Chromosomal Gains at 9q Characterize Enteropathy-Type T-Cell Lymphoma

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Genetic alterations in enteropathy-type T-cell lymphoma (ETL) are unknown so far. In this series, 38 cases of ETL were analyzed by comparative genomic hybridization (CGH). CGH revealed chromosomal imbalances in 87% of cases analyzed, with recurrent gains of genetic material involving chromosomes 9q (in 58% of cases), 7q (24%), 5q (18%), and 1q (16%). Recurrent losses of genetic material occurred on chromosomes 8p and 13q (24% each), and 9p (18%). In this first systematic genetic study on ETL, chromosomal gains on 9q (minimal overlapping region 9q33-q34) were found to be highly characteristic of ETL. Fluorescence *in situ* hybridization analysis on four cases of ETL, using a probe for 9q34, indicated frequent and multiple gains of chromosomal material at 9q34 (up to nine signals per case). Among 16 patients with ETL who survived initial disease presentation, patients with more than three chromosomal gains or losses ($n = 11$) followed a worse clinical course than those with three or less imbalances ($n = 5$). The observation of similar genetic alterations in ETL and in primary gastric ($n = 4$) and colonic ($n = 1$) T-cell lymphoma, not otherwise specified, is suggestive of a genetic relationship of gastrointestinal T-cell lymphomas at either localization. (*Am J Pathol* 2002, 161:1635–1645)

Enteropathy-type T-cell lymphoma (ETL) is an extranodal T-cell non-Hodgkin's lymphoma characterized by a differentiation of tumor cells toward the phenotype of intestinal intraepithelial T cells.¹ ETL arises in the jejunum or ileum as multiple circumferential ulcers, frequently leading to intestinal perforation. ETLs have a dismal prognosis with most patients dying of disease within a few months after diagnosis.² Histologically, ETLs show a wide spectrum of appearances, ranging from highly pleomorphic or anaplastic tumors to cases composed of mono-

morphic small- to medium-sized tumor cells.³ Most ETLs have a CD3⁺CD4[−]CD8[−]CD7⁺CD5[−] immunophenotype and co-express cytotoxic markers TIA-1 and granzyme B. ETLs mainly consisting of monomorphic small- to medium-sized tumor cells frequently show a CD8⁺CD56⁺ phenotype.^{4,5}

ETLs show a strong association with celiac disease.⁶ Clonal intraepithelial T-cell populations with an aberrant immunophenotype are found in the intestinal mucosa of patients with therapy-refractory celiac disease and are clonally related to subsequent ETL.^{7–10} In contrast to primary gastrointestinal B-cell lymphoma,^{11–13} only few data are available on genetic alterations occurring in ETL. Only four karyotypes of ETL have been published so far, with no recurrent genetic alterations revealed.^{14–16} In contrast to ETL arising in the small intestine, the biological derivation and exact classification of primary gastric and colonic T-cell lymphomas remains controversial.¹

To investigate genetic alterations in ETL, we performed a comprehensive study on genetic imbalances in 38 clinically and immunohistochemically well-characterized cases of ETL applying comparative genomic hybridization (CGH) and fluorescence *in situ* hybridization (FISH). To investigate the genetic relationship to ETL, four gastric and one colonic T-cell lymphoma, not otherwise specified (NOS), were additionally included into this series.

Materials and Methods

Specimen Selection and DNA Extraction

Seventy-five cases of ETL, all of them arising in the small intestine, were selected from the files of the Lymph Node Reference Center at the Department of Pathology, University of Würzburg, Würzburg, Germany, and from the files of the Department of Clinical Pathology, University of Vienna, Vienna, Austria. All cases were classified as ETL according to the current World Health Organization Classification of Tumors of Hematopoietic and Lymphoid Tissues.¹⁷ To assure a sufficiently high content of tumor cells for CGH, only cases with <40% nonneoplastic bystander cells were included for DNA extraction. In four

Supported by the Interdisziplinäres Zentrum für Klinische Forschung (IZKF), Würzburg, Germany.

Accepted for publication July 18, 2002.

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cases, fresh-frozen material was available for DNA extraction. In the remaining cases, DNA was extracted from formalin-fixed, paraffin-embedded tissue blocks using the phenol-chloroform extraction method according to previously published protocols.¹⁸ In 32 cases, DNA extracted from paraffin-embedded material was not suitable for further genetic analysis because of DNA degradation (DNA fragment size <1000 bp). Except for three cases (cases 5, 7, and 23), all of the specimens analyzed genetically stemmed from initial diagnosis of ETL. In three cases (cases 7, 25, and 27), material from disease relapse could be additionally analyzed.

In addition, for the purpose of comparison of genetic alterations, four cases of primary gastric and one case of primary colonic T-cell lymphoma were included into the study. In all of these cases, there was no clinical evidence of generalized lymphoma with manifestation in the stomach or colon. Based on clinical findings, the cases were considered as of primary gastric/colonic origin. Because the exact classification of the latter lymphomas and their relationship to enteropathy, in particular to ETL remains controversial, in the following, the term ETL will be limited to designate primary, enteropathy-associated T-cell lymphomas arising in the small intestine, whereas the five cases arising in the stomach or colon will be referred to by primary gastric and primary colonic T-cell lymphoma, not otherwise specified (NOS), respectively (extranodal peripheral T-cell lymphoma, not otherwise specified, according to the World Health Organization classification¹⁷).

