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## Hypomethylation therapy for hemoglobin disorders is back at center stage

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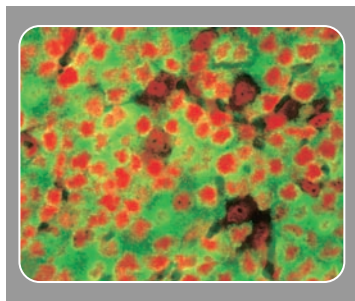
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## Mediastinal B-cell lymphoma, classical Hodgkin disease, and the winds of change

For many years, the bifurcation between Hodgkin disease and non-Hodgkin lymphomas appeared settled: the former comprised an easily recognizable lymphoma category and the latter was a heterogeneous group of T- and B-cell neoplasias. The clinically aggressive B-cell lymphomas were regarded as a rather fuzzy group of tumors composed of more or less large neoplastic cells. As is often the case in pathology, this view turned out to be naive. Hodgkin disease is presently grouped into 2 subcategories: “classical,” which current opinion regards as germinal-center-derived in nearly all cases (rare T-cell cases excluded), and “lymphocyte predominant,” which comprises cases that are believed to always be of germinal-center origin. Large-scale gene expression studies profiling diffuse, large B-cell lymphomas revealed different expression patterns, called signatures, that mimicked the steps in normal B-cell ontogeny.



Molecular cytogenetics strongly suggest that primary mediastinal B-cell lymphoma is a disease of its own in the large B-cell lymphoma group, and studies recently produced the startling idea that mediastinal B-cell lymphoma and classical Hodgkin disease might be pathogenetically related because they share characteristic chromosomal aberrations, especially gains on 2p and 9p.

Two very recent studies based on gene-expression profiling, including the work of Savage and colleagues (in this issue, page 3871), give substantial support to this emerging concept. Rosenwald et al<sup>1</sup> define mediastinal B-cell lymphoma as a recognizable entity different from the “germinal center” and from the “activated” group, as previously defined by these authors by a characteristic signature. Surprisingly, probing cell lines on a previously published Lymphochip comprising 12 196 cDNAs revealed a similarity between Hodgkin cell lines and both the putatively mediastinal cell line Karpas1106 and whole tumor tissue-derived RNA from mediastinal B-cell lymphomas. In essence, the conclusions drawn from this gigantic set of data are confirmed by conclusions drawn by Savage and colleagues from their own data based on the U133A and U133B Affymetrix chips. Again, mediastinal B-cell lymphoma had a signature differing from those of other large B-cell lymphomas, including several with secondary mediastinal involvement. In addition, the profiles of mediastinal B-cell lymphoma resembled those of Hodgkin cell lines. Both studies constitute an important step forward. Both papers contain a wealth of new genes of interest that may now be studied in detail, including confirmation on the protein level. Proof-of-principle examples are given by Rosenwald et al (eg, the MAL protein, previously published as a characteristic of mediastinal B-cell lymphomas, in Reed-Sternberg cells) and by Savage et al (eg, the nuclear localization of c-REL protein in mediastinal B-cell lymphoma, a feature that had been recently shown in Hodgkin cases with gains of 2p, including the REL locus).

The next step to be taken is the expression profiling of Reed-Sternberg cells isolated from the *ex vivo* situation. This is essential in order to discern the “true” situation in classical Hodgkin disease, uninfluenced by effects of clonal progression and selection *in vitro*. Only then will it be worth-

while to embark on molecular biologic studies further illuminating the common mechanisms in tumorigenesis and mechanisms explaining the unquestionable differences such as histology, gender predominance, and spread, which for so long have clouded the relationship between mediastinal B-cell lymphoma and classical Hodgkin disease.

—Peter Möller

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1. Rosenwald A, Wright G, Leroy K, et al. Molecular diagnosis of primary mediastinal B cell lymphoma identifies a clinically favorable subgroup of diffuse large B cell lymphoma related to Hodgkin lymphoma. *J Exp Med*. 2003;198:851-862.

## Hypomethylation therapy for hemoglobin disorders is back at center stage

The discovery of the inverse relationship between methylation of cytosine residues and gene expression provided an early glimpse of the important role of epigenetic modifications in the regulation of globin gene expression. The use of 5-azacytidine to induce hypomethylation of silenced fetal globin genes to reactivate their expression in adult life heralded a new era in molecular therapy.<sup>1</sup> Although the induction of fetal hemoglobin (HbF) by 5-azacytidine was impressive, serious concerns about its potential short- and long-term side effects prevented its widespread use in sickle cell disease and/or  $\beta$ -thalassemia. Interestingly, controversy over the proposed mechanism of induction of HbF by 5-azacytidine led to the identification of hydroxyurea as another chemotherapeutic agent that can activate HbF expression in adult life. A large, multicenter, randomized, placebo-controlled study demonstrated a reduction by about half in vasoocclusive crises and acute chest syndrome in hydroxyurea-treated patients.<sup>2</sup> Longer follow-up of patients enrolled in this study demonstrated a survival advantage of treatment with hydroxyurea in sickle cell disease.<sup>3</sup>

