Genomic Alterations in Blastic Natural Killer/Extranodal Natural Killer-Like T Cell Lymphoma with Cutaneous Involvement

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N atural killer and natural killer-like T cell lymphomas represent a rare type of non-Hodgkin’s lymphoma originally described to involve the upper aerodigestive tract. This malignancy has been increasingly observed in other extranodal sites, particularly in the skin. Patients with cutaneous natural killer cell lymphoma generally have a poor prognosis; however, the etiology and the underlying molecular pathogenesis remain unclear. This study aimed to investigate comprehensively genomic changes in blastic natural killer and extranodal natural killer-like T cell lymphomas with cutaneous involvement. Comparative genomic hybridization showed chromosome imbalances in six of eight cases studied (75%). The mean number of chromosome imbalances per sample was 2.18 ± 1.63 with similar number of gains (1.18 ± 1.17) and losses (1.00 ± 1.34). The most frequent DNA copy number changes observed were losses of 9p9p (83%), followed by loss of 13q and gain of 7 (67%). Similar patterns of chromosome imbalances were observed in both blastic natural killer and cutaneous natural killer-like T cell lymphomas. Loss of the RB1 gene at 13q14.2 was detected in one blastic natural killer cell lymphoma with 13q loss using a gene dosage assay, and in one cutaneous natural killer-like T cell lymphoma without 13q loss using fluorescent in situ hybridization. Genomic microarray analysis identified oncogene copy number gains of PAK1 and JUNB in three of four cases studied, and gains of RAF1, CTBS, FGFR1, and BCR in two cases. Real-time polymerase chain reaction detected amplification of CTBS and RAF1 in four of five cases analyzed, JUNB and MYCN in three cases, and REL and YES1 in two cases, respectively. In conjunction with this study, an extensive literature search for the published G-band karyotypes of four subsets of natural killer cell lymphomas was conducted, which showed a nonrandom pattern of multiple chromosome aberrations. These results reveal consistent genetic alterations in cutaneous natural killer cell lymphomas, and provide a basis for further investigation of molecular pathogenesis in this malignancy.


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receptors, such as CD158a, CD158b, p40-KIR3DL1, and CD94/natural killerG2A or not remain debating (Bagot et al., 1998, 2001; Nikolova et al., 2002). Clinically, patients with cutaneous natural killer cell lymphomas usually present with widespread smooth-surfaced nodules and plaques with a characteristic violaceous color. The tumors have a predilection for the trunk but also involve the extremities, the head and neck, and central nervous system (Ansal et al., 1997; Savoia et al., 1997; Natkunam et al., 1999, 2000; Cho et al., 2000; El Shabrawi-Caelen et al., 2000; Mraz-Gernhard et al., 2001; Child et al., 2003). Histologically, there is a bottom-heavy dermal infiltrate without significant epidermotropism (Child et al., 2003). Angiocentricity and red cell extravasation are present together with numerous blast-like mononuclear cells (Ansal et al., 1997; Savoia et al., 1997; Natkunam et al., 1999, 2000; Cho et al., 2000; El Shabrawi-Caelen et al., 2000; Mraz-Gernhard et al., 2001; Siu et al., 2002; Child et al., 2003). The prognosis of natural killer and natural killer-like T cell lymphomas is very poor (Ansal et al., 1997; Savoia et al., 1997; Natkunam et al., 1999, 2000; Cho et al., 2000; El Shabrawi-Caelen et al., 2000; Mraz-Gernhard et al., 2001; Siu et al., 2002; Child et al., 2003). Despite recent progress in defining the immunopathologic features of this disease, the etiology and underlying molecular pathogenesis remain unclear.

Previous cytogenetic studies have shown a variety of nonrandom chromosomal abnormalities in natural killer lymphomas (Fernandez et al., 1986; Bassan et al., 1988; Taniwaki et al., 1990; Hart et al., 1992; Oshimi et al., 1993; Gelb et al., 1994; Brody et al., 1995; Callet-Bauchet et al., 1997; Tien et al., 1997; Kameoka et al., 1998; Petrella et al., 1999; Wong et al., 1999; Zhang et al., 1999; Hamaguchi et al., 2001; Leroux et al., 2002); however, unlike nodal non-Hodgkin’s lymphoma, no recurrent chromosome translocations have been identified in this malignancy.

