Incidence of TCR and TCL1 Gene Translocations and Isochromosome 7q in Peripheral T-Cell Lymphomas Using Fluorescence In Situ Hybridization

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Abstract

Translocations involving the T-cell receptor (TCR) and TCL1 genes occur in T-cell precursor lymphoblastic leukemia/lymphoma and prolymphocytic leukemia; isochromosome 7q has been associated with hepatosplenic T-cell lymphoma. However, the incidence of these abnormalities in peripheral T-cell lymphomas (PTCLs) as a whole has not been well defined.

We studied genetic abnormalities in 124 PTCLs seen at the Mayo Clinic, Rochester, MN, between 1987 and 2007. Tissue microarrays were screened using 2-color break-apart fluorescence in situ hybridization probes flanking the TCRα (TCRA, 14q11), TCRβ (TCRB, 7q35), and TCRγ (TCRG, 7p15) genes and the TCL1 gene (14q32). Isochromosome 7q was analyzed by using a 2-color probe to 7p and 7q32.1.

Translocations involved TCRA in 3 (2.9%) of 102 cases and TCRB in 1 (1%) of 88. Isochromosome 7q was detected in 2 cases of extranodal NK/T-cell lymphoma, nasal type, and 2 cases of anaplastic lymphoma kinase-negative anaplastic large cell lymphoma. One of the latter cases also had a translocation of TCRA, and further studies confirmed a novel t(5;14) translocation.

Peripheral T-cell lymphomas (PTCLs) are aggressive malignancies that cause death within 3 years of diagnosis despite combination chemotherapy. Improvements in patient outcomes might result from novel targeted therapies; such therapies often are directed at proteins that are overexpressed as a result of recurrent genetic events such as chromosomal translocations (eg, BCR/ABL in chronic myelogenous leukemia) or gene amplifications (eg HER2/neu in breast cancer). Unfortunately, the genetics of PTCLs remain poorly characterized, hindering the development of new therapeutic strategies to target key cellular pathways.

The most well-established recurrent translocation among PTCLs is the t(2;5) and its variants, leading to anaplastic lymphoma kinase (ALK) protein overexpression in a subset of patients with systemic anaplastic large cell lymphoma (ALCL). The discovery of this translocation has greatly facilitated understanding of ALCL, which has been considered a “model” disease entity with characteristic morphologic, phenotypic, and clinical features. However, ALK+ ALCL represents a minority of mature T-cell lymphomas.

Recently, the first recurrent genetic abnormality in PTCL, unspecified (PTCL-U), was identified: the t(5;9)(q33;q22), resulting in overexpression of the SYK gene under the control of the ITK promoter. Preliminary data suggest that the t(5;9) is present in only a small minority of PTCLs, but the discovery offers promise that additional recurrent genetic events exist. Other genetic events in PTCLs include inversions or translocations involving the TCL1 gene in T-cell prolymphocytic leukemia (T-PLL) and isochromosome 7q [i(7q)] in hepatosplenic T-cell lymphoma (HSTCL). The incidence of these abnormalities in PTCLs other than T-PLL and HSTCL has not been well characterized. Translocations involving the
T-cell receptor (TCR) gene loci also occur in T-cell lymphomas. They are seen in about one third of cases of precursor T-cell lymphoblastic leukemia/lymphoma (T-LBLL) but have been reported only sporadically in PTCLs other than T-PLL. The overall incidence of these translocations in PTCLs has not been defined.

Screening of paraffin sections of tissue microarrays (TMAs) using fluorescence in situ hybridization (FISH) recently has emerged as a high-throughput method for assessing chromosomal abnormalities in lymphoma tissue samples. In the present study, we screened TMAs containing samples of PTCLs using FISH probes to identify the incidence of translocations involving the TCR gene loci and TCL1 gene and the presence of i(7q).

