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Whole-Genome Analysis and HLA Genotyping of Enteropathy-Type T-Cell Lymphoma Reveals 2 Distinct Lymphoma Subtypes

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Background & Aims: **Enteropathy-type T-cell lymphoma (ETL) is an aggressive extranodal T-cell non-Hodgkin lymphoma assumed to arise in the setting of celiac disease.** ***Methods:*** **To precisely define the genetic alterations underlying the pathogenesis of ETL, 30 ETL samples were profiled for genetic copy number alterations using high-resolution whole-genome tiling path array comparative genomic hybridization. To investigate the potential association of genetic alterations in ETL with celiac disease, HLA-DQB1 genotyping was performed.** ***Results:*** **By array comparative genomic hybridization, 13 novel recurrent minimal regions of chromosomal alteration were identified on multiple chromosome arms. ETL is characterized by frequent complex gains of 9q31.3-qter (70% of cases), or by an almost mutually exclusive 2.5-megabase loss of 16q12.1 (23% of cases). Two distinct groups of ETL could be delineated morphologically and genetically: type 1 ETL is characterized by nonmonomorphic cytomorphology, CD56 negativity, and chromosomal gains of 1q and 5q. Type 1 ETL also appears to be linked pathogenetically to celiac disease, sharing genetic alterations and HLA-DQB1 genotype patterns with (refractory) celiac disease. Type 2 ETL shows monomorphic small- to medium-sized tumor cell morphology, frequently shows CD56 expression, *MYC* oncogene locus gain, and rare gains of chromosomes 1q and 5q. In contrast to type 1 ETL, type 2 ETL shows a HLA-DQB1 genotype pattern more resembling that of the normal Caucasian population.** ***Conclusions:*** **Contrary to current clinical classification, ETL comprises 2 morphologically, clinically, and genetically distinct lymphoma entities. In addition, type 2 ETL may not be associated with celiac disease.**

Enteropathy-type T-cell lymphoma (ETL) is a primary extranodal T-cell non-Hodgkin lymphoma arising in the gastrointestinal tract that shows a differentiation of tumor cells toward the phenotype of intestinal intraepithelial T cells. ETL tends to affect multiple segments of the small intestine, most notably the jejunum, leading to intestinal ulcerations and perforations.[1] The clinical course of ETL is highly aggressive, with most patients dying from the disease within months of diagnosis.[2]

ETL shows a wide range of histologic and immunophenotypical features. Approximately 80%–90% of ETLs (subsequently referred to as *nonmonomorphic* or *type 1 ETL*) are composed of pleomorphic, anaplastic, or immunoblastic tumor cells that usually have a $CD3^+CD4^-CD8^-CD7^+CD5^-CD56^-$ immunophenotype, a phenotype akin to that of the majority of normal intestinal intraepithelial T-cell receptor$\alpha/\beta+$ T lymphocytes. About a third of these ETLs are associated with a previous clinical history of celiac disease. In 85% of these tumors there is histologic evidence of enteropathy-like alterations in the gastrointestinal mucosa adjacent to the invasive tumor. In contrast, about 10%–20% of ETLs (subsequently referred to as *monomorphic* or *type 2 ETL*) are characterized by frequent expression of CD8 and CD56, and are composed commonly of monomorphic small- to medium-sized tumor cells. These likely are derived from activated $CD8^+CD56^+$ intraepithelial T lymphocytes, which make up around 15% of intraepithelial T-cell receptor$\alpha/\beta+$ T lymphocytes. This subtype appears to be associated rarely with a previous clinical history of celiac disease. In addition, only 50% of tumors show histologic evidence of enteropathy adjacent to the invasive neoplasm.[3]

We previously showed by conventional comparative genomic hybridization (CGH) that ETL is characterized by recurrent gains of 1q, 5q, 7q, and 9q, and recurrent losses of 8p, 9p, and 13q, with gains of 9q33-q34 being the most frequent alteration, occurring in 58% of cases analyzed.[4] Similar regions of allelic imbalance were identified by Baumgartner et al,[5] using a microsatellite marker study of 26 loci. In their study, monomorphic ETL was associated with a lower level of microsatellite

Abbreviations used in this paper: **BAC, bacterial artificial chromosome; ETL, enteropathy-type T-cell lymphoma; CGH, comparative genomic hybridization; Mb, megabase; ROA, regions of alteration; SSP, sequence specific primer.**

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instability, 3q27 allelic imbalances, and a trend toward lack of allelic imbalances at several loci.