Most malignancies accumulate a series of genetic events, including activation of oncogenes and loss of tumor suppressor genes, leading to a malignant phenotype. Comparative genomic hybridization (CGH) is a molecular cytogenetic technique used to identify chromosomal imbalances representing regions of gain and loss. Previous CGH studies have shown a variety of DNA copy number changes in nodal and extranodal lymphomas (Knuttila et al., 2000; Siu et al., 1999, 2002; Ko et al., 2001). We have identified consistent and distinctive patterns of chromosome imbalances in mycosis fungoides/Sézary syndrome, primary cutaneous B cell lymphoma, and primary cutaneous CD30+ anaplastic large cell lymphoma (CD30+ anaplastic large cell lymphoma) (Mao et al., 2002a,b, 2003b,c). Genetic microarray is a novel genomic analysis technology used to screen rapidly for genomic imbalances in a tumor genome (Pinkel et al., 1998). Previous studies have shown oncogene copy number changes in several epithelial cancers (Pinkel et al., 1998; Daigo et al., 2001; Hui et al., 2001, 2002). We have also detected gains and losses of various different oncogenes in mycosis fungoides/Sézary syndrome, primary cutaneous B cell lymphoma and CD30+ anaplastic large cell lymphoma using genomic microarray technique (Mao et al., 2002b, 2003a,c). There are no data available, however, on cutaneous natural killer cell lymphomas.

To address the issues of whether there are consistent genomic changes in cutaneous natural killer cell lymphomas, we have investigated eight cases using CGH, genetic microarray and real-time polymerase chain reaction (real-time PCR). In addition, we have analyzed the RBI gene in three cases with 1q loss and one case without 1q loss using gene polymorphism and gene dosage assays, and fluorescence in situ hybridization. We have then correlated these findings with the results of an extensive literature review.

**MATERIALS AND METHODS**

**Samples** The study was approved by St. Thomas’ Hospital Research Ethics Committee. A total of 11 DNA samples from eight patients with cutaneous natural killer cell lymphomas were selected for this study based on clinico-pathologic features, immunophenotype, extensive staging investigations, and the results of T cell receptor (TCR)/immunoglobulin (IGH) gene analysis (Table I) (Child et al., 2003). This included four cases of blastic natural killer lymphoma with a germline configuration of the TCR gene, and four cases of extranodal natural killer-like T cell lymphoma with a clonal TCR gene rearrangement. Two patients (cases 3 and 5) had lymph node and bone marrow involvement, and case 3 also had nasal involvement. Two other patients (cases 1 and 8) had leukemia. Immunophenotypic studies demonstrated that all eight cases studied were CD56 positive. In addition, case 3 had a clonal B cell population with a rearrangement of the IGH gene, a t(4;18) translocation, and BCL2 positive staining. Only case 4 was positive for EBV detected with in situ hybridization (Child et al., 2003). This study was approved by the St Thomas’ Hospital Research Ethics Committee for sampling (EC02/069).

CGH DNA was extracted from the skin lesions, peripheral blood mononuclear cells, lymph nodes, and bone marrow from the patients with cutaneous natural killer cell lymphomas using standard procedures. CGH was performed as previously described (Kallioniemi et al., 1994; Mao et al., 2001, 2002a,b, 2003a,c). At least 10 metaphases were captured and analyzed on a Leica DMRXA fluorescence microscope (Leica Mikroskopie und System, Solms, Germany) with a COHU charged-couple device camera using MacProbe 4.0 software (Perceptive Scientific Instruments International Ltd, Chester, UK). The threshold set for gains corresponded to a mean hybridization ratio between tumor and normal of greater than 1.25:1, and losses of less than 0.75:1 (Fig I) (Kallioniemi et al., 1994; Mao et al., 2001, 2002a,b, 2003a,b).