In spite of the remarkable benefits of hydroxyurea in sickle cell disease, approximately half of the treated patients do not respond to this therapy. An earlier study by Koshy et al<sup>4</sup> showed that in all patients who did not respond to hydroxyurea, HbF levels increased in response to intravenous decitabine, a new analogue of 5-azacytidine that has more potent hypomethylation activity and a more favorable safety profile. The article by Sauntharajah and colleagues (page 3865) represents an important advance that brings hypomethylation therapy back to center stage. The authors extend their earlier observations and show a more impressive increase in HbF levels following intermittent subcutaneous administration of low-dose decitabine. All 8 patients enrolled in this study had a significant increase in their HbF levels, including those with low baseline HbF levels who failed to respond to hydroxyurea. Such patients would probably be butyrate unresponsive too since a low baseline HbF level appears to predict failure to respond to butyrate.<sup>5</sup> Just as importantly, the studies of Sauntharajah et al demonstrate, for the first time, that the observed induction of HbF is associated with decreased methylation at key cytosine residues in the promoters of the  $\gamma$ -globin genes. In addition, this study demonstrates significant improvements in the parameters of red cell adhesion, endothelial damage, and activation of the coagulation pathway following decitabine exposure. The only short-term toxicity that was observed was grade 4 neutropenia in 1 patient and grade 3 neutropenia in 2 others. It is still not clear whether the high HbF levels would persist following long-term subcutaneous therapy with decitabine and what the potential toxicity of such long-term treatment might be. It is also not clear whether decitabine would also be active in  $\beta$ -thalassemia where hydroxyurea has been rather disappointing. Like all good studies, this one raises as many new questions as it answers. Nonetheless, the resurrection of hypomethylation therapy for hemoglobin disorders opens several new avenues of clinical investigation into the potential benefits of new therapeutic

agents that target epigenetic modifications that regulate gene expression.

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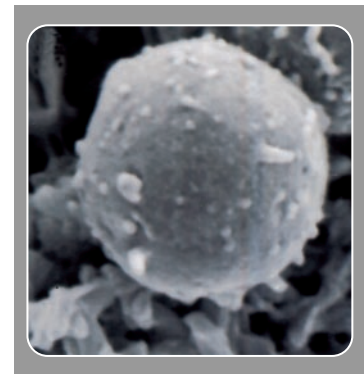
1. DeSimone J, Heller P, Hall L, Zwiers D. 5-azacytidine stimulates fetal hemoglobin synthesis in anemic baboons. *Proc Natl Acad Sci U S A*. 1982; 79:4428-4431.
2. Charache S, Terrin ML, Moore RD, et al. Effect of hydroxyurea on the frequency of painful crisis in sickle cell anemia: investigators of the Multicenter Study of Hydroxyurea in sickle cell anemia. *N Engl J Med*. 1995;332:1317-1322.
3. Steinberg MH, Barton MS, Castro O, et al. Effect of hydroxyurea on mortality and morbidity in adult sickle cell anemia [Erratum appears in *JAMA*. 2003;290:756]. *JAMA*. 2003;289:1645-1651.
4. Koshy M, Dorn L, Bressler L, et al. 2-deoxy 5-azacytidine and fetal hemoglobin induction in sickle cell anemia. *Blood*. 2000;96:2379-2384.
5. Atweh GF, Sutton M, Nassif I, et al. Sustained induction of fetal hemoglobin by pulse butyrate therapy in sickle cell disease. *Blood*. 1999;93:1790-1797.

### Microvillar loss: when your pERM won't hold

Circulating lymphocytes exhibit an intriguing segregation of adhesion proteins with respect to microvillar membrane domains. Proteins such as L-selectin that mediate initial tethering to endothelial cells are enriched at the tips of microvilli, a distribution that has been shown to enhance the frequency at which rolling is initiated.<sup>1</sup> In contrast, integrins such as leukocyte function-associated antigen 1 (LFA-1) that are involved in strong adhesion are distributed randomly or are enriched on the lymphocyte cell body. Endothelium-displayed chemokines trigger a rapid up-regulation of integrin avidity through a process involving release of integrins from the cortical actin cytoskeleton.<sup>2</sup> This leads to the arrest of the lymphocyte on the endothelium and, ultimately, to transmigration across the endothelium. Scanning electron microscopy studies have shown that initial contact between the lymphocyte and the endothelium is made by contacts with the tips of microvilli, whereas strong adhesion is associated with both a loss of microvilli and the formation of contacts with irregular surface folds on the lymphocyte cell body. It has been proposed that the relative paucity of

LFA-1 in the microvillar tips functions to diminish nonspecific adhesion to blood vessels and that chemokine-induced loss of microvilli is important for the formation of strong adhesive contacts.<sup>1</sup>

In this issue, Brown and colleagues (page 3890) address the mechanisms regulating microvillar remodeling in chemokine-stimulated T cells. This study draws from lessons learned in cell biology, where members of the ezrin/radixin/moesin (ERM) family are known to link cell surface and cytosolic proteins to the actin cytoskeleton and to recruit these proteins into microvilli.<sup>3</sup> Brown and coworkers show that in human peripheral blood T cells, the loss of microvilli upon chemokine stimulation is accompanied by dephosphorylation of moesin and ezrin at a specific C-terminal threonine known to control ERM linker activity by regulating an intramolecular autoinhibitory interaction. ERM dephosphorylation is accompanied by loss of these proteins from the cytoskeletal fraction, in keeping with the idea that dephosphorylation inhibits linker activity. As expected for chemokine-induced signaling, ERM dephosphorylation is very



rapid and sensitive to G-protein inhibitors. Further substantiation of the role of ERM proteins in organizing T-cell microvilli comes from experiments showing that expression of a dominant-negative ERM mutant leads to complete loss of microvilli whereas expression of a constitutively active form of moesin leads to increased microvillar structures.

This work convincingly demonstrates that ERM proteins are key regulatory