To the editor:

The PRDX2 gene is transcriptionally silenced and de novo methylated in Hodgkin and Reed-Sternberg cells of classical Hodgkin lymphoma

In a recent issue of Blood, Agrawal-Singh et al reported the identification of PRDX2 (peroxiredoxin 2) as a novel tumor suppressor gene in acute myeloid leukemia (AML).1 The authors observed recurrent histone H3 deacetylation and DNA hypermethylation at the PRDX2 promoter region in primary leukemia samples. Epigenetic alterations correlated with low expression of PRDX2 protein and were associated with poor prognosis in AML patients. Moreover, the authors demonstrated that this gene acts as a growth inhibitor.

Figure 1. Promoter hypermethylation and lack of PRDX2 expression in Hodgkin and Reed-Sternberg (HRS) cells. (A) Localization of the 27K BeadArray (Illumina) array tags cg03860466 and cg00155609 (indicated by arrows) with respect to the PRDX2 TSS. (B) PRDX2 mRNA expression (Affymetrix U95 microarray, tag 39729_at) in chL cell lines, normal B-cell populations (10 × GCB cells, 5 × naive B cells, 5 × memory B cells), 15 diffuse large B-cell lymphomas (DLBCLs), 11 Burkitt lymphomas (BLs), 6 follicular lymphomas (FLs), 11 B-cell chronic lymphocytic leukemias (CLLs), and 5 large cell lymphomas (LCLs). Mean PRDX2 promoter methylation level based on 27K BeadArray (Illumina) array (tags: cg03860466, cg00155609) in the chL cell lines is given in parentheses. Methylation and expression data were taken from Ammerpohl et al2 and Kuppers et al,3 respectively. (C) Bisulfite pyrosequencing of PRDX2 promoter region in chL cell lines. Bars represent the cytosine guanine dinucleotide (CpG) sites analyzed within the sequenced region (genomic position of the CpG sites: black bar, chr19:12912528; gray bar, chr19:12912542). No bar indicates a methylation level of 0%. (D) Bisulfite pyrosequencing of PRDX2 promoter region in microdissected HRS cells (nodular sclerosis, cases 1, 2, 4, 5; mixed cellularity, 3) and nontumoral bystander cells (Lymph) (in duplicate) (genomic position of the CpG sites: black bar, chr19:12912528; gray bar, chr19:12912542). No bar indicates a methylation level of 0%. M, methylated DNA control (Millipore); POOL, whole blood DNAs, pooled; U, unmethylated DNA control (whole genome amplified DNA).
in myeloid cells. Here, we provide evidence that PRDX2 methylation and transcriptional silencing is recurrent not only in AML but also in classical Hodgkin lymphoma (cHL), further emphasizing the importance of loss of this tumor suppressor in hematologic malignancies.

By analyzing methylation profiles of 5 cHL cell lines (27K BeadArray; Illumina), we observed hypermethylation of the PRDX2 gene compared with normal B-cell populations, including germinal center B (GCB) and a lymphoblastoid B-cell line. Elevated PRDX2 methylation was present in cHL cell lines L428, KMH2, L1236, and UH01, but not HDLM2, and was indicated by tags flanking the PRDX2 transcription starting site (TSS) (cg03860466, −217 bp from TSS; cg00155609, +196 bp from TSS) (Figure 1A). As observed in AML, elevated methylation correlated with lack of PRDX2 messenger RNA (mRNA) in the cHL cell lines (Figure 1B). In contrast, expression was present in germinal center, naive, and memory B cells and other lymphoma and leukemia specimens (except 3/11 Burkitt lymphomas) showing consistent expression of PRDX2 in normal B-cell populations and the majority of other hematologic malignancies (Figure 1B). This is consistent with the observation that the PRDX2 promoter region is rich in motifs recognized by transcription factors widely expressed in B cells, including PAX5, ELF1, TCF4, OCT-1, and nuclear factor κB. Together, these findings suggest epigenetic silencing as the mechanism responsible for the downregulation of PRDX2 in cHL (Figure 1B).