**Analysis of the RBI gene with molecular and fluorescent in situ hybridization assays** To confirm CGH findings on 13q in this study, two blastic natural killer cell lymphomas (sample nos 1231 and 1683) and one cutaneous natural killer-like T cell lymphoma sample (no. 1733) with 13q loss were analyzed using gene polymorphism assay. In this experiment, 100 ng of tumor DNA was amplified with the fluorescently labeled primer sets for Rho.2 (microsatellite) and RBL1.20 (variable number tandem repeat, VNTR) (Onadim et al., 1992, 1997), and the PCR products were then analyzed for intragenic RBI polymorphism using ALFExpress automatic sequencer (Amersham Biosciences, Bucks, UK).

To further characterize the further loss of these 13q gains and losses of the 13q, a fluorescent dosage assay was conducted in sample 1231. Briefly, 100 ng of tumor DNA was amplified with 32 pairs of fluorescently labeled primers, in 11 multiplex reactions, covering all exons with associated splice sites, the promoter region, 3’ untranslated region and the poly(A) site of the entire RBI gene (NCBI LocusLink, 2002). The PCR products were analyzed using ALFExpress (Amersham Biosciences). The RBI gene dosage was determined by the dosage quotient. The dosage quotient value between 0.76 and 1.28 was set as the level for disomy (normal gene dosage), whereas the value between 0.36 and 0.68 was regarded as monosomy (hemizygosity).

In addition, single-color fluorescent in situ hybridization was carried out on the available slide containing metaphase chromosomes and interphase nuclei from case 8 (sample 2634). Briefly, SpectrumOrange labeled RBI probe, LSI 13 (Vysis Inc., Downers Grove, Illinois), was applied to the patient slide, and codenatured on a hot block at 70°C for 5 min, and hybridized at 37°C overnight. Posthybridization washing and fluorescent image analysis were as described previously (Mao et al., 2001, 2002a, 2003a).

**Genomic microarray** To define genomic imbalances, four cases of blastic natural killer cell lymphoma (nos 1, 2, 4, and 6) with noticeable chromosome imbalances detected by CGH were selected for genomic microarray analysis. This experiment was conducted as previously described (Pinkel et al., 1998; Daigo et al., 2001; Hui et al., 2001, 2002; Mao et al., 2002b, 2003a,c). After probe labeling, hybridization and posthybridization washes, fluorescent images of the hybridized microarray chips, AmpliOnc I DNA array (Vysis Inc.), which contains 59 clones from 57 oncogenes (BCL2 and AR are represented by both 5’ and 3’ genomic clones) representing genomic regions that have been reported to be amplified in human tumors, were captured and analyzed using the GenoSensor Reader System (Vysis Inc). The fluorescent ratio threshold for controls and gains and losses of oncogene copy number were set according to our control experiments (Mao et al., 2002b, 2003a,c). The measured fluorescent ratio of oncogenes on autosomes ranged from 0.8 to 1.2 with a mean ratio of 1 and SD of 0.2 in testing samples. The ratios on AR located at Xq11q12 were beyond 1.25 in normal female to male control sets due to the extra copy of X chromosome from normal female DNA, and below 0.75 in normal male to female control sets due to one copy of X chromosome from male DNA. Therefore a value of 1±0.2 was set as the level for disomy (normal gene copy number), whereas ratios greater than 1.25 were...
regarded as cut-off for trisomy (gain of gene copy number), and ratios less than 0.75 as cut-off for monosomy (loss of gene copy number) (Mao et al., 2002b, 2003a,c).

Real time-PCR To validate further and clarify chromosome imbalances and genomic imbalances identified by CGH and genomic microarray in this study, four cases of blastic natural killer lymphoma (nos 1, 2, 4, and 6) and one cutaneous natural killer-like T cell lymphoma (case 3) showing noticeable genomic changes were analyzed using real-time PCR. A total of seven oncogenes (targets), namely MYCN, REL, RAF1, CTSB, YES1, BCL2, and JUNB, and two housekeeping genes, B2M and ALB (reference) were tested (MWG Biotech, High Point, North Caroline) (Table II). This experiment, based on the Taqman assay (Bieche et al., 1998), was carried out using the ABI Prism 7700 Sequence Detector System (ABI/Perkin Elmer, Foster City, California) as previously reported (Mao et al., 2002b,c, 2003b).

