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Identification of novel *STAT5B* mutations and characterization of TCRβ signatures in CD4+ T-cell large granular lymphocyte leukemia

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CD4+ T-cell large granular lymphocyte leukemia (T-LGLL) is a rare subtype of T-LGLL with unknown etiology. In this study, we molecularly characterized a cohort of patients ($n = 35$) by studying their T-cell receptor (TCR) repertoire and the presence of somatic *STAT5B* mutations. In addition to the previously described gain-of-function mutations (N642H, Y665F, Q706L, S715F), we discovered six novel *STAT5B* mutations (Q220H, E433K, T628S, P658R, P702A, and V712E). Multiple *STAT5B* mutations were present in 22% (5/23) of *STAT5B* mutated CD4+ T-LGLL cases, either coexisting in one clone or in distinct clones. Patients with *STAT5B* mutations had increased lymphocyte and LGL counts when compared to *STAT5B* wild-type patients. TCRβ sequencing showed that, in addition to large LGL expansions, non-leukemic T cell repertoires were more clonal in CD4+ T-LGLL compared to healthy. Interestingly, 25% (15/59) of CD4+ T-LGLL clonotypes were found, albeit in much lower frequencies, in the non-leukemic CD4+ T cell repertoires of the CD4+ T-LGLL patients. Additionally, we further confirmed the previously reported clonal dominance of TRBV6-expressing clones in CD4+ T-LGLL. In conclusion, CD4+ T-LGLL patients have a typical TCR and mutation profile suggestive of aberrant antigen response underlying the disease.

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INTRODUCTION

T-cell large granular lymphocyte leukemia (T-LGLL) is a rare lymphoproliferative disease characterized by chronic expansion of clonal, mature cytotoxic T cells in the peripheral blood and bone marrow [1]. Two subtypes of T-LGLL are commonly recognized: the most common CD8+ T-LGLL (70% of cases) and the less frequent CD4+ T-LGLL (30% of cases) [1]. Clinically, CD4+ T-LGLL is usually an indolent disease and unlike in its CD8+ counterpart, these patients rarely have cytopenias or autoimmune symptoms. However, CD4+ T-LGLL has been reported more frequently associated with secondary neoplasms such as monoclonal B-cell lymphocytosis and plasma cell disorders [2, 3]. In CD4+ T-LGLL, the leukemic T-LGLs express CD4 (either alone or together with CD8) and the α/β T-cell receptor (TCR) together with a typical mature cytotoxic (Granzyme B+, CD56+, CD57+) and activated/memory T-cell ($CD2^{bright}$, $CD7^{dim}$, $CD11a^{bright}$, CD28−, CD62L−) phenotype [3].

Up to 55% of CD4+ T-LGLL patients have been shown to harbor *STAT5B* mutations [4, 5]. In CD8+ T-LGLL, the most common mutated gene is *STAT3* [6], whereas *STAT5B* mutations are rare and often associated with an aggressive disease form [7–9]. All reported *STAT5B* mutations in CD4+ T-LGLL are point mutations within the SH2 or transactivation domains of *STAT5B*. N642H and Y665F are the most common *STAT5B* mutations, and they both have been shown to increase STAT5B protein activity [4, 10, 11].

The etiology of CD4+ T-LGLL remains unknown. An initial antigen-driven expansion of CD4+ T cells, followed by the occurrence of oncogenic events (i.e., somatic mutations), has been suggested to lead to the persistence of abnormal T-cell clones [11]. Non-self-antigen(s) instead of autoantigens [5] are proposed targets of CD4+ T-LGL clones as CD4+ T-LGLL is not associated with autoimmune diseases. In some earlier reports, CD4+ T-LGL clones have been implied to recognize cytomegalovirus (CMV) antigens [12, 13]. In CD4+ T-LGLL [3], the enrichment of the Vβ13.1 gene usage has also been reported, differentiating it from

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