**Materials and Methods**

**Patient Samples**

Paraffin-embedded tissue specimens diagnosed as mature T-cell neoplasms dated from 1987 to 2007 were reviewed from the archives of the Mayo Clinic, Rochester, MN. All specimens were obtained in-house from patients seen at the Mayo Clinic. Specimens referred for pathologic consultation were not included. H&E-stained and immunohistochemically stained slides, clinical information, and other laboratory studies (e.g., TCR gene rearrangement, Epstein-Barr virus–encoded RNA in situ hybridization) were used to classify cases according to the current World Health Organization classification system.

Inclusion criteria included the following: (1) an unequivocal diagnosis of PTCL; (2) patient consent for research use of pathologic material; and (3) adequate paraffin material for the study. B-5– and formalin-fixed cases were included, based on prior data from our laboratory suggesting similar FISH results with both fixatives. The original diagnostic material was not available in all cases, and some patients received treatment before specimens were obtained. Primary T-cell lymphoproliferative diseases occurring in skin were not studied, other than subcutaneous panniculitis-like T-cell lymphoma (SCPTCL). The study was approved by the Mayo Clinic Institutional Review Board and the Biospecimens Committee.

**Fluorescence In Situ Hybridization**

TMAs were constructed from paraffin tissue blocks as described previously. Briefly, an H&E-stained section (and immunostained sections as appropriate) from each block was examined by a pathologist (A.L.F.), and areas for TMA sampling were marked on the corresponding paraffin block. Three cylindrical cores of 0.6 mm diameter were removed from each donor paraffin block and transferred to a recipient paraffin block at defined array positions. Three cores were sampled from each block to control for possible tumor heterogeneity.

In some cases, insufficient tissue was available for inclusion on TMAs, and tissue sections were analyzed. TMA sections were cut at a thickness of 5 µm and placed on charged slides.

The slides were immersed in Citrisolve (Fisher Scientific, Pittsburgh, PA) for 15 minutes, jet air dried, immersed in Lugol solution for 5 minutes, and immersed in 2.5% sodium thiosulfate for 30 seconds. The slides were then placed in 10 mmol/L of citrate/citric acid solution (pH 6.0) and microwaved on the high setting for 5 minutes, followed by 15 to 45 minutes in 0.4% pepsin solution (pepsin A/0.9% sodium chloride at pH 1.5) at 37°C. Ten microliters of FISH reagent (7 µL LSI buffer [Vysis, Downers Grove, IL] and 3 µL probe) were placed on each slide, and the slides were coveredslipped, denatured in a Hybrite (Vysis) set at a melt temperature of 80°C for 5 minutes, and incubated in a humidified chamber at 37°C for 12 hours. The slides were then washed in 2× saline sodium citrate/0.1% NP40 (US Biological, Swampscott, MA) at 70°C for 2 minutes and counterstained with 4',6-diamidino-2-phenyl indole dihydrochloride. The cells were analyzed by a microscopist (M.L.) using a fluorescent microscope equipped with appropriate filter sets. A minimum of 50 cells and a maximum of 200 cells were scored per case. A minimum of 20 abnormal cells was required for a sample to be considered abnormal.

**FISH Probes**

Clones to cover the regions of interest were identified by using the University of California Santa Cruz Genome Browser (http://wwwgenome.ucsc.edu). Clones were chosen to avoid low-copy repeat sequences that could lead to cross-hybridization and misinterpretation of results. Glycerol stocks of each bacterial artificial chromosome (BAC) clone were received from ResGen Invitrogen (Carlsbad, CA). Clones were plated and propagated immediately on arrival. DNA isolation and purification was performed using the QIAGEN (Valencia, CA) Plasmid Maxi Kit according to the manufacturer’s instructions. Primers were designed to unique sequences within each BAC clone, and polymerase chain reaction was performed under standard conditions to verify the presence of the appropriate sequence within each clone.