The parameter CT is defined as the fractional cycle number at which the fluorescence generated by cleavage of the probe passes a fixed threshold above baseline. The target gene copy number is quantified by measuring CT and by using a standard curve to determine the starting copy number. The ratio of the target gene copy number to the reference gene copy number normalizes the amount and quality of genomic DNA. The ratio defining the level of increased copy number of the target gene was termed as "N" and was determined as follows: N = copy number of target gene/copy number of reference gene. An N-value greater than 2 was set for gene amplification (Mao et al., 2002b, 2003b,c).

Literature review To compare our findings with previous published cytogenetic data, we have collated the G-banded karyotypes of peripheral

<table>
<thead>
<tr>
<th>Case</th>
<th>Sample</th>
<th>Diagnosis</th>
<th>Immunophenotype</th>
<th>TCR/IgH²</th>
<th>CGH³</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1231S</td>
<td>Blastic NK lymphoma</td>
<td>CD2⁺,CD4⁺,CD8⁺,CD30⁺,CD56⁺,TIA-1⁺</td>
<td>pc/pc</td>
<td>enh(7),dim(9p13p22,12p13,13q14q21)</td>
</tr>
<tr>
<td>2</td>
<td>1683S</td>
<td>Blastic NK lymphoma</td>
<td>CD2⁺,CD3⁺,CD4⁺,CD8⁺,CD20⁺,CD43⁺,CD56⁺,TIA-1⁺</td>
<td>pc/pc</td>
<td>enh(7p),dim(2q35q37,13q12q34)</td>
</tr>
<tr>
<td>3</td>
<td>1733S</td>
<td>Cutaneous NK/T cell lymphoma</td>
<td>CD2⁺,CD3⁺,CD4⁺,CD8⁺,CD20⁺,CD43⁺,CD56⁺,TIA-1⁺</td>
<td>c/c</td>
<td>dim(3p12p21,9,13q)</td>
</tr>
<tr>
<td>4</td>
<td>1734B</td>
<td>Cutaneous NK/T cell lymphoma</td>
<td>CD2⁺,CD3⁺,CD4⁺,CD8⁺,CD20⁺,CD43⁺,CD56⁺,TIA-1⁺</td>
<td>c/c</td>
<td>enh(18)</td>
</tr>
<tr>
<td>5</td>
<td>1801S</td>
<td>Cutaneous NK/T cell lymphoma</td>
<td>CD2⁺,CD3⁺,CD4⁺,CD8⁺,CD20⁺,CD43⁺,CD56⁺,TIA-1⁺</td>
<td>c/c</td>
<td>N</td>
</tr>
<tr>
<td>6</td>
<td>1860S</td>
<td>Blastic NK lymphoma</td>
<td>CD4⁺,CD43⁺,CD56⁺</td>
<td>pc/-</td>
<td>enh(6p21p22,7q),dim(9p23p24)</td>
</tr>
<tr>
<td>7</td>
<td>2022S</td>
<td>Cutaneous NK/T cell lymphoma</td>
<td>CD2⁺,CD3⁺,CD4⁺,CD8⁺,CD20⁺,CD30⁺,CD43⁺,CD56⁺,TIA-1⁺,CD5⁺,CD7⁺</td>
<td>c/pc</td>
<td>N</td>
</tr>
<tr>
<td>8</td>
<td>2412S</td>
<td>Blastic NK lymphoma</td>
<td>CD2⁺,CD3⁺,CD4⁺,CD8⁺,CD20⁺,CD43⁺,CD56⁺,Alk¹,CD56⁺</td>
<td>pc/-</td>
<td>dim(9,13q14q34)</td>
</tr>
<tr>
<td>9</td>
<td>2592S</td>
<td>Cutaneous NK/T cell lymphoma</td>
<td>CD56⁺</td>
<td>c/-</td>
<td>N</td>
</tr>
<tr>
<td>10</td>
<td>2634B</td>
<td>Cutaneous NK/T cell lymphoma</td>
<td>CD56⁺</td>
<td>c/-</td>
<td>enh(5q33q35,7q33q36,9q),dim(12p11p12)</td>
</tr>
</tbody>
</table>

1 S, skin lesion; B, blood; L, lymph nodes; M, bone marrow.
2 pc, polyclonal (normal); c, clonal (rearrangement); -, not available.
3 enh, enhanced (gain); dim, diminished (loss); N, normal.