We therefore hypothesized that among ETL, genetically distinct tumor subtypes may be distinguishable. Thus, alternate pathogenetic pathways may be responsible for the different subtypes of ETL. To test this hypothesis we used whole-genome tiling-path array CGH to precisely delineate genetic alterations in a series of 30 histologically and clinically well-characterized ETL cases. Furthermore, to test the potential association of the tumors with celiac disease, HLA-DQB1 genotyping was performed. Because 95% of celiac disease patients carry HLA-DQ2 and most individuals who are not HLA-DQ2 positive express HLA-DQ8, celiac disease is extremely rare in patients not carrying either HLA-DQ molecule.[6,7] In addition, HLA-DQ homozygosity confers an increased susceptibility for celiac disease.[8,9] Therefore, although the presence of HLA-DQ2/-DQ8 cannot be used as a positive indicator of celiac disease, their absence can be used as a negative surrogate marker for celiac disease. Here, we show that ETL comprises 2 morphologically, clinically, and genetically distinct lymphoma entities, one of which, despite its current name, may not be associated with celiac disease in a substantial proportion of cases.

Materials and Methods

Morphologic and Clinical Features of ETL Cases

Thirty cases of ETL were selected from the archive of the Lymph Node Reference Center at the Department of Pathology, University of Würzburg, Germany, and from the archive of the Department of Pathology, University of Vienna, Austria. All cases had been classified as ETL based on the criteria defined by the World Health Organization classification of tumors of hematopoietic and lymphoid tissues.[10] All cases were reviewed before inclusion in the study. Before the array CGH study, 18 of the 30 ETL cases had been analyzed by conventional CGH.[4]

Morphologically, 15 ETLs were classified as monomorphic small- to medium-sized type (type 2 ETL), and 15 as type 1 ETL, composed of 4 anaplastic large-cell type, 2 immunoblastic type, and 8 pleomorphic medium- to large-cell type, whereas 1 case was unclassifiable (tumorous ascites) according to the criteria previously provided by one of the authors (A.C.).[11]

Clinical follow-up information was available for all patients (Table 1). In brief, there were 19 male and 11 female patients, with the patients' ages ranging from 39 to 85 years (average, 63 y). Twenty-seven of the 30 patients already had died from the disease, with survival time ranging from 1 to 25 months after the initial diagnosis (median survival time, 3 mo). Because the specimens stemmed from a period of 20 years, very limited reliable information on pre-existing celiac disease was available. Therefore, typing for HLA-DQB1 was used as a surrogate marker for potential celiac disease association.

Immunophenotype

Immunohistochemical analysis was performed for all cases on formalin-fixed paraffin-embedded tissue sections according to previously published protocols[12] (Table 1). Immunostains included markers CD2 (Novocastra, Newcastle, UK; dilution 1:20), CD3 (Dako, Copenhagen, Denmark; 1:400), CD4 (Novocastra; 1:10), CD8 (Dako; 1:30), CD30 (Dako; 1:80), CD56 (Sanbio, Uden, Netherlands; 1:200), and TIA1 (Coulter; Hialeah, FL; 1:800).

DNA Extraction

In all cases, tumor tissue sections were screened for tumor cell content and only cases that contained greater than 60% neoplastic cells were included in this study. DNA was extracted as previously described.[13] In 28 cases, DNA was extracted from formalin-fixed, paraffin-embedded tissue, whereas in 2 cases fresh material was available (cases 15 and 30). In all but 1 case, the material stemmed from the initial surgical resection specimen. In the 1 case, tumorous ascites from a patient with known ETL was used for DNA extraction (case 30).

BAC Array CGH

Bacterial artificial chromosome (BAC) array CGH was conducted as previously described.[14–17] Briefly, for BAC array CGH, 300 ng of sample or pooled reference male DNA (Novagen, Mississauga, Ontario) was labeled with cyanine-3 deoxycytidine triphosphate or cyanine-5 deoxycytidine triphosphate (Perkin Elmer Life Sciences Inc., Boston, MA), respectively. Labeled probe was purified, precipitated with C_ot-1 DNA (Invitrogen, Burlington, Ontario), and resuspended in hybridization buffer.[14–17] Probe mixtures then were denatured, allowed to block at 45°C, and applied to tiling-path arrays composed of 26,819 duplicate spotted BAC clones (53,638 elements) selected from the previously described submegabase resolution tiling set to give optimal genome coverage (available at: http://www.bccrc.ca/arraycgh/).[17–19] These arrays were batch quality-controlled by hybridization and analysis of cell-line material with known alterations. The arrays were hybridized at 45°C for approximately 40 hours, washed with 0.1× standard saline citrate/0.1% sodium dodecyl sulfate, and rinsed with 0.1× standard saline citrate.

Imaging and Analysis

Imaging and analysis were conducted as previously described.[14–17] Briefly, a charged-couple device camera system was used to capture the cyanine-3 and cyanine-5 channels of hybridized arrays (Applied Precision, Issaquah, WA). The images then were analyzed using SoftWoRx analysis software (Applied Precision). The resultant data were normalized using a stepwise normalization algorithm.[20] Custom software *SeeGH* was used to visualize all data.[21]

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