Mutations in the JAK/STAT and RAS signaling pathways are common in intestinal T-cell lymphomas

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Primary intestinal T-cell lymphomas (ITCLs) comprise mainly the enteropathy-associated T-cell lymphomas (EATLs). EATL is an aggressive, rare lymphoma, which represents ~5% of mature T-cell lymphomas.1 Two subtypes are recognized based on distinct morphology, immunophenotype and epidemiology. EATL type I (EATL I) is more common in Western countries, is highly associated with celiac disease (CD) and shows a phenotype akin to that of the majority normal TCRAβ+ intraepithelial lymphocytes (IEL). EATL type II (EATL II) is more frequent in Asia, is uncommon in patients with CD and is usually derived from TCRAγδ+ IELs.2,3 Both are CD3 positive and express cytotoxic markers, but though EATL I is typically CD8 and CD56 negative, EATL II is generally CD8 and CD56 positive.

The mechanisms and genetic aberrations responsible for malignant transformation are largely unknown, owing to the rarity of these lymphomas. Comparative genomic hybridization microarray studies show multiple genomic imbalances, with common gains on chromosome 1q and 5q in EATL I, gains of 8q24 in EATL II and a high prevalence of 9q gains/16q losses in both subtypes.4,5 Until recently, there were few genetic/genomic studies of these lymphomas with the exceptions of a study of natural killer (NK)/T and γδ T-cell lymphomas that included cases of γδ EATL II,6 and a second more comprehensive study of EATL II.8 Both groups reported a high incidence of STAT5B mutations in EATL II, whereas the second group also identified frequent mutations of JAK3 and the α G-protein subunit GNAI2, as well as some less common mutations. To further understand the molecular pathogenesis of these rare lymphomas, we analyzed our own series of primary ITCL, which included EATL I, EATL II and peripheral T-cell lymphoma not otherwise specified (PTCL-NOS), by targeted next generation sequencing (NGS) of genes associated with T-cell neoplasia and proliferation.

A total of 34 ITCLs with formalin-fixed paraffin-embedded tissue were retrieved from the consultation files of the Hematopathology Section of the National Cancer Institute under an institutional review board approved protocol. All cases were reviewed by four co-authors (ESJ, SP, MR, AN) and a consensus diagnosis was reached. Cases were classified as EATL I (10), EATL II (20) and PTCL-NOS (4), and were further subdivided as CD8, γδ, silent or indeterminate, according to their expression of CD8, CD56, CD123, CD45RO (clone 8A3, ThermoFisher Scientific, Rockford, IL, USA) or TCRγδ (clone γ3.20 ThermoFisher Scientific) (Supplementary Table S1). Cases were diagnosed as PTCL-NOS if they did not meet the morphological and/or immunophenotypical WHO criteria for EATL type I or EATL type II, but had confirmed involvement of the intestine. Such cases typically lacked the mucosal involvement and epitheliotropism of EATL. These criteria were proposed by a recent workshop on Peripheral T-cell and NK-cell lymphomas.7 A targeted NGS strategy was used to analyze extracted tumor DNA for somatic mutations in 38 genes. These included genes previously reported to be mutated in T-cell lymphomas, components of the JAK/STAT pathway and selected genes involved in T-cell receptor signaling and proliferation. In all, 31 and 33 samples were tested for mutations within JAK1 (codon 1097) and GNAI2 (codons 179 and 182) by targeted pyrosequencing, respectively, as these recently described mutational hotspots were not covered in the NGS panel.8 Further details of the pyrosequencing and NGS methods, and the list of genes analyzed are included in the Supplementary Methods and Supplementary Table S2.

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A total of 49 mutations were identified in the 34 ITCL cases, including 46 nonsynonymous single-nucleotide variants and 3 deletions. All mutations were predicted to be deleterious based on the results of computational algorithms PolyPhen-2 and SIFT, and/or available literature. A total of 82.4% of cases showed ≥1 mutation with only 6 samples (17.6% (2 γδ and 2 silent EATL I, 1 αβ EATL II and 1 silent PTCL-NOS)) showing no mutations. The most common alterations involved members of JAK/STAT pathway found in 67.6% of cases, followed by RAS pathway gene alterations in 24.2% of cases. Less common mutations included TET2 (12.1%), EZH2, FYN, NOTCH1 and CD247 (3% each) (Figure 1a). Other mutations previously reported in T-cell lymphoma subtypes or in other JAK/STAT pathway genes, including IDH2, DNMT3A, RHOA, GNB1, PLCG1, CCR4, JAK2, IL7R and CD247 (IL6ST), were not detected.

GNAI2 mutations were not detected in 33 cases studied, including 20 EATL II cases. Within the JAK/STAT cascade, STAT5B and JAK3 were the most frequently mutated genes present in 26.5% and 27.3% of cases, respectively. These were followed by JAK1 (14.7%), STAT3 (12.1%), TYK2 and SOCS1 (3% each). STAT5B and STAT3 mutations were mutually exclusive, as were STAT3, and JAK1 or JAK3. In contrast, 4/9 STAT5B mutated cases showed additional mutations of the JAK3 gene. The STAT5B mutations all occurred at same hotspot, N642H; the STAT3 mutations were S614R (2), E616G (1) and D661Y (1). The two most common JAK3 variants were M511I (4) and A573V (4), followed by A572V (1), R657Q (1) and V674F (1). JAK3 M511I co-existed with A573V in one case, and with V674F in a second case. For JAK1, G1097D (2), G1097S (1), S703I (1) and S729C (1) alterations were identified (Figure 1b). Mutations in RAS pathway genes involved KRAS (12.1%), NRAS (6.1%) and BRAF (6.1%). KRAS mutations included G12A (2), G13D (1) and Q61H (1); NRAS mutations were G12R (1) and Q61K (1). The two BRAF mutations were the common V600E variant. Interestingly, six of the eight RAS pathway mutated cases had an accompanying mutation in the JAK/STAT pathway (STAT5B (3), JAK3 (2) and JAK1 (2)).