Figure 1. Illustration of the original CGH fluorescent image (left) and ratios (right) of case 2 (sample 1683). In the left figure light gray-colored chromosomes 9 (short arrow) and 13q (long arrow) were clearly visible, and in the right the fluorescent ratios of chromosomes 9 and 13q were below 0.75 indicating losses of 9 and 13q.

RESULTS

CGH Of the eight cases (11 DNA samples) analyzed in this study, six showed chromosome imbalances (75%). Overall the mean number of chromosome imbalances per sample was 2.18 ± 1.63 with similar number of gains (1.18 ± 1.17) and losses (1.00 ± 1.34). The most frequent DNA copy number changes (minimum three cases) were losses of 9p (five of six, 83%), followed by loss of 13q and gain of 7 (four of six, 67%) (Table I) (Figs 1–5). Less frequent chromosome imbalances (< 3 cases), including losses of 2q, 3p, and 12p, and gains of 4, 5q, 6p, 9q, 18, and 19 were also seen (Table I) (Fig 3). A similar pattern of chromosome imbalances was present in both blastic natural killer and cutaneous natural killer-like T cell lymphomas. There was only one case (no. 3) from which multiple DNA samples were analyzed. This case showed intratumoral variation in the pattern of chromosome imbalances, namely, blood (1734) and lymph node (1802) revealing gain of 18, skin (1733) demonstrating losses of 3p, 9, and 13q, and bone marrow (1781) having a normal copy number (Table I).

RBI assays All three samples (nos 1231, 1683, and 1733) analyzed using the two intragenic RBI polymorphic markers, Rbi.2 microsatellite and RBI.20 VNTR, exhibited single alleles with these polymorphisms. In addition, sample 1231, which was further analyzed for all RBI regions (including the promoter and the β untranscribed region) using fluorescent dosage screening, showed hemizygosity across all regions of RBI tested (dosage quotient value ranged from 0.32 to 0.63), indicating the presence of only one copy of the RBI gene. Furthermore, fluorescent in situ hybridization analysis of sample 2634 revealed that 67% of cells had two copies of RBI, and 33% of cells showed only one copy of RBI (Fig 4).

Table II. A summary of the primers and the probes used in real-time PCR

<table>
<thead>
<tr>
<th>Gene</th>
<th>Forward primer</th>
<th>Reverse primer</th>
<th>Probe</th>
</tr>
</thead>
<tbody>
<tr>
<td>MYCN</td>
<td>CCGCAAGCAGCCCTCCTCGG</td>
<td>TCTTTAACCACCTGGGCACG</td>
<td>5′-(FAM)-CGCAGTTTCTCAGCCTAGGGACC-(TAMRA)-3′</td>
</tr>
<tr>
<td>REL</td>
<td>CCCAGCCTCAGGGAATACA</td>
<td>CAATCTCAGACCACCTGTACAGTA</td>
<td>5′-(FAM)-CCACACCTGGCAGAGCTAC-(TAMRA)-3′</td>
</tr>
<tr>
<td>RAFI</td>
<td>CCGTTCGCCGGCACATCC</td>
<td>GTGCAGTGGTACAGGTAGTTTTC</td>
<td>5′-(FAM)-CATCGGTCGACCCACAGTGGG-(TAMRA)-3′</td>
</tr>
<tr>
<td>CTSB</td>
<td>CATTCCGACAGCCGTA</td>
<td>TCTCAGACCCACCCGACCTCAG</td>
<td>5′-(FAM)-CTCTGGTGGTTGCTGTGTTGC-(TAMRA)-3′</td>
</tr>
<tr>
<td>YESI</td>
<td>CAGTTGAGGGCGGATACGCC</td>
<td>GAGTCTGGTTCTCAGGGAG</td>
<td>5′-(FAM)-CCCTCGTGCTCAGCTGCT-(TAMRA)-3′</td>
</tr>
<tr>
<td>BCL2</td>
<td>TGTTGTTGACACTTCTAGAGA</td>
<td>AGGTTCGTTCATTCTCTTC</td>
<td>5′-(FAM)-AGGTTGACTGCGGACAGATCATCACC-(TAMRA)-3′</td>
</tr>
<tr>
<td>JUNB</td>
<td>CTACCGGGATGCGGCG</td>
<td>AGGTCGTTGTTCTCAGGGAG</td>
<td>5′-(FAM)-AGGTTGACTGCGGACAGATCATCACC-(TAMRA)-3′</td>
</tr>
<tr>
<td>B2M</td>
<td>GPAATTGATTGGGGAGGACATC</td>
<td>CAGTGCTTTGCTTCAATT</td>
<td>5′-(FAM)-GAATATACGGCATCATTCAACCCACCTGTCAGATC-(TAMRA)-3′</td>
</tr>
<tr>
<td>ALB</td>
<td>AGGGTTAAGAGTCCTGCTGATATGCT</td>
<td>CAATCTCAGACCACCTGTACAGTA</td>
<td>5′-(FAM)-CCACACCTGGCAGAGCTAC-(TAMRA)-3′</td>
</tr>
</tbody>
</table>