CGH

CGH was performed at the Department of Pathology, University of Würzburg, according to a standard protocol.¹⁹ Tumor DNA was labeled by nick translation with biotin-16-dUTP (Roche Diagnostics, Mannheim, Germany). Reference DNA, extracted from placental tissue of a healthy newborn, was labeled with digoxigenin-11-dUTP (Roche Diagnostics). The amount of DNase I (Roche Diagnostics) and DNA polymerase I (Promega, Mannheim, Germany) were adjusted to obtain DNA fragment sizes of ~500 to 1000 bp. After inactivation of DNase I, unincorporated nucleotides were removed by gel filtration on Sephadex G50-packed columns (Sigma, Tarkirchen, Germany). Equal amounts of tumor and reference DNA (1 µg each), together with 70 µg of Cot-1 DNA (Roche Diagnostics) for blocking repetitive sequences, were co-hybridized on commercially available metaphase slides (Vysis, Downers Grove, IL), which had been denaturated in 70% deionized formamide/2× standard saline citrate (SSC)/50 mmol/L sodium phosphate, pH 7.0, for 5 minutes at 70°C, followed by dehydration in cold ethanol. After overnight hybridization at 37°C, the slides were washed four times with 50% formamide/2× SSC, pH 7.0, at 42°C and three times at 60°C with 0.1× SSC. Biotin- and digoxigenin-labeled DNA were detected by addition of fluorescein-isothiocyanate avidin (Vector Laboratories, Burlingame, CA) or Cy3-conjugated anti-digoxigenin antibodies (Dianova, Hamburg, Germany),

respectively. A 4,6-diamidino-2-phenylindole counterstain was performed for chromosomal banding.

Signals were visualized with a Zeiss Axiophot fluorescence microscope and analyzed with ISIS digital image analysis system (MetaSystems, Altlussheim, Germany). Ratio values of 1.25 and 0.8 were used as upper and lower thresholds for identification of chromosomal gains or losses. A high-level amplification was defined as an overrepresentation of genetic material with the fluorescence ratio values exceeding 2.0 or based on the observation of strong focal signals in the fluorescein-isothiocyanate fluorescence.

FISH

For further analysis of the most frequent genetic imbalance detected by CGH (gain of chromosomal region 9q33-q34), FISH was performed in four ETL cases, in three of them on cells isolated from the identical frozen tissue blocks that had been used for DNA extraction for CGH according to previously published protocols.²⁰ One case was only analyzed by FISH, but not by CGH. Briefly, 10 to 20 10-µm cryostat sections were minced and suspended in C50T (0.05 mol/L citric acid monohydrate and 0.5% Tween 20) for 10 minutes. After washing in phosphate-buffered saline, the pellet was resuspended and fixed in a -20°C Carnoy solution. For hybridization, the cell suspension was dropped onto glass slides and dried at room temperature.

Dual-color FISH experiments with fluorescence-dye labeled DNA probes were performed using a probe for chromosome 9q34 (locus-specific *abl* probe labeled with SpectrumOrange) together with a probe for 22q11 as a control (locus-specific *bcr* probe labeled with SpectrumGreen) according to the manufacturer's instructions (LSI *bcr/abl* ES Dual Color Translocation probe, Vysis, Stuttgart, Germany). Briefly, the slides were incubated for 1 to 3 minutes at 38°C in 10 µg/ml of pepsin (4500 U/mg; Sigma, Germany)/0.01 mol/L HCl and dehydrated with ethanol, followed by denaturation in 70% formamide/2× SSC at 73°C for 5 minutes. The hybridization mixture containing 1 µl of probe mixture, 2 µl of purified H₂O₂, and 7 µl of hybridization buffer was denaturated at 73°C for 5 minutes. The slides were incubated for 12 to 16 hours at 37°C. After hybridization, the slides were washed in 0.4× SSC/0.3% Nonidet P-40 at 73°C and in 2× SSC/0.1% Nonidet P-40 for 2 minutes each at room temperature. Counterstaining was performed with 4'6-diamidino-2-phenylindole. Signals were visualized with a Zeiss Axiophot fluorescence microscope (Zeiss, Jena, Germany). Images were captured using the ISIS imaging system (MetaSystems, Altlussheim, Germany).

For signal evaluation, nuclear signals from at least 100 cells were analyzed per case. Nuclear signals had to be observed after hybridization in >90% of cells. Only cells with clearly discernible nonoverlapping nuclei were counted. Signals should have the same intensity and split signals were counted as one signal. Cutoff levels for gains of 9q and 22q were determined for the probes at 8% (mean ± 3 SD).

