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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 (ITCL) comprise mainly the enteropathy-associated T-cell lymphomas (EATL). EATL is an aggressive, rare, lymphoma, which represents approximately 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 TCRαβ$^+$ intraepithelial lymphocytes (IEL). EATL type II (EATL II), is more frequent in Asia, is uncommon in patients with CD, and is usually derived from TCRγδ$^+$ IELs [2, 3]. Both are CD3 positive and express cytotoxic markers, but while 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, due to the rarity of these lymphomas. CGH 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 [3, 4]. Until recently there were few genetic/genomic studies of these lymphomas, with the exceptions of a study of NK/T and γδ T-cell lymphomas that included cases of γδ EATL II [5], and a second more comprehensive study of EATL II.[6] Both groups reported a high incidence of *STAT5B* mutations in EATL II, while 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 PTCL-NOS, by targeted next generation sequencing (NGS) of genes associated with T-cell neoplasia and proliferation.

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Thirty-four ITCL with formalin-fixed paraffin-embedded tissue were retrieved from the consultation files of the Hematopathology Section of the National Cancer Institute under an IRB approved protocol. All cases were reviewed by four co-authors (EJ, 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 αβ, γδ, silent or indeterminate, according to their expression of βF1 (clone 8A3, ThermoFisher Scientific Rockford, IL) or TCRγ (clone γ 3.20 ThermoFisher) (supplemental 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. Thirty-one and thirty-three samples, respectively, were also tested for mutations within *JAK1* codon 1097, and *GNAI2* codons 179 and 182 by targeted pyrosequencing, as these recently described mutational hotspots were not covered in the NGS panel [6, 8]. Further details of the pyrosequencing and NGS methods, and the list of genes analyzed are included in the supplemental methods and supplemental Table S2.

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 computational algorithms PolyPhen-2 and SIFT, and/or available literature. 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 *CD130 (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 coexisted 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 2 *BRAF* mutations were

the common V600E variant. Interestingly, 6 of the 8 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 ITCL 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 to 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. These mutations occurred in 20% of EATL I (2/10, 1 γδ and 1 silent), 31.6% of EATL II (6/19; 2 αβ, and 3 γδ, 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 [9]. 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 *STAT5B* mutated cases showed an unexpected co-occurrence of *JAK3* mutations. Coexistence 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 [8]. Similarly, it is possible that the *STAT5B/JAK3* double mutants act synergistically by direct augmentation of STAT5B activity, or alternatively the *JAK3* mutations may be involved in activating additional STAT proteins, such as STAT3. In addition, a high percentage of cases showed co-occuring mutations in both the JAK/STAT and RAS pathway. As RAS is involved in cytokine pathway signaling, mutations of this branch of the pathway may further augment lymphomagenesis.

Alterations of the JAK/STAT and RAS signaling molecules were seen across all ITCL, independent of lymphoma subtype, or origin from αβ or γδ-T-cell. This may reflect derivation from cells that share similarities in their immune response and dependency on cytokine signaling, where the JAK/STAT and downstream RAS pathways play critical roles in signal transduction. Likewise a dependency on cytokine signaling may also explain the high frequency of mutations reported in *STAT5B* in hepatosplenic γδ T-cell lymphoma, [5, 10], *STAT3* in T-cell large granular lymphocytic leukemia and NK lymphoproliferative disorders [11, 12] and *JAK3* in NK/T cell lymphomas [13], all lymphoproliferations that utilize these signaling pathways.

Similar to the Nairismagi study,[6] 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 *GNA12* mutations in that report, we did not identify any *GNA12* 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 a 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. While 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. Meanwhile, BRAF and/or MEK inhibitors may be worth considering for some patients.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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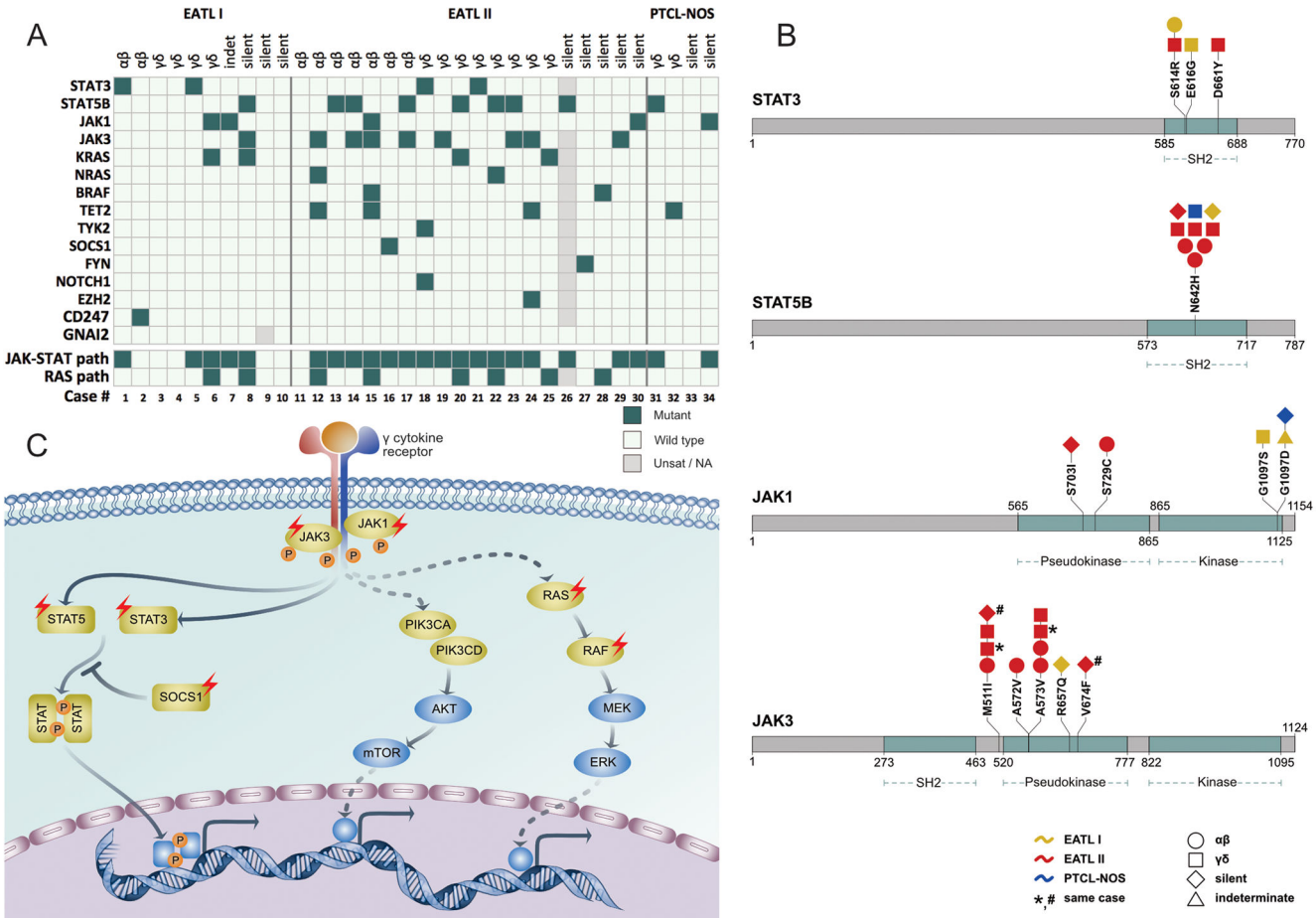

Fig. 1.

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 wither the JAK/STAT signaling pathway 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 the lightening symbol.