JAK/STAT pathway mutations were found in all ITCL subtypes, regardless of αβ and γδ origin. These were present in 50% of EATL I (5/10), 80% of EATL II (16/20) and 50% (2/4) of PTCL-NOS cases. JAK/STAT pathway mutations were found in 77.7% of ITCLs expressing αβ (7/9), 71.4% expressing γδ (10/14) and 50% of silent cases (5/10). STAT5B and JAK3 mutations were primarily seen in EATL II as compared with EATL I (7/20 vs 1/10, and 8/19 vs 1/10, respectively; P = 0.20, Fisher’s exact test), and it will be of interest to see if this difference becomes statistically significant in a larger study group. RAS pathway mutations were also detected in both EATL I and EATL II cases, irrespective of cell origin.

Figure 1. (a) Summary of all mutations by ITCL subtype (EATL type I, EATL type II, PTCL-NOS). Genes containing mutations are listed in the first 14 rows. The final two rows are summary data of mutations involving either the JAK/STAT or the RAS/RAF signaling pathway. (b) Location of STAT3, STAT5B, JAK1 and JAK3 mutations in ITCL cases. (c) γ-cytokine signaling pathway showing JAK/STAT, and associated signaling pathways. Members of the pathway analyzed for mutations are colored gold, those not analyzed are colored blue and those with mutations are identified with a lightning symbol.
These mutations occurred in 20% of EATL I (2/10, 1 γδ and 1 silent), 31.6% of EATL II (6/19; 2 αβ, 3 γδ and 1 silent) and in none of the 4 PTCL-NOS cases.

The occurrence of such a high frequency of JAK1/3, STAT3/5 mutations and of RAS/RAF mutations might suggest that ITCL arises, in part, through subversion of cytokine signaling pathways, which are critical for the development and homeostasis of normal αβ and γδ intestinal T-cells. The γ-cytokine receptors, in particular, utilize both JAK3 and JAK1, and most frequently signal through activation of STAT5B or STAT3. Engagement of these receptors, not only activates the JAK/STAT pathway directly, but also leads to activation of the PI3K/AKT and RAS/RAF/MAPK pathways (Figure 1c).

Interestingly, 44.4% of the mutations in two genes of the JAK/STAT pathway has recently been described in systemic ALCL, ALK negative, where the presence of both JAK1 and STAT3 mutations resulted in hyperactivated STAT3 with sustained cell transformation. Similarly, it is possible that the unexpected co-occurrence of JAK3 mutations. Co-existence of mutations in two genes of the JAK/STAT pathway has recently been described in systemic ALCL, ALK negative, where the presence of both JAK1 and STAT3 mutations resulted in hyperactivated STAT3 with sustained cell transformation. Similarly, it is possible that the unexpected co-occurrence of JAK3 mutations. Co-existence of mutations in two genes of the JAK/STAT pathway has recently been described in systemic ALCL, ALK negative, where the presence of both JAK1 and STAT3 mutations resulted in hyperactivated STAT3 with sustained cell transformation. Similarly, it is possible that the unexpected co-occurrence of JAK3 mutations. 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Co-existence of mutations in two genes of the JAK/STAT pathway has recently been described in systemic ALCL, ALK negative, where the presence of both JAK1 and STAT3 mutations resulted in hyperactivated STAT3 with sustained cell transformation. Similar to the study by Nairismägi et al., we confirm the presence of a high incidence of JAK/STAT pathway mutations in EATL II (76 and 80%, respectively). In contrast to the 24% prevalence of GNAI2 mutations in that report, we did not identify any GNAI2 mutations in our 20 cases; rather we found a high percentage of RAS/RAF pathway mutations (31.6%), previously not reported in EATL II. Whether this is a statistical aberration, or reflects differences in the study group compositions is unclear at this time.

Our study further extends the investigation of ITCL to EATL I and PTCL-NOS, and suggests that these lymphomas also have a high incidence of JAK/STAT and RAS/RAF pathway mutations, although the predominant JAK/STAT mutations in EATL I appears to be JAK1 and STAT3, rather than JAK3 and STAT5B.

Identification of both JAK/STAT mutations and RAS pathway mutations is the first step toward understanding ITCL pathogenesis, and in developing targeted therapies for these aggressive lymphomas. New drugs targeting both JAKs and STATs are in clinical trials, and these may be worth considering for patients with ITCL. Although there are no effective drugs targeting RAS-driven cancers, there are major efforts underway in RAS-mediated tumorigenesis, and it is anticipated that these new initiatives and approaches will lead to effective treatments in the future.

Supplementary Information accompanies this paper on the Leukemia website (http://www.nature.com/leu)