Immunohistochemistry

Immunohistochemical analysis was performed in all cases on formalin-fixed paraffin-embedded tissue sections in the laboratory of one of the authors (AC) according to previously published protocols.⁴ Immunostains included markers CD2 (dilution 1:20; Novocastra, Newcastle, UK), CD3 (1:400; DAKO, Copenhagen, Denmark), CD4 (1:10; Novocastra), CD5 (1:20; Novocastra), CD7 (1:40; Novocastra), CD8 (1:30; DAKO), CD20 (1:200; DAKO); CD30 (1:80; DAKO), CD56 (1:200; Sanbio, Uden, The Netherlands), β F1 (1:10; T-Cell Sciences, Woburn, MA), EMA (1:100; DAKO), and TIA1 (1:800; Coulter, Hialeah, FL).

T-Cell Clonality Analysis

For molecular clonality studies, polymerase chain reaction for T-cell receptor rearrangement was performed using a mixture of specific and consensus primers for the T-cell receptor γ -chain.²¹ Aliquots of polymerase chain reaction products were mixed with size standard and formamide, denatured, and subjected to electrophoresis on a 373 DNA Sequencer (Perkin-Elmer, Weiterstadt, Germany). Data were automatically collected and analyzed using Genescan software as described in the manufacturer's manual.

Clinical Data

Medical records were reviewed for the patient's history, type and duration of symptoms at initial presentation, and type of treatment. Follow-up information was obtained via clinical records, attending physicians or autopsy files.

Statistical Analysis

Statistical correlation between immunophenotype and genetic alterations was performed using Fisher's exact test. Comparison of genetic alterations between ETL and gastrointestinal B-cell lymphoma was based on chi-square test. Survival analysis (impact of the number of genetic imbalances on survival) was performed using Kaplan-Meier statistics; for significance testing, a log rank test was used.

Results

Histological Characterization, Immunophenotyping, and TCR- γ Analysis of ETL

On histology, 41% cases of ETL were composed of monomorphic small- to medium-sized tumor cells, 26% of pleomorphic medium-sized to large cells, and 23% of anaplastic large cells (Table 1). Two cases were composed of immunoblasts and one case of pleomorphic small cells. Immunohistochemically, 87% of cases were CD3-positive (Table 1). All tumors analyzed were CD4-, CD5-, and CD20-negative, but CD7-positive (data not

shown). Seventy-six percent of cases analyzed expressed cytotoxic marker TIA-1. Sixteen ETLs (41%) expressed CD56, among them 14 cases with monomorphic small- to medium-sized tumor cell morphology. T-cell receptor γ -chain rearrangement studies were performed in 21 lymphomas, all of which exhibited a monoclonal rearrangement (data not shown).

CGH of ETL

Thirty-three of 38 ETLs (87%) showed gains and/or losses of genetic material (Table 2; Figure 1; Figure 2, a to d; Figure 3, a and b). In the entire group, gains of chromosomal material ($n = 88$) occurred slightly more frequently than losses ($n = 69$). Per case showing genetic imbalances, one to eight gains (median three gains) and one to seven losses (median two losses) were found.

The most frequent recurrent gain of genetic material was observed on chromosome 9q (22 of 38 cases, 58%), with 9q33-q34 representing the minimal overlapping region (Figure 2, b, d, and f; and Figure 3, a and b). Further recurrent gains were detected on 7q (9 cases, 24%); 5q (7 cases, 18%); 1q (6 cases, 16%); 6p, 12p, and 8q (4 cases each, 11%); 11q, 16p, and 17q (3 cases each, 8%). Minimal overlapping regions on 7q, 5q, 1q, and 6p were 7q21-q22, 1q21-q23, 1q41, 5q34-q35, and 6p22-pter, respectively.

The most frequent recurrent loss of genetic material occurred on 8p (9 of 38 cases, 24%) with 8cen-p21 representing the minimal overlapping region, and on 13q (9 of 38 cases, 24%). Further recurrent losses were observed on 9p (7 cases, 18%); 4q (5 cases, 13%); 7p, 11q14-q23 (4 cases, 11%); 3p12, 6q21-22, 10p12-p13, 16q, Xq, and 18q22-q23 (3 cases each, 8%). Minimal overlapping regions on 13q and 9p were 13q21 and 9p21, respectively.

In three cases, recurrent ETL could be compared genetically to primary ETL. In two cases, the recurrent ETL shared genetic alterations with the primary tumor (cases 7 and 27, period between initial disease presentation and relapse 7 and 8 months, respectively), whereas in case 25, recurrent ETL did not share genetic alterations with the primary tumor, suggesting a *de novo*, second ETL rather than lymphoma recurrence in this patient's relapse (period between initial disease presentation and relapse 3 years).

Multivariate statistical analysis did not show correlations between genetic alterations, the immunophenotype, and the lymphoma morphology.

FISH for Chromosomal Gain of 9q in ETL

To confirm the frequent gain of chromosomal material on 9q in ETL, four cases of ETL in which fresh material was available were studied by dual-color FISH, including one case in which only FISH was performed (case 33). A probe located in the minimal overlapping region 9q33-q34 (probe for *abl* locus at 9q34) was co-hybridized with a control probe for 22q11 (*bcr* locus) (Table 3, Figure 3, c and d).

