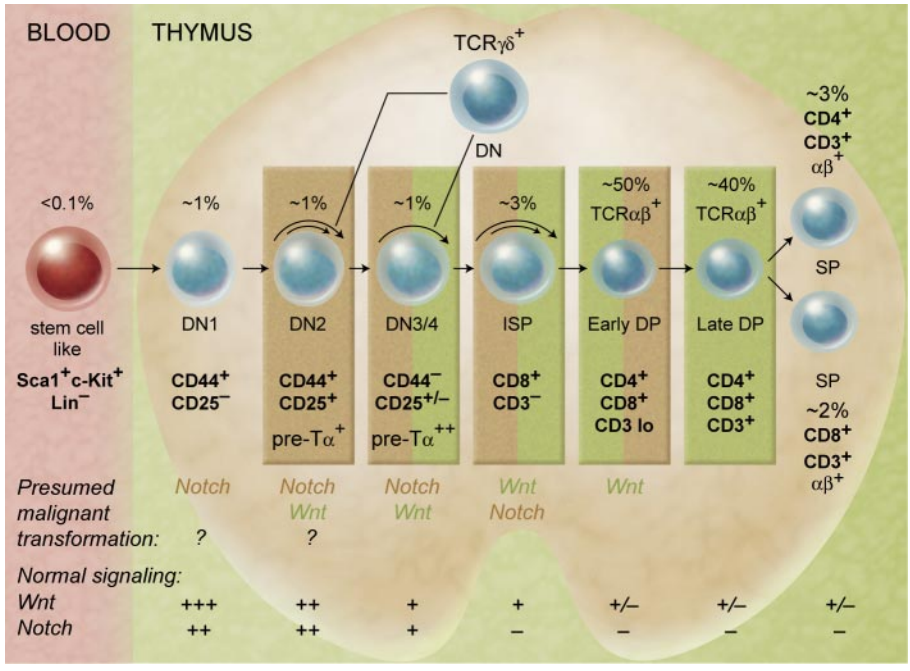
In this issue of *Blood*, Guo and colleagues provide the first solid evidence that thymic overexpression of stabilized β-catenin, the central player in the Wnt signal transduction route, leads to the development of T-cell lymphomas. The development of T-cell lymphomas in the mouse is an excellent model for human T-cell acute lymphoblastic leukemia (T-ALL). Similar to human T-ALL, the murine lymphomas often arise due to overexpression of oncogenic forms of factors that regulate normal T-cell development in the thymus.

Normal T-cell development in the thymus is an ordered and tightly controlled process (see figure). Two exogenously activated signaling pathways, the Notch and the Wnt signaling cascades, are crucial at the earliest stages of normal T-cell development.[1] Both act as molecular switches: the executing transcription factor that is normally bound to the DNA as a transcriptional repressor is converted into an activator. For the Notch pathway, set in motion by ligands of the Delta and Jagged family, this transcription factor is CSL. For the Wnt pathway, which is activated by secreted Wnt proteins, these are factors of the Tcf/Lef family, in thymocytes mainly Tcf-1. There now is compelling evidence that uncontrolled Notch signaling is a causative factor in the development of many T-ALLs.[2] For Wnt signaling, strong evidence that deregulated Wnt signals lead to T-ALL was lacking until now. There were good reasons to believe that uncontrolled Wnt signals could cause leukemias, as many solid tumors have somatic mutations in key regulatory genes of the Wnt pathway. Given the importance of Wnt signals in normal T-cell development, we have previously proposed that by analogy with Notch signaling, constitutively active Wnt signals should lead to ALL.[4] Such evidence has now been provided, making use of powerful mouse genetics.

The elegant study by Guo and colleagues makes use of various transgenic and tissue-specific conditional-knockout mouse models. Key is the conditional deletion of exon 3 in β-catenin, leading to a constitutively active form of β-catenin that can no longer be phosphorylated and broken down. When expressed using the Lck or CD4 promoters driving the cre recombinase, the activated form of β-catenin is specifically expressed in the thymus from early in the double negative (DN) 2 and DN4 stages and onwards. As canonical Wnt signals are absent to low from the double-positive (DP) stage and onwards,[3] there is massive uncontrolled Wnt signaling during late thymocyte differentiation in both of these types of mice. This leads to the development of aggressive T-cell lymphomas that can invade the bone marrow and are transplantable into irradiated recipient mice. Although the Lck-cre and CD4-cre mice develop tumors with somewhat different latency and incidence, the phenotype of these lymphomas is very similar: TCRαβ + CD4 + CD8 + DP. Interestingly, when



Wnt and Notch signaling during murine T-cell development in the thymus. The roles of Wnt and Notch signaling in both normal and malignant development are indicated. The stages in which constitutively activated forms of molecules in the Wnt or Notch pathways lead to malignant transformation are still somewhat speculative. Data from various sources combining human and mouse models are summarized.[1-3] Illustration by A. Y. Chen.