Nick translation was performed using the Abbott Molecular Nick Translation Kit (Abbott Molecular, Des Plaines, IL) to fluorescently label the BAC DNA using SpectrumOrange deoxyuridine triphosphate (dUTP) and SpectrumGreen-dUTP (Abbott Molecular). Probe details are given in Table 1. Probe validation was conducted based on previously described “familiarization” procedures. To determine criteria for positivity using each probe, the upper boundary of the normal range was determined using a 95% confidence interval as described previously. Normal cutoffs ranged from 6% to 9%.

**Table 1**

<table>
<thead>
<tr>
<th>Probe</th>
<th>Normal Cutoff</th>
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<tr>
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Cases were screened for translocations involving TCRA (14q11), TCRB (7q35), TCRG (7p15), and TCL1 (14q32) using break-apart DNA probes that hybridize to regions that flank the breakpoint regions of these genes. The presence of extra break-apart fusion signals without separation was considered indicative of aneuploidy. Cases were screened for abnormalities of chromosome 7 using a SpectrumOrange-labeled 7q deletion probe corresponding to 7q32.1 and a SpectrumGreen-labeled 7p probe (7p22.3). Cells with at least 2 more 7q signals than 7p signals were considered consistent with the presence of isochromosome 7q. Other anomalies of 7q were not counted. Cases that were positive with any FISH probe on TMAs were confirmed by performing FISH on whole tissue sections. In this article, SpectrumOrange-labeled signals are referred to as red, SpectrumGreen-labeled signals as green, and SpectrumOrange-SpectrumGreen fusion signals as fusion (F).

**Conventional Cytogenetics**

Results of karyotype analysis prepared at the time of biopsy using previously described methods were reviewed retrospectively for patients with abnormalities detected by FISH. Additional FISH studies were performed on archived metaphase spreads from a concurrent bone marrow specimen in 1 case (case 2) using the methods described in the preceding text.

**Results**

**Clinicopathologic Features**

There were 129 cases involving 124 patients that met the inclusion criteria. The patients included 73 males and 51 females, with a mean age of 61 years (range, 10-88 years). By the World Health Organization classification criteria, 35 (28.2%) were angioimmunoblastic T-cell lymphoma, 52 (41.9%) were PTCL-U, and 12 (9.7%) were ALCL. The remaining cases
included nodal involvement by mycosis fungoides (4 [3.2%]); enteropathy-associated T-cell lymphoma (3 [2.4%]); extranodal NK/T-cell lymphoma, nasal type (NKTL, 9 [7.3%]); HSTCL (5 [4.0%]); SCPTCL (2 [1.6%]); large granular lymphocyte leukemia (1 [0.8%]); and T-PLL (1 [0.8%]).

**Genetic Features**

FISH results are summarized in **Table 2** and **Table 3**. Three cases had translocations involving TCRA (2.9%). The first (case 1) was a case of nodal PTCL-U with some features resembling angioimmunoblastic T-cell lymphoma. This case additionally had a translocation involving TCRA, but no additional material was available to characterize these translocations further.

The second case with a TCRA translocation was a case of ALK– ALCL involving a lymph node (case 2). Repeated FISH using the TCRA probe on a metaphase spread from the patient’s bone marrow indicated a partner gene on chromosome 5, and conventional cytogenetics from the bone marrow showed 46-48,X,–X,add(2)(q37),t(5;14)(q11.2;q11.2),add(6)(p21.3),t(9;18)(p22;q12.2),add(11)(q13),+22,+1-2mar. The lymph node additionally demonstrated i(7q) by FISH.

The third case with a TCRA translocation was the single case of T-PLL (case 6), which also had a translocation involving the TCL1 gene consistent with the presence of t(14;14) (q11;q32) or inv(14)(q11;q32).