Genomic microarray All four cases studied by genomic microarray showed genomic imbalances (100%). Oncogene copy number gains of PAK1 and JUNB were observed in three cases, and RAF1, CTSB, FGFR1, and BCR in two cases (Figs 5 and 6). There was broad concordance between the results of CGH and genomic microarray in two cases (Figs 3 and 6). Case 2 (sample 1683) revealed losses of 9 and ABL1 (9q34.1). Case 4 (sample 1860) had gains of 6p21p22 and MYB (6q22). These two methods also revealed discordant results in case 6, however, which showed loss of chromosome 9 by CGH without loss of ABL1 (9q34.1) by genomic microarray (Figs 3 and 6).

Real-time PCR Of the five cases analyzed by real-time PCR, four showed amplification of CTSB and RAF1 (80%), three had amplifications of JUNB and MYCN (60%), and two revealed amplifications of REL and YESI (40%) (Table III). The pattern of oncogene amplification in both blastic natural killer and cutaneous natural killer-like T cell lymphomas was similar. The real-time PCR results were consistent with the CGH and genomic microarray findings in all these cases (Tables I and III; Figs 3 and 6). For example, case 2 showed amplifications of RAF1, CTSB, and JUNB by both real-time PCR and genomic microarray. Case 3 revealed amplifications of YESI and BCL2 (18q21.3) by genomic microarray, and gain of 18 by CGH. Case 4 had real-time PCR, and genomic microarray evidence of MYCN, REL, RAF1, CTSB, and JUNB amplification. Case 6 showed amplifications of JUNB by both real-time PCR, and genomic microarray. Discrepancies between these three techniques, however, were present in case 1, which showed amplifications of RAF1 and CTSB by real-time PCR without corresponding chromosomal or oncogene gains by either CGH or genomic microarray (Tables I and III; Figs 3 and 6).

Literature review The major nonrandom chromosome abnormalities (same aberration presenting in more than five cases) identified in peripheral T cell lymphoma/natural killer cell lymphoma nonspecific (Mitelman Database of Chromosome Aberrations in Cancer, 2002), CD4+ CD56+ DC2 (review of 32 published cases), natural killer/TCL (review of 19 published cases) and aggressive natural killer cell lymphoma/leukemia (review of 34 published cases) were summarized in Table IV. Among these subsets of natural killer cell lymphomas, peripheral T cell lymphoma/natural killer cell lymphoma nonspecific showed multiple aberrations affecting each individual chromosome, and the most common chromosome changes (> 10) cases were gains of chromosomes 3, 5, 7, X, losses of 9, 14, 6q, and 17p, and a translocation involving 6p and 6q (Table IV). Similar chromosome abnormalities were
present in CD4⁺ CD56⁺ DC2, natural killer/TCL and aggressive natural killer cell lymphoma/leukemia but with involvement of only a few chromosomes/ chromosomal regions (Table IV). The most frequent chromosome aberrations observed in CD4⁺ CD56⁺ DC2 (>5 cases) were losses of 5/5q, 9/9p, 12/12p, 13/13q, and 15, and gain of 6/6q. In natural killer/TCL the most common chromosome changes (>5 cases) were losses of 2/2q, 6/6q, and Y, and gain of X. Whereas losses of 7 and 10/10p were frequently seen in aggressive natural killer cell lymphoma/leukemia (>5 cases) (Table IV). Overall our CGH
results, such as losses of 2q, 9, 12p, and 13q, and gain of 7 identified in both blastic natural killer and cutaneous natural killer-like T cell lymphomas (Table I) (Fig 3), were consistent with previously cytogenetic findings (Table IV).