FISH results for 22q11 revealed disomic signal distribution of tumor cells in three cases and a signal constel-

Table 1. Comparative Genomic Hybridization of ETLs: Clinical Data, Morphology, and Immunohistochemical Phenotype

Clinical data					Immunohistochemical phenotype						
No.	Age	Sex	Survival (months)	Stage	Morphology	CD3	CD4	CD8	CD30	CD56	TIA1
1	51	m	DOD 1	EII2	pleo m&l	+	—	—	—	—	+
2	53	f	DOD 19	EI	pleo m&l	+	—	—	—	—	+
3	50	f	NED 48+	ns	pleo m&l	+	—	—	—	—	—
4	65	m	DOD 6	EII	pleo m&l	+	—	+	+	—	—
5	64	f	DOD 12	EII	pleo m&l	+	—	—	+	—	+
6	63	m	NED 7+	ns	pleo m&l	+	—	—	+	—	+
7	29	m	AWD 60+	ns	pleo m&l	+	—	+	—	—	—
8	57	f	DOD1		pleo m&l	—	—	—	+	—	—
9	81	f	Lost		pleo m&l	+	—	+	+	—	+
10	61	f	DOD 2	EI	pleo m&l	+	—	+	—	+	+
11	59	f	DOD 9	EIII	Pleo sm	+	—	+	—	—	+
12	51	m	Lost	ns	ALCL	nd	nd	—	+	—	—
13	66	f	DOD 5	EIV	ALCL	—	—	—	+	—	—
14	71	m	DOD 1	EIV	ALCL	+	—	—	+	—	+
15	62	m	DOD 3	EIV	ALCL	+	—	+	+	—	+
16	45	m	DOD 3	EII	ALCL	+	—	+	+	—	+
17	65	f	DOD 15	Ns	ALCL	+	—	—	+	—	+
18	65	f	DOD 1	EIV	ALCL	+	—	—	+	—	—
19	53	f	DOD 1	EIV	ALCL	+	—	—	+	—	+
20	66	f	NED 29+	EI2	IB	+	—	+	+	—	+
21	77	m	DOD 1	ns	IB	+	—	—	+	—	+
22	55	m	DOD 2	EIIA	mono sm	+	—	+	—	+	+
23	70	m	NED 87+	EI	mono sm	+	—	+	—	+	+
24	51	m	DOD 1	EIV	mono sm	+	—	—	—	+	+
25	61	m	AWD 37+	EIIB	mono sm	+	—	—	—	+	+
26	39	m	DOD 5	EIV	mono sm	+	—	+	nd	+	+
27	63	m	DOD 16	EIIB	mono sm	+	—	+	—	+	+
28	85	m	DOD 4	ns	mono sm	+	—	+	—	+	+
29	61	m	DOD 5	EIV	mono sm	+	—	+	—	+	+
30	64	m	AWD 6	EI	mono sm	+	—	+	—	+	+
31	43	m	NED 5+	EI2	mono sm	—	—	—	—	+	+
32	77	m	Lost	ns	mono sm	+	—	+	—	+	nd
33	84	f	DOD 1	ns	mono sm	+	—	—	—	+	—
34	66	m	Lost		mono sm	+	—	—	—	+	+
35	56	m	Lost		mono sm	+	—	+	+	+	—
36	75	m	DOD 1	EIV	mono sm	—	—	—	—	—	+
37	68	f	DOD 1	EIIA	mono sm	+	—	—	—	—	+
38	68	f	Lost	ns	unclass.	+	—	—	+	+	+
39*	66	m	Lost		ALCL	+	—	—	+	—	nd

Abbreviations: age, age at diagnosis of ETL; m, male; f, female; DOD, died of disease; NED, alive with no evidence of disease; AWD, alive with disease; lost, lost to follow-up; ALCL, morphology of anaplastic large cell lymphoma; pleo m&l, lymphoma composed of pleomorphic with medium and large cells; ib, immunoblastic lymphoma; mono sm, monomorphic with small- to medium-sized cells; TCR- γ , T-cell receptor γ chain rearrangement; m, monoclonal; nd, not done; ns, not staged or stage unknown. Stage according to the Ann Arbor System as modified by Musshof.⁴⁵

*Only FISH hybridization performed.

lation indicating tetraploidy in one case. In none of the four cases that had been analyzed by CGH were gains of 22q observed. In accordance with CGH profiles indicating gains at 9q34, up to seven FISH signals were observed in the diploid cases (Table 3, Figure 3, case 23330/92 not analyzed by CGH). In the tetraploid case, up to nine signals were observed at 9q34 with the number of signals at 9q34 exceeding those with the probe for 22q11. In none of the cases was co-localization of signals of both probes observed.

Correlation between Genetic Imbalances in ETL and Prognosis

Clinical follow-up data could be obtained from 34 of the 44 patients (Table 1). The common disease presentation

of the 32 clinically followed patients with ETL (19 males, 13 females) was small bowel perforation or obstruction. Only three patients had a clinical history of celiac disease. Twenty-four patients had already died: thirteen patients had succumbed to peritonitis and/or septicemia because of bowel perforation at initial disease manifestation with a median survival time of less than 1 month, whereas 11 patients had died of recurrent or disseminated disease with a median survival time of 6 months after initial diagnosis. In contrast, eight patients were still alive, among them five surviving more than 24 months (cases 3, 7, 20, 23, 25). Three patients were still alive but had only been followed clinically for less than 7 months (cases 6, 30, and 31).