Encouraged by these results, we designed a bisulfite pyrosequencing assay to measure DNA methylation at 2 CpG sites (chr19:12912528 corresponding to the cg00155609 array tag and chr19:12912542) in the 5′ region of PRDX2. The sequenced region encompassed the position chr19:12912503-12912544 (hg19) (forward primer: 5′-GTTTATGGTTATTTTG-3′; reverse primer: b19-CCACACCACTATAAACTAA-3′; sequencing primer: 5′-TGTTTAAAGTATGATGATAA-3′). The assay was validated on cHL cell lines showing similar methylation levels of PRDX2 as measured by the microarray (Figure 1C).

We applied the assay to primary HRS cells from cHL patients. Because HRS cells constitute usually ~1% of the cellular infiltrate in an affected lymph node, we used laser microdissection to obtain a pure population of HRS cells. For each patient, 200 HRS cells, 200 nontumoral bystander cells, and a membrane control were microdissected. DNA extracted from these samples was bisulfite converted and pyrosequenced, including methylated and nonmethylated technical controls. Samples from 5 patients were analyzed in duplicate.

In 2 patients (#2 and #4), we indeed observed hypermethylation of the PRDX2 promoter region in microdissected HRS cells compared with corresponding bystander cells (Figure 1D). This demonstrates that PRDX2 is recurrently hypermethylated in primary HRS cells and provides a rationale for our observation based on U133 plus 2.0 expression profiles that primary microdissected HRS cell populations (n = 12) show a 0.58-fold downregulation of PRDX2 expression compared with GCB cells (n = 10). Under physiological conditions, PRDX2 scavenges reactive oxygen species modulating their intracellular levels. Interestingly, we recently reported that genetic as well as epigenetic alterations in cHL frequently target genes related to reactive oxygen species homeostasis, putatively contributing to loss of B-cell identity and survival of HRS cells. Here we propose PRDX2 as a new player among these genes. Moreover, our results suggest epigenetic silencing of this gene in cHL extending the findings by Agrawal-Singh et al.1

References
Type II refractory celiac disease (RCD II) is a rare condition characterized by a massive accumulation of intraepithelial lymphocytes with a natural killer/T phenotype and containing clonal T-cell rearrangements.1 Its severe prognosis is largely due to the development of an aggressive enteropathy-type–associated T-cell lymphoma (EATL). RCD II is a diffuse digestive but also extradigestive disease.2 Various pulmonary manifestations have been reported in celiac patients3-6 but little, if anything, is known about their mechanisms, even though the presence of a T lymphoid alveolitis had been described. One study has shown the presence of aberrant T cells in the lungs of some celiac patients.7 We confirm and extend these data by reporting here 7 patients with RCD II in whom the presence of specific aberrant T lymphocytes and clonal T-cell receptor gamma (TCR-γ) rearrangements were detected in bronchoalveolar lavage (BAL), and/or in bronchial biopsies, or in mediastinal lymph nodes, all of which argue for the lung as an overt extradigestive site of the disease.

All patients exhibited a massive digestive intraepithelial infiltration by cCD3+, CD3+, CD4+, CD8+, and CD103+ T cells containing clonal TCR-γ rearrangements associated in some way with the presence of similar T cells in skin and/or bone marrow (Table 1). Five of the 7 also exhibited the same phenotypically aberrant cells in BAL and/or bronchial biopsy T cells, accounting by fluorescence-activated cell sorter analysis for 0.5% to 74% of total BAL lymphocytes. Lung cells also contained rearranged TCR-γ T-cell clones, which were not always identical to those detected in the digestive mucosa and/or in blood.

EATL developed in 4 of 7 patients. Two of them died of cutaneous lymphoma and 2 from digestive lymphoma.

Various clinical respiratory manifestations (chronic cough, hemoptysis, organized pneumonia) can occur in celiac patients, including the intrapulmonary location of RCD II–specific T cells in clinically asymptomatic patients.7 We confirm and extend the latter observation by showing the presence of clonal T cells in the lungs of 7 patients with RCD II in alveolar and/or bronchial epithelium or mediastinal lymph nodes. Interestingly, these clonal T-cells in some instances harbored different TCR-γ rearrangements from those detected in the digestive mucosa or in circulating blood, ruling out their detection in the lung as mere blood contamination. Taken together, these data strongly argue for airway epithelium as an additional specific target of RCD II with its potentially most severe complication—aggressive local EATL. This should alert the clinicians in charge of such patients about the importance of including pulmonary evaluation in their routine workups. Indeed, these patients can be clinically and/or radiologically asymptomatic and yet exhibit abnormal T cells, with a potential for transformation into aggressive malignant cells.