DISCUSSION

Our comprehensive genomic studies of blastic natural killer and cutaneous natural killer-like T cell lymphoma presenting in the skin have identified consistent patterns of genetic alterations. CGH showed losses of 9/9p in 83% of cases, and loss of 13q and gain of 7 in 67% of cases. In addition, RB1 assays revealed loss of this tumor suppressor gene in one blastic natural killer cell lymphoma and in one cutaneous natural killer-like T cell lymphoma. Furthermore, genomic microarray demonstrated oncogene copy number gains of PAK1 and JUNB in three cases, and gains of RAF1, CTSB, FGFR1, and BCR in two cases. This was supported by real-time PCR, which detected amplification of CTSB, RAF1, MYCN, JUNB, REL, and YES1 in more than 40% of cases analyzed. There was a concordance between the results of these techniques, although some discrepancies were also noted, which are likely to be due to either the different sensitivity of the techniques or intratumoral heterogeneity. Our extensive literature review also revealed nonrandom chromosome aberrations in four subsets of natural killer cell lymphomas, including losses of 2/2q, 5/5q, 6/6q, 9, 12/12p, and 13/13q, and gain of 7 (see the references cited in Materials and Methods). Our findings suggest that these genomic alterations may be critical in the pathogenesis of both blastic natural killer and cutaneous natural killer-like T cell lymphomas.

There have been two reports describing genome-wide DNA copy number changes in natural killer cell lymphomas from the high incidence areas (Siu et al., 1999; Ko et al., 2001). One study showed frequent losses of chromosomes 6q, 11q, 13q, and 17p, and gains of 1p, 6p, 11q, 12q, 17q, 19p, 20q, and Xq in Chinese patients with nasal natural killer cell lymphoma and aggressive natural killer cell lymphoma/leukemia (Siu et al., 1999). Another study revealed frequent gains of 2q, 3q, 5q, 10q, 13q, 17q, and
and losses of 1p, 6q, 12q, 13q, and 17p in Korean patients with nasal natural killer/TCL (Ko et al., 2001). East Asia, however, is one of the areas with the highest rates of EBV infection in the world (de-The et al., 1975; Henle and Henle, 1980). In addition, EBV nonrandomly integrates into the human genome, and the human chromosome integration sites of EBV include chromosome bands of 1p31, 1q31, 1q43, 2p22, 2q32, 3q13, 3q28, 4q13, 5p14, 5q12, 6q24, 7q31, and 11p15 (Lestou et al., 1993; Wuu et al., 1996). Therefore, it is difficult to exclude the possibility that these genetic changes detected in natural killer cell lymphomas are a result of EBV infection. In contrast, all the patients analyzed in this study came from the UK, a low incidence area for both EBV infection and natural killer cell lymphomas, and only one of eight patients studied was EBV positive. The most intriguing findings in this study were the identification using CGH of losses of chromosomes 9/9p and 13q, and gain of 7, which were present in both blastic natural killer and cutaneous natural killer-like T cell lymphomas. These results are consistent with the previous cytogenetic reports (see the references cited in Materials and Methods), suggesting that the disruptions of genes on these chromosomes may be associated with cutaneous natural killer cell lymphomas. In addition, these findings are distinct from our previous observations in mycosis fungoides/Sezary syndrome, primary cutaneous B cell lymphoma and CD30⁺ anaplastic large cell lymphomas.

Figure 6. A summary of genomic microarray profiling of four blastic natural killer cell lymphomas. This diagram illustrated gains of PAK1, JUNB, RAF1, CTSB, FGFR1 and BCR.