The number of genetic alterations detected in CGH was correlated with survival comparing the five patients

Table 2. Comparative Genomic Hybridization of ETL: Chromosomal Gains and Losses

No.	rev ish enh	rev ish dim
1	—	—
2	+7q22-q31, +9q33-qter	—9p21-pter
3	+7p, +7q32-qter	—9p21-pter
4	+1p36 (high-level)	—2q22-q23, —13q21-q22
5	+1p32-p34, +1q12-q25, +1q32-q42, +7p, +7cen-q22, +8q24, +9q33-q34, +12p	—4p14-pter, —4q22-q27, —8p, —16q
6	+1q12-q24, +12p, +12q23-qter, +15	—3p12, —4q26-q28
7	+9q22-qter	—
7R	+5, +6p, +9q31-qter	—9cen-q22, —14
8	ampl 7q22, +9q33-q34, +12p12-pter	—13q21-q22
9	+5q35-qter, +9q33-q34, +13cen-q13, +13q31-qter	—4q23-q24, —13q14-q21
10	+17q21-qter	—8p, —9p21, —10q, —13q21-qter
11	—	—
12	+1p36 (high-level), +9q34, +16p, +17q	—1p21-1p31, —3p12, —9p23-p24; —13q14-q22
13	—	—
14	—	—6q
15	+1cen-q24, +1q32-q42, +5q13-q15, +6p, +7q11-q32, +9q	—10p12-p14
16	—	—
17	+1q, +1p32-36, +2p21-pter, +5q35, +6p21-pter, +7q22-qter, +8q24, +9	—3q26-qter, —6q22-q25, —8p, —13q14-q22
18	+5, +7q35-qter, +18q	—13q, —16q12-q21, —8p22-pter, —X
19	+11q23-qter	—
20	+9q34	—18q22-qter
21	+1q, +5q32-qter, +9q32-qter	—4p15-pter, —10, —11q14-q22
22	—	—18
23	+9q, +14q31-32	—6q15-q21
24	+9q21-qter	—9p21-pter, —13q22-qter
25	+16p13	—
25R	+7q, +8q, +9q22-qter, +Xp21-pter	—8p
26	+6p23-pter, +7q32-q33, +9q, +11q13, +16p, +17, +21	—3cen-p14, —8p
27	+5q31-qter, +8q, +9q22-qter, +21, +X	—8p, —11q14-q21
27R	+3p21-pter, +7q21-qter, +8q, +9q22-qter, +X	—5q14-q31, —7p15-pter, —8p, —11q14-q21
28	+9q, +21, +22	—10cen-p14, —11q14-qter, —12p, —13q13-qter, —16q, —18p
29	+Xq	—7p, —8cen-p21
30	+9q	—
31	+9q22-qter, +11q15, +12q23-qter	—2q14-q24, —5q21-q22, —Xq, —Xcen-p11, —7p, —8p, —9p13-pter
32	+7q, +9q33-q34, +Y	—7p
33	+9q34, +11cen-q13, +12p, +22	—
34	+9q	—17p13
35	+8q, +9q, ampl9q33-q34, +Xq23-qter	—4cen-q21, —9p21-p22
36	+1q21-q22, +5q31-qter, +6p, +9q, +11q13	—4q26-q28, —12q21-q22, —18q22-qter
37	—	—7p, —8p, —11q21-q23, —Xq22-qter
38	—	—

Abbreviations: R, recurrent disease; rev ish enh, chromosomal gains detected by CGH; rev ish dim, chromosomal losses detected by CGH. Case 39 was only analyzed by FISH.

who survived more than 24 months to the 11 patients who died of lymphoma but not of peritonitic/septic complications of lymphoma-induced bowel perforation at disease manifestation. Among this albeit limited group of 16 patients, Kaplan-Meier survival analysis showed that patients with more than three genetic imbalances detected by CGH had a significantly worse outcome than those with three or less alterations ($P < 0.05$, log-rank test; Figure 4). No correlation was found between the number of chromosomal imbalances and patient survival when including patients dying of immediate peritonitic/septic complications because of bowel perforation at initial disease presentation. Whereas a significant correlation was found between patient survival and stage of disease (data not shown), no significant correlation between the number of chromosomal imbalances and stage of disease was observed.