To the editor:

Phenotypically aberrant clonal T cells in the lungs of patients with type II refractory celiac disease

Type II refractory celiac disease (RCD II) is a rare condition characterized by a massive accumulation of intraepithelial lymphocytes with a natural killer/T phenotype and containing clonal T-cell rearrangements.1 Its severe prognosis is largely due to the development of an aggressive enteropathy-type–associated T-cell lymphoma (EATL). RCD II is a diffuse digestive but also extradigestive disease.2 Various pulmonary manifestations have been reported in celiac patients3-6 but little, if anything, is known about their mechanisms, even though the presence of a T lymphoid alveolitis had been described. One study has shown the presence of aberrant T cells in the lungs of some celiac patients.7 We confirm and extend these data by reporting here 7 patients with RCD II in whom the presence of specific aberrant T lymphocytes and clonal T-cell receptor gamma (TCR-γ) rearrangements were detected in bronchoalveolar lavage (BAL), and/or in bronchial biopsies, or in mediastinal lymph nodes, all of which argue for the lung as an overt extradigestive site of the disease.

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Table 1. Characteristics of patients with refractory celiac disease type II and specific pulmonary involvement

<table>
<thead>
<tr>
<th>Patient</th>
<th>Sex</th>
<th>Age (years)</th>
<th>Aberrant T cells in digestive sample (%)</th>
<th>Extradigestive localization of aberrant T cells</th>
<th>Aberrant T cells in BAL (%)</th>
<th>Clonal TCR-γ rearrangement in the lung</th>
<th>Site</th>
<th>Follow-up (months)</th>
<th>Outcome</th>
</tr>
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<tbody>
<tr>
<td>1 F</td>
<td>36</td>
<td>60</td>
<td>Skin, bone marrow, lung</td>
<td>NA</td>
<td>+</td>
<td>BAL, BB</td>
<td>60</td>
<td>Died of digestive lymphoma</td>
<td></td>
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<tr>
<td>2 M</td>
<td>39</td>
<td>65</td>
<td>Bone marrow, lung</td>
<td>0.5</td>
<td>+</td>
<td>BAL, BB</td>
<td>96</td>
<td>Alive</td>
<td></td>
</tr>
<tr>
<td>3 F</td>
<td>60</td>
<td>85</td>
<td>Bone marrow, lung</td>
<td>1</td>
<td>–</td>
<td>–</td>
<td>72</td>
<td>Alive</td>
<td></td>
</tr>
<tr>
<td>4 F</td>
<td>68</td>
<td>60</td>
<td>Skin, bone marrow, lung</td>
<td>0.5</td>
<td>+</td>
<td>BAL, BB</td>
<td>36</td>
<td>Died of skin lymphoma</td>
<td></td>
</tr>
<tr>
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<td>51</td>
<td>80</td>
<td>Bone marrow, lung</td>
<td>1</td>
<td>+</td>
<td>BAL, BB</td>
<td>36</td>
<td>Alive</td>
<td></td>
</tr>
<tr>
<td>6 F</td>
<td>57</td>
<td>70</td>
<td>Skin, bone marrow, blood, lung</td>
<td>74</td>
<td>+</td>
<td>BAL, BB</td>
<td>24</td>
<td>Died of skin lymphoma</td>
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</tr>
<tr>
<td>7 F</td>
<td>31</td>
<td>40</td>
<td>Bone marrow, lung</td>
<td>NA</td>
<td>+</td>
<td>Mediastinal lymph nodes</td>
<td>28</td>
<td>Died of digestive lymphoma</td>
<td></td>
</tr>
</tbody>
</table>

BB, bronchial biopsy; F, female; M, male; NA, not available.
*Percentage of total sample lymphocytes.
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