Correspondence

To the editor:

Use of PLS3, Twist, CD158k/KIR3DL2, and NKp46 gene expression combination for reliable Sézary syndrome diagnosis

Several publications, including ours, reported that T-plastin (PLS3) is specifically expressed in patients with Sézary syndrome (SS), the erythrodermic and leukemic form of cutaneous T-cell lymphoma (CTCL).1-5 Besides PLS3, several other molecular SS-restricted markers, including the transcription factor Twist,6 have been identified by gene expression analysis on whole peripheral blood mononuclear cells. We also previously reported SS malignant cells characterized by their membrane expression of CD158k/KIR3DL22,8 and CD335/NKp46.10 Here we investigated whether PLS3, Twist, KIR3DL2, and NKp46 gene expression profiling by quantitative PCR (qRT-PCR) can be used for an efficient molecular SS diagnosis. A well-defined unicentric cohort of 81 patients (47 males, 34 females, with a median age of 69.5 years [22-88]), all with SS diagnosed according to international criteria including a median absolute number of Sézary cells of 4740 (1171-62 240) and a T-cell clone characterized by a specific Vβ expression (median 87% of CD3+ T-cells [22-99.5]), was investigated. Quantification of mRNA expression profiling was established in CD4+ purified T cells from SS patients’ blood samples by positive sorting (Miltenyi) and use of SYBR Green PCR Core (ABI PRISM7300 Real-time PCR System) with specific primer pairs (available upon request).

For each marker, an mRNA level threshold was established using qPCR mean values (± SEM) of PLS3, Twist, KIR3DL2, and NKp46 mRNA levels detected in blood CD4+ - purified T cells from healthy donors (Table 1). A threshold of 95% significance was set up for each marker and any value less than the respective threshold was considered as negative. As positive controls were used mRNA mean levels detected in SS HuT-78 and CD56+-purified T cell SS patients’ samples; 53% of patients’ samples exhibited mRNA combination of 3 markers, mainly PLS3+Twist+KIR3DL2 (98%), 20% showed a 2 marker combination (9% Twist+KIR3DL2, 6% PLS3+Twist, 5% PLS3+KIR3DL2) and 7% did express only one marker (4%Twist,2%PLS3,1%NKp46). Our data clearly indicate that combination of Twist and PLS3 or KIR3DL2 positive mRNA values from CD4+ -isolated T-cells accounted for the diagnosis of 98% SS patients, and designed Twist as the strongest biologic SS marker from the combination with positivity in 91% SS patients. It is noteworthy that mRNA extracts from whole peripheral blood mononuclear cells (PBMCs) gave mean values (± SEM, n = 48) of 620 ± 427 and 538 ± 219, respectively, and thus can be used in routine for PLS3 and Twist mRNA detection using identical thresholds than those for mRNA detection in CD4+ isolated T cells. It should be mentioned that KIR3DL2 and NKp46 must be detected carefully in whole PBMC mRNA extracts since circulating NK cells should hamper distinction between healthy donors and SS patients, but both of these transmembrane receptors could contribute to ascertain the SS diagnosis by multi-color flow cytometry analysis.

In conclusion, we show for the first time that our combination of 4 biomarkers in PCR allows easy diagnosis of SS in 100% of cases.

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Table 1. PLS3, Twist, KIR3DL2, and NKp46 mRNA expression in CD4+ isolated T cells from patients with SS (n = 81) and from healthy donors (n = 12)

<table>
<thead>
<tr>
<th>Markers</th>
<th>T-plastin (PLS3)</th>
<th>Twist</th>
<th>KIR3DL2</th>
<th>NKp46</th>
</tr>
</thead>
<tbody>
<tr>
<td>2(-ΔΔCt) value (AU) in HuT-78 Sézary cell line</td>
<td>128</td>
<td>316</td>
<td>13 150</td>
<td>7.3</td>
</tr>
<tr>
<td>2(-ΔΔCt) value (AU) in CD56+ NK/T malignant cell line</td>
<td>0.01</td>
<td>0.12</td>
<td>0.5</td>
<td>181</td>
</tr>
<tr>
<td>2(-ΔΔCt) mean value (AU) ± SEM CD4+ T cells isolated from healthy donors</td>
<td>2.4 ± 2.5</td>
<td>6.6 ± 8</td>
<td>22.5 ± 20.4</td>
<td>2.6 ± 2.5</td>
</tr>
<tr>
<td>Threshold (95%)</td>
<td>5</td>
<td>10</td>
<td>25</td>
<td>5</td>
</tr>
<tr>
<td>2(-ΔΔCt) mean value (AU) ± SEM (min-max) in CD4+ isolated T cells from CTCL patients</td>
<td>726 ± 144 (0-6385)</td>
<td>1511 ± 313 (0-1441)</td>
<td>878 ± 102 (1-3746)</td>
<td>7.4 ± 1.8 (0-95)</td>
</tr>
<tr>
<td>Fold/threshold</td>
<td>×145</td>
<td>×150</td>
<td>×35</td>
<td>×1.5</td>
</tr>
<tr>
<td>% positive patients/Marker</td>
<td>87</td>
<td>91</td>
<td>84</td>
<td>28</td>
</tr>
</tbody>
</table>
To the editor:

Exome sequencing identifies an MLL3 gene germ line mutation in a pedigree of colorectal cancer and acute myeloid leukemia

Recently, the frequently mutated gene MLL3 was found to be related to the pathogenesis of hepatocellular carcinoma (HCC), fluke-associated cholangiocarcinoma, gastric cancer, and transitional carcinoma of the bladder,\(^1\) raising the possibility that the MLL3 gene and its encoded chromatin remodeling protein MLL3 are etiologically related to cancers. We performed exome sequencing for 4 patients in a multigenerational pedigree with colorectal cancers and acute myeloid leukemia (AML) and identified an insertion mutation in the MLL3 gene on chromosome 7, producing a frame shift leading to a premature truncation at codon 827. To our knowledge, it was the first germ line MLL3 mutation found in a cancer pedigree. Because MLL3 is an enzyme for histone methylation, pharmacologic intervention may be possible.

Of the 4 patients we analyzed in this multigenerational pedigree (Figure 1), II-1 was diagnosed with rectal cancer at age 43 and still alive at age 56; II-3 was diagnosed with colon cancer at age 59 and still alive at age 67; III-2 was diagnosed with AML-M2 at age 40 and still alive at age 45 in complete remission state; and III-3 was diagnosed with AML-M1 at age 43 and died at age 43. All subjects gave informed consent, and the protocol was approved by the Committee on Studies Involving Human Beings at Tianjin Medical University and Southwest Hospital of Third Military Medical University. Genomic DNA was extracted from whole blood samples; exome sequencing was carried out for these 4 patients, respectively. A heterozygous insertion mutation in the MLL3 gene on chromosome 7 (151,945,071 bp, ins T; Human Genome Database Build 36) was identified by exome sequencing.

Figure 1. A Chinese pedigree with colorectal cancers and acute myeloid leukemia. Solid symbols indicate affected individuals. Open symbols indicate unaffected individuals. The arrow indicates the proband, and slashes indicate deceased persons. I-1, lung cancer. II-1, rectal cancer. II-2, rectal cancer. II-3, colon cancer. III-2 and III-3, acute myeloid leukemia, M2 and M1.

Conflict-of-interest disclosure: The authors declare no competing financial interests.

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