Genetic Analysis of Gastric and Colonic T-Cell Lymphomas, NOS

For purpose of comparison, cases of gastric ($n = 4$) and colonic ($n = 1$) T-cell lymphoma, NOS, were analyzed by CGH and FISH (Table 4). Morphologically, three cases were classified as anaplastic large cell lymphoma, two as pleomorphic medium-sized to large cell lymphoma. Immunohistochemically, all were CD56-negative. Three patients were male; the mean age at diagnosis was 61 years. Two of the patients were lost to follow-up, and three died of local complications a few days after surgery. CGH showed recurrent gains on 9q in four cases, among them one with an amplicon located at 9q33-q34, on 1q and 7q (two cases), and recurrent losses on chromosome 18 (two cases) (Figure 2, e and f). FISH was

Figure 1. Genetic gains and losses in ETL. Summation profile of genetic gains and losses occurring in ETL ($n = 38$) as detected by CGH. **Left bar:** loss of chromosomal material; **right bar:** gain of chromosomal material; **thick bars:** high level amplifications.

performed for 9q34 on three of five cases in which fresh material was available. In all cases, gain of signal for 9q34 was observed. In one case (case 42), FISH analysis detected gain at 9q34, whereas the CGH profile showed a clear shift to a gain of genetic material, but did not trespass the upper threshold.

Genetic Imbalances in ETL Differ from Those in Gastrointestinal B-Cell Lymphoma

Genetic imbalances detected in the 38 ETLs were compared to those in 58 gastrointestinal diffuse large B-cell lymphomas with previously published CGH profiles.^{11,12} Chromosomal gains of 9q, 7q, and 5q and losses of 8p, 9p, and 11q occurred significantly more often in ETLs, whereas gains of 11q were significantly more frequent in gastrointestinal diffuse large B-cell lymphomas ($P < 0.05$, chi-square test).

Discussion

Modern lymphoma classification is strongly related to genetic alterations in neoplastic lymphocytes that define lymphoma as disease entities.¹⁷ Among the few T-cell lymphoma entities characterized genetically so far, chro-

mosomal imbalances exceed reciprocal translocations as characteristic genetic alterations.²²⁻²⁵ Yet, in contrast to B-cell lymphoma, genetic alterations associated with specific T-cell lymphoma entities are still unknown for many types of T-cell lymphomas, among them ETL, an extranodal T-cell non-Hodgkin's lymphoma derived from gastrointestinal intraepithelial T-lymphocytes. In this first systematic genetic study on ETL, CGH and FISH were applied to characterize genetic alterations in a large series of clinically and immunohistochemically well-characterized cases of ETL.

Gain of whole or parts of the long arm of chromosome 9 was the most frequent genetic imbalance in ETL, occurring in more than half of the cases, with 9q33-q34 being the minimal overlapping region. The terminal part of 9q harbors many genes potentially involved in lymphomagenesis, such as *Notch1/Tan1*, a transmembrane receptor mediating cell fate decisions in hematopoiesis, the cyclin-dependent kinase *CDK9*, the oncogenes *ABL1* and *VAV2*, and the homeobox gene *LHX2*. 9q34 is a locus involved in chromosomal translocations in T-lymphoblastic lymphoma, with *Notch1* among the rearranged genes.^{26,27} *LHX2*, a homeobox gene with regulatory function in adult hematopoiesis, is expressed in lymphoid malignancies, and aberrant expression of genes of the homeobox gene family has been implicated in lym-

Figure 2. CGH of ETL and primary gastric T-cell lymphoma, NOS: morphological-genetic correlation. **a:** Histology of a case of CD56⁺ ETL composed of monomorphic medium-sized tumor cells (case 31). CGH showed gains at chromosomes 9q, 11q, and 12q, and losses at 2q, 5q, 7p, 8p, 9p, and X (**b**). **c:** Histology of a CD56⁺ ETL with immunoblastic tumor cell morphology (case 21). CGH revealed gains of genetic material at chromosomes 1q, 5q, and 9q, and losses at 4p, 10, 11q (**d**). **e:** Histology of a case of primary gastric T-cell lymphoma, not otherwise specified (case 40). CGH detected gains of genetic material on chromosomes 1q, 5p, 6p, and 9q, and losses on X and 18 (**f**). Gains of 9q, with minimal overlapping region 9q33-34, were the most frequent alteration observed (**b**, **d**, **f**).

phomagenesis (eg, *HOX11* activation in T-lymphoblastic lymphoma).²⁸ Translocation-mediated transcriptional activation of tyrosine kinase gene *ABL1* is involved in the pathogenesis of chronic myeloid leukemia, but also of a proportion of adult B-lymphoblastic leukemia. However, the role of all of these genes in the pathogenesis of ma-

ture T-cell lymphomas, such as ETL, is unknown. Of note, Wright and colleagues¹⁴ reported abnormalities of chromosome 9 as a clonal alteration in T cells from a patient with adult-onset celiac disease, an early intraepithelial form of ETL. So far, gains of 9q have only occasionally been reported in cytogenetic and CGH studies of B- and

Figure 3. FISH of ETL: gain of 9q as a frequent and characteristic genetic alteration. **a:** CGH result for chromosome 9 in case 7. Digital image analysis (**b**) indicates a chromosomal gain of 9q22-qter (**bar** on right). FISH with probes for 9q34 (**c**, red signals) and 22q11 (**c**, green signals) confirms the chromosomal gain at 9q in this case of a pleomorphic medium to large cell ETL. **d:** FISH of a monomorphic ETL (case 36) using the same probes, again confirming chromosomal gains at 9q (red signals) detected by CGH.