No additional cases had translocations involving TCL1 or TCRB, and no cases showed an abnormality of TCRG. Three additional cases (cases 3-5) showed evidence of i(7q), for a total of 4 (4%) of 93. The 4 positive cases included 2 cases of ALK– ALCL and 2 cases of NKTL. In one of the cases of NKTL Image 21, conventional cytogenetics showed 45-48,XY,+X,i(7)(q10),+21. None of the 4 HSTCL cases had evidence of i(7q). Three HSTCL cases had a documented αβ TCR phenotype, and the fourth was negative by immunohistochemical staining for βF1, but the TCR phenotype was not established definitively.

### Table 2

**Incidence of Cyto genetic Abnormalities in the Present Study**

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>TCRA</th>
<th>TCRB</th>
<th>TCRG</th>
<th>TCL1</th>
<th>i(7q)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Angioimmunoblastic TCL</td>
<td>0/32</td>
<td>0/22</td>
<td>0/30</td>
<td>0/12</td>
<td>0/15</td>
</tr>
<tr>
<td>Peripheral TCL, unspecified</td>
<td>1/45†</td>
<td>1/27†</td>
<td>0/43</td>
<td>0/29</td>
<td>0/45</td>
</tr>
<tr>
<td>Anaplastic large-cell lymphoma, ALK+</td>
<td>0/2</td>
<td>0/2</td>
<td>0/2</td>
<td>0/1</td>
<td>0/2</td>
</tr>
<tr>
<td>Anaplastic large-cell lymphoma, ALK–</td>
<td>1/3†</td>
<td>0/4</td>
<td>0/6</td>
<td>0/4</td>
<td>2/3†</td>
</tr>
<tr>
<td>Mycosis fungoides (nodal involvement)</td>
<td>0/4</td>
<td>0/4</td>
<td>0/4</td>
<td>0/2</td>
<td>0/4</td>
</tr>
<tr>
<td>Enteropathy-associated TCL</td>
<td>0/1</td>
<td>0/1</td>
<td>0/1</td>
<td>0/1</td>
<td>0/3</td>
</tr>
<tr>
<td>Extranodal NK/TCL, nasal type</td>
<td>0/4</td>
<td>0/3</td>
<td>0/3</td>
<td>0/1</td>
<td>2/3</td>
</tr>
<tr>
<td>Hepatosplenic TCL</td>
<td>0/2</td>
<td>0/2</td>
<td>0/2</td>
<td>0/1</td>
<td>0/4</td>
</tr>
<tr>
<td>Subcutaneous panniculitis-like TCL</td>
<td>0/1</td>
<td>0/1</td>
<td>0/0</td>
<td>0/0</td>
<td>0/1</td>
</tr>
<tr>
<td>Large granular lymphocyte leukemia</td>
<td>0/1</td>
<td>0/1</td>
<td>0/1</td>
<td>0/1</td>
<td>0/1</td>
</tr>
<tr>
<td>T-cell prolymphocytic leukemia</td>
<td>1/12</td>
<td>1/88</td>
<td>0/93</td>
<td>1/63</td>
<td>4/93</td>
</tr>
</tbody>
</table>

ALK, anaplastic lymphoma kinase; i(7q), isochromosome 7q; TCL, T-cell lymphoma; TCRA, T-cell receptor α; TCRB, T-cell receptor β; TCRG, T-cell receptor γ; TCRL, T-cell receptor ϒ.

† A single case of peripheral TCL, unspecified, had translocations involving the TCRA locus and the TCRB locus.

‡ A single case of ALK– anaplastic large-cell lymphoma had a translocation of the TCRA locus and i(7q).