Table III. Real-time PCR findings in 5 cutaneous NK lymphomas

<table>
<thead>
<tr>
<th>Case no.</th>
<th>Sample no.</th>
<th>N valueᵃ</th>
<th>MYCN</th>
<th>REL</th>
<th>RAF1</th>
<th>CTSB</th>
<th>YES1</th>
<th>BCL2</th>
<th>JUNB</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1231</td>
<td>0.78</td>
<td>1.95</td>
<td>2.44</td>
<td>4.66</td>
<td>1.51</td>
<td>1.22</td>
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<tr>
<td>2</td>
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<td>2.88</td>
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<td>3.40</td>
<td>1.45</td>
<td>1.66</td>
<td>4.24</td>
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<td>4.59</td>
<td>4.97</td>
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<td>1.67</td>
<td>3.34</td>
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<td>2.22</td>
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<td>2.00</td>
<td>0.95</td>
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<td>0.63</td>
<td>0.48</td>
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<td>3.34</td>
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study detected natural killer cell lymphomas in the literature. For example, one cell lymphomas. (Mao et al, 2003a). The pattern of genomic imbalances in cutaneous natural killer cell lymphomas was also distinctive from that in primary cutaneous B cell lymphoma (Mao et al, 2002b), further implying different genetic pathway involved. RAFl (v-raf-1 murine leukemia viral oncogene homolog 1) is a mitogen-activated protein kinase, which acts downstream of RAS, and is regulated by BCL2 and other apoptosis-related proteins (NCBI LocusLink, 2002). CTBS encodes cathepsin B, a lysosomal cysteine protease that cleaves amyloid precursor protein and is involved in tumor invasion (NCBI LocusLink, 2002). Previous studies have shown amplifications of RAFl and CTBS in esophageal and urinary bladder carcinomas, respectively (Hughes et al, 1998; Simon et al, 2001). We have also identified amplifications of these oncogenes in mycosis fungoides/Sezary syndrome and CD30+ anaplastic large cell lymphoma (Mao et al, 2003a,c). Further functional studies are now required to assess the potential role of these oncogenic alterations in the pathogenesis of cutaneous natural killer cell lymphomas.

Intratumoral variation of the pattern of chromosome imbalances has been reported in multiple tumor samples from the individual patients as evidence of genetic heterogeneity (Jung et al, 1999). In this study, such variation was seen in case 3. DNA samples from blood and lymph nodes showed gain of 18, and lymph nodes also had amplification of BCL2 by real-time PCR. In contrast, skin sample revealed losses of 3p, 9, and 13q. Whereas the bone marrow sample did not demonstrate chromosome imbalances. In addition, conventional cytogenetic analysis of bone marrow samples detected trisomies 3, 13, and 18 in small number of cells (data not shown). Interestingly identical V(T)2(TCR) and VH (IGH) clonal rearrangements were identified in all samples from skin, blood, lymph node, and bone marrow. This was further complicated by the detection of a t(14;18) translocation, in both skin and lymph node. The t(14;18) translocation is known to occur in the majority of follicular lymphomas and some diffuse large B cell lymphomas (Weiss et al, 1987). The clinical and pathologic features in this case were in keeping with a blastic natural killer cell lymphoma, and B cell markers were negative but 10% of the neoplastic cells within the infiltrate were CD3, CD8, and TIA-1 positive (Child et al, 2003). These results suggest the presence of a transformed extranodal natural killer-like T cell lymphoma with cytotoxic features (Child et al, 2003). The presence of a clonal IGH gene rearrangement could be explained by an anomalous IGH gene rearrangement in a T cell lymphoma but this would not explain the detection of a t(14;18) translocation, although this can be detected at very low levels in healthy individuals (Limpens et al, 1995). Previous studies have suggested that natural killer-like T cell lymphoma originate from a T cell lineage (Ng et al, 1987; Macon et al, 1996; Chan et al, 1997). Our data suggest, however, that both T and B lineage malignant cells presenting in this cutaneous natural killer-like T cell lymphoma may result from the molecular defects occurring at the stage of common lymphoid progenitors with the capacity to develop into T and B cells (Kondo et al, 1997); this may represent a genuine composite lymphoma as three additional cases also showed coexistence of T and B cell lymphomas (data not shown). Further study is therefore appropriate to clarify this hypothesis.

In summary, we have identified a consistent pattern of genomic alterations in cutaneous natural killer cell lymphomas, and further studies are underway to characterize the candidate genes using comprehensive genomic and proteomic approaches.

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REFERENCES