T-cell non-Hodgkin's lymphomas. Offit and colleagues²⁷ report a case of an extranodal high-grade T-cell lymphoma (large cleaved cell, diffuse) with a gene amplification on 9q34 [hsr(9)(q34)]. In classical cytogenetic analysis of high-grade T-cell lymphomas and among the few entities of T-cell lymphoma investigated by CGH so far, gain of 9q was neither a typical nor a recurrent or frequent finding.^{25,29–33} Gains of 9q were observed in ETL irrespective of lymphoma morphology or immunophenotype. Furthermore, it was also present in gastric and colonic T-cell lymphoma, NOS, indicating that chromosomal gains of terminal parts of 9q are highly characteristic of T-cell lymphomas arising from the gastrointestinal mucosa-associated lymphoid tissue.

Figure 4. Impact of number of chromosomal imbalances on patient survival in ETL. Kaplan-Meier survival curve of patients dying of ETL not related to perforation-associated complications ($n = 11$) as compared to patients surviving more than 24 months ($n = 5$). Patients with more than three genetic imbalances detected by CGH had a significantly worse outcome than those with three or less alterations.

Recurrent chromosomal gains in ETL were also demonstrated on chromosomes 7q and 1q. In contrast to gains of 9q, gains of 7q and 1q are among the most frequent genetic alterations detected both in classical cytogenetic studies and in CGH analysis of different types of T- and B-cell lymphomas.^{25,30,31,33} Similar to other lymphomas, the minimal overlapping region on 7q in ETL was 7q21-q22, harboring *CDK6* and *ASK/DBF4*, genes involved in cell cycle control, *TRRAP*, a transcriptional co-activator, and the multidrug resistance gene *MDR1*.^{30,31} Gains on chromosome 1q in ETL covered large chromosomal regions and occurred at similar frequency as reported previously by CGH for natural killer cell lymphoma/leukemia and adult T-cell leukemia/lymphoma.^{25,33} Thus, in contrast to chromosomal gains of 9q, gains of 1q and 7q are frequent and recurrent, but less specific genetic alterations in a broad spectrum of T-cell lymphomas, including ETL.

The most frequent recurrent losses of genetic material in ETL occurred on chromosomes 8p, 13q, and 9p. Losses of 8p were found in 24% of cases of ETL. 8p harbors the genes encoding proapoptotic TRAIL recep-

Table 3. ETL: Comparison of Data Obtained by CGH and FISH with Probes for 9q34

Case no.	CGH rev ish enh	CGH rev ish dim	FISH 9q34 Number of signals (percentage of cells)	FISH 22q11 Number of signals (percentage of cells)
7	+9q22-qter	–	7 (18%) 9 (14%) 4 (42%)	3 (18%) 4 (14%) 2 (100%)
13	+1q, +1p32-36, +2p21-pter, +5q35, +6p21-pter, +7q22-qter, +8q24, +9	–3q26-qter, –6q22-q25, –8p, –13q14-q22	5 (4%) 6 (23%) 7 (4%) 3 (16%)	2 (100%)
36	+1q21-q22, +5q31-qter, +6p, +9q, +11q13	–4q26-q28, –12q21-q22, –18q22-qter	4 (41%) 3 (11%) 4 (7%)	2 (100%)
39	nd	nd		

Table 4. Histology and Genetics of Gastric and Colonic T-Cell Lymphoma, Not Otherwise Specified

No.	Patient	Histology	CGH rev ish enh	CGH rev ish dim	FISH 9q34 Number of signals (percentage of cells)	FISH 22q11 Number of signals (percentage of cells)
40*	76, f, no follow-up	pleo m&l	+1cen-q23, +5p, +6p25, +9q31-q34, amp19q34	-Xp, -Xcen-q21, -18q	6 (8%) 7 (37%)	3 (8%) 4 (37%)
41*	85, m, DOD 2	CD3+, CD4+, CD8- CD30+, CD56-, TIA1+ pleo m&l	+1q24-qter, +3p25-pter, +5q33-qter, +7p, +7cen- q31, +9q, +19	-3p12, -10p11, -15q25-q26	nd	nd
42*	62, m, lost	CD3+, CD4-, CD8+, ALCL CD3+, CD4-, CD8+, CD30+, CD56-, TIA1+	- (borderline gain of 9q, below upper threshold in CGH)	-18	3 (9%) 4 (19%) 5 (16%) 6 (34%)	2 (45%) 3 (42%)
43*	41, f, DOD 1	ALCL CD3+, CD4-, CD8- CD30+, CD56-, TIA1+	+2cen-q14, +3q27-qter +6p22-pter, +7q22-qter, +9q +11q23-qter, +12cen- p11, +12cen-q13	-	3 (5%) 4 (22%)	2 (100%)
44†	60, m, lost	ALCL CD3-, CD4-, CD8-, CD30+, CD56-, TIA1+	+9q, +14q22-qter	-4p, -4cen-q26, -6cen-q25, -13q14-q22	nd	nd

Abbreviations: m, male; f, female; DOD, died of disease; lost, lost to follow-up; ALCL, morphology of anaplastic large cell lymphoma; pleo m&l, lymphoma composed of pleomorphic with medium and large cells.