### Table 3

**Cases With Abnormal Cytogenetic Findings by FISH in the Present Study**

<table>
<thead>
<tr>
<th>Case No./ Sex/Age (y)</th>
<th>Diagnosis</th>
<th>Tissue Site</th>
<th>Previously Treated</th>
<th>FISH Abnormalities</th>
<th>Karyotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>1/M/37</td>
<td>PTCL-U</td>
<td>Lymph node</td>
<td>Unknown</td>
<td>TCRA, TCRB, i(7q)</td>
<td>46,XY</td>
</tr>
<tr>
<td>2/F/50</td>
<td>ALCL, ALK–</td>
<td>Lymph node, bone marrow*</td>
<td>Yes</td>
<td>TCRA, i(7q)</td>
<td>46-48,X,–X,add(2)(q37),t(5;14)(q11.2;q11.2),add(6)(p21.3),t(9;18)(p22;q12.2),add(11)(q13),+22,+1-2mar,[cp17]/46,XX[3]</td>
</tr>
<tr>
<td>3/M/75</td>
<td>ALCL, ALK–</td>
<td>Chest wall</td>
<td>Yes</td>
<td>i(7q)</td>
<td>46,XY</td>
</tr>
<tr>
<td>4/M/37</td>
<td>NKTL</td>
<td>Skin and soft tissue</td>
<td>No</td>
<td>i(7q)</td>
<td>45-48,XY,+X,i(7)(q10),+21[cp17]</td>
</tr>
<tr>
<td>5/F/53</td>
<td>NKTL</td>
<td>Nasal</td>
<td>No</td>
<td>i(7q)</td>
<td>46,XX</td>
</tr>
<tr>
<td>6/M/76</td>
<td>T-PLL</td>
<td>Nasopharynx</td>
<td>No</td>
<td>TCRA, TCL1</td>
<td>ND</td>
</tr>
</tbody>
</table>

ALCL, anaplastic large-cell lymphoma; ALK, anaplastic lymphoma kinase; FISH, fluorescence in situ hybridization; i(7q), isochromosome 7q; ND, not done; NKTL, extranodal NK/T-cell lymphoma, nasal type; PTCL-U, peripheral T-cell lymphoma, unspecified; TCL, T-cell lymphoma; TCRA, T-cell receptor α; TCRB, T-cell receptor β; T-PLL, T-cell prolymphocytic leukemia.

* In case 2, FISH was performed on lymph node material, and the karyotype was obtained from a concurrent bone marrow specimen.
Discussion

There are 3 genetic loci for TCR genes in T cells: αδ (TCRA), β (TCRB), and γ (TCRG). The δ locus is contained entirely within the α locus, and, thus, these loci are considered together. Translocations involving TCR genes in T-cell lymphomas may drive transcription of an oncogene on the partner chromosome, as has been shown in T-LBLL\(^6\) and T-PLL.\(^1\) Our data, derived from a spectrum of PTCL types, suggest the incidence of translocations involving TCR genes is low, around 3%, but real. Three PTCLs with translocations involving the TCRA gene locus were found. One was a case of T-PLL with the likely partner being TCL1; translocations involving TCL1 have been associated specifically with T-PLL,\(^1\) and the present study provides additional evidence that TCL1 translocations are not seen in other mature T-cell neoplasms. The partner genes in the other 2 cases are unknown. One of these cases also had a translocation involving the TCRB locus, but no additional TCRB abnormalities were detected. No case had a TCRG translocation, in keeping with previously published data.\(^8\) Among T-LBLLs, however, Gesk et al\(^7\) reported a case with a TCRG translocation and another case with translocations involving TCRA and TCRB.

The case in the present series that demonstrated translocations involving the TCRA and the TCRB loci was a case of PTCL-U. Because the “unspecified” designation of PTCL-U incorporates a spectrum of lymphomas with

![Image 1](Case 2) Representative findings from a case of anaplastic lymphoma kinase (ALK)–negative anaplastic large cell lymphoma showing evidence of a translocation involving TCRA (T-cell receptor α). The tumor effaces the lymph node architecture (A, ×10) and shows cytologic features of “hallmark cells” (B, ×40; inset, ×100). The tumor cells are positive for CD30 (C, ×40) and negative for ALK (D, ×40).
varying morphologic, immunophenotypic, and clinical characteristics, discovery of potentially distinguishing genetic features in PTCL-U offers the possibility of defining new, more homogeneous disease entities within this broad category, such as the recently described cases with ITK/SYK translocations. Characterization of such translocations may lead to identification of novel oncogenic events, even though the translocation may not be present in all tumors affected (eg, the BCL2 gene was found in association with the t(14;18) translocation in follicular lymphoma, but bcl-2 protein also is overexpressed in most cancers that lack this translocation). Unfortunately, in the PTCL-U in the present series, additional material was not available to characterize the translocations further.