*Lymphomas with primary gastric location.

†Lymphoma with primary colonic location.

tors death receptor 4 (*DR4/TNFRSF10A/TRAILR1*) and death receptor 5 (*DR5/TNFRSF10B/TRAILR2*), located at 8p21, the minimal overlapping region. TRAIL-DR4/DR5 interactions are involved in regulating cell suicide and tissue homeostasis and have been implicated in activation-induced cell death of T lymphocytes.³⁴⁻³⁶ Because ETL is frequently accompanied or preceded by gluten-sensitive enteropathy and thus by chronic T-cell stimulation and activation,³⁷ loss of regulatory circuits preventing excessive intraepithelial T-cell activation after antigen challenge might contribute to autonomous T-cell proliferation and the development of ETL. In active celiac disease, decreased apoptosis of intraepithelial lymphocytes has been observed and hypothesized to play a role in lymphoma development in celiac disease.³⁸ In contrast, apoptosis of intraepithelial T cells maintains T-lymphocyte homeostasis in the normal intestine.³⁹ Losses of 8p have been observed with high frequency in CGH and cytogenetic studies of T-PLL, but not in cytogenetic analysis of other T-cell lymphomas.³⁰⁻³²

Chromosomal regions 9p (harboring p15^{INK4a}/p16^{INK4b}) and 13q, where losses of chromosomal material were found in 18% and 24% of ETL, respectively, are deleted in various types of T- and B-cell lymphomas.^{30-33,40-43} In contrast, notably, only 8% of ETL showed loss of genetic material on 6q, contrasting cytogenetic reports on losses of 6q in up to one third of cases of high-grade T-cell lymphoma.^{30,31}

The overall pattern of genetic imbalances detected in ETL allows for several conclusions. The comparison of

genetic imbalances in ETL to those of the few other T-cell lymphoma entities characterized genetically so far indicates distinct pathways in T-cell lymphomagenesis. Genetic alterations shared among different types of T-cell lymphomas (such as gains of 1q and 7q and losses of 13q) may abrogate the function of genetic loci controlling basic regulatory mechanisms of T-lymphocyte proliferation or may be acquired during lymphoma progression, conferring growth advantage to subclones of neoplastic T cells. In contrast, entity-characteristic genetic alterations (such as gain at 9q in ETL) may overcome growth regulation mechanisms in specific T-cell subpopulations (such as in intraepithelial T lymphocytes) giving rise to distinct T-cell lymphoma entities (such as ETL).

Furthermore, B- and T-cell lymphomagenesis in the same anatomical location, namely the gastrointestinal tract, clearly follow different genetic pathways. The comparison of genetic imbalances in ETL to those of primary gastrointestinal diffuse large B-cell lymphoma (DLBL) previously published^{11,12} revealed significant genetic differences between both types of lymphoma.

Clinically, ETLs follow a dismal clinical course with most patients dying within a few months after the diagnosis; however, long-term survivors are reported.^{2,4} Similar to other types of lymphoma, death of ETL can be because of lymphoma recurrence, dissemination and/or progression, however, in contrast to other types of lymphoma, a substantial proportion of patients with ETL die of septic or peritonitic complications because of bowel perforation at initial disease manifestation. Based only on

the 16 patients with clinical follow-up available who survived the initial disease manifestation, we found a significant correlation between the total number of chromosomal gains and losses and patient survival. These findings (in an albeit small group of only 16 patients) suggest that the number of chromosomal imbalances in ETL may be a stage-independent risk factor of patient survival in ETL in those patients who do not succumb to the immediate consequences of bowel perforation at initial disease manifestation.

The precise classification of the very rare cases of T-cell lymphomas arising in the stomach and colon is controversial, in particular, the relationship of these lymphomas to ETL is unknown.^{1,44} Although only five cases of primary gastric and colonic T-cell lymphoma, NOS, could be analyzed in this study, the genetic profiles are suggestive of similar oncogenetic pathways and a genetic relationship of these lymphomas to ETL.⁴⁴ All of the cases analyzed showed chromosomal gain of 9q, characteristic of ETL. In addition, two cases displayed gains at 1q, 7q, and 6p along with loss of 18q that were among the more frequent genetic imbalances detected in ETL.

In summary, in this first systematical CGH and FISH analysis of ETL, gains of terminal parts of 9q (minimal overlapping region 9q33-q34) were found to be characteristic of ETL, potentially harboring key genes involved in the pathogenesis of this T-cell lymphoma entity. Among 16 patients with ETL who survived initial disease presentation, patients with more than three chromosomal gains or losses ($n = 11$) followed a worse clinical course than those with three or less imbalances ($n = 5$). Comparison of the genetic alterations in 38 cases of ETL to 5 cases of primary gastric and colonic T-cell lymphoma, NOS, suggests that primary T-cell lymphoma of the gastrointestinal tract may be genetically be related to each other. They are, however, genetically distinct from gastrointestinal B-cell lymphoma.

Acknowledgments

We thank all referring pathologists providing the tumor samples examined in the presented series and Erwin Schmitt for excellent photographic work.

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