The second case with a translocation involving the TCRA locus was an ALK– ALCL. The pathogenesis of this subset of systemic ALCLs has been elusive, despite the morphologic and immunophenotypic (except for ALK) similarity to ALK+ ALCL. However, ALK– ALCL tends to affect an older patient population and have a poorer prognosis than ALK+ ALCL. Therefore, identification of potential pathogenetic

**Image 1** Interphase fluorescence in situ hybridization (FISH) using a break-apart probe to TCRA (E) shows a tumor cell with a normal fusion signal (yellow) and a split red and green signal. On metaphase FISH (F), the split green signal is localized to a derivative chromosome 5, indicative of a t(5;14)(?;q11).

**Image 2** Representative findings from a case of extranodal NK/T-cell lymphoma, nasal type, and isochromosome 7q. The tumor cells infiltrate the soft tissue of the arm (A, H&E, ×40; inset, ×100) and are positive for CD2 (B, ×40), negative for CD5 (C, ×40), and positive for TIA-1 (D, ×40) and Epstein-Barr virus–encoded RNA by in situ hybridization (E, ×40). (cont next page)
events may be key in understanding this disease. The availability of metaphase spreads in this case allowed further evaluation of this translocation by FISH, confirming the presence of a t(5;14)(q11.2;q11.2). To our knowledge, this translocation has not been reported in lymphomas, although rare cases of acute lymphoblastic leukemias carry a translocation t(5;14)(q31;q32). The present case also showed evidence of i(7q), discussed further in the next paragraph.

The genetics of NKTLs have not been well characterized. The most commonly reported cytogenetic abnormality is deletion of 6q. The i(7q) has been reported in a previous case of NKTL; in the present study, we observed i(7q) in 2 (22%) of 9 cases of NKTL. The i(7q) can be seen in a variety of hematopoietic neoplasms, but among the peripheral T- and NK-cell lymphomas it has been characterized as a recurrent abnormality seen in HSTCL. Although originally thought to be pathognomonic for this entity, recent studies have shown that not all cases of HSTCL have i(7q) and that this abnormality seems to be more common in cases with a γδ phenotype than in cases with an αβ phenotype. In our study, none of the 4 cases of HSTCL had an i(7q), but at least 3 of these were of the αβ phenotype. Two additional lymphomas found to have i(7q) were ALK– ALCLs. These data indicate that the presence of i(7q) among PTCLs is neither specific nor required for HSTCL. The biologic significance of i(7q) in ALK– ALCL and NKTL is unknown.

Image 21 (cont) Interphase FISH (F) using probes to 7p (green) and 7q (red) show 3 copies of 7q and only 1 copy of 7p, consistent with the presence of i(7q).
Because some patients whose specimens were studied were referred to our institution from other centers, 2 comments are warranted regarding the incidence of genetic abnormalities reported herein. First, although cases referred for pathologic consultation were not included, oncology referral practices might influence the patient population we studied. Second, some patients underwent initial biopsies at other centers, and some received treatment before the specimens analyzed in this study were obtained. Therefore, the incidences we observed might not reflect those at the time of first diagnosis.

TCR gene translocations and i(7q) can be detected in paraffin-embedded PTCLs by FISH. Subjects for further study include the identification of genes that may be up-regulated in lymphomas with i(7q), characterization of the novel translocation t(5;14)(q11;q11) in ALK-ALCL, and investigation of the biologic significance of the genetic abnormalities described herein.

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References


