Gene expression profile of adult T-cell leukemia/lymphoma with human T-cell lymphotropic virus type 1-infected Hodgkin and Reed-Sternberg-like cells is more similar to classic Hodgkin lymphoma than typical adult T-cell leukemia/lymphoma

Adult T-cell leukemia/lymphoma (ATLL) is a mature T-cell neoplasm associated with human T-cell leukemia virus type 1 (HTLV-1) infection.[1] The most common histological pattern is diffuse proliferation of pleomorphic tumor cells, but several other histological variations have been recognized.[1] Classic Hodgkin lymphoma (CHL) is a B-cell lymphoma with characteristic Hodgkin and Reed-Sternberg (HRS) cells. Using a new sensitive method that can detect HTLV-1 viral products on formalin-fixed paraffin-embedded (FFPE) samples, *in situ* hybridization for HTLV-1 basic zipper factor (HBZ-ISH),[2] we identified a new histological pattern termed ATLL with HTLV-1-infected HRS-like cells (ATLL-HH).[2] ATLL-HH is considered a unique histological pattern of ATLL, with neoplastic HTLV-1-positive HRS-like cells and non-neoplastic inflammatory cells, histologically mimicking CHL.[2] Similar to HRS cells in CHL, HRS-like cells in ATLL-HH express CD30 and CD15, but they are usually negative for PAX5 and often CD4-positive.[2] The tumor microenvironment (TME) differs in terms of both quantity and quality between ATLL and CHL.[3,4] In this study, we explored the pathological characteristics of both tumor cells and the TME of ATLL-HH based on their comprehensive gene expression profiles to elucidate where ATLL-HH is pathologically located on the spectrum between typical ATLL and CHL.

We analyzed 12 samples from 11 patients (1 recurrent case) of ATLL-HH. The diagnosis of ATLL-HH was based on the detection of HBZ-ISH-positive signals in HRS-like cells using FFPE samples. The clinicopathological findings in 6 cases (patient numbers 1, 3-7; see *Online Supplementary Table S1A*) were reported in a previous study.[2] We also analyzed 8 CHL cases and 6 typical ATLL cases. The CHL cases included 3 HTLV-1 carriers and 5 HTLV-1-negative patients (*Online Supplementary Table S1B*). Among 3 cases of CHL arising from HTLV-1 carriers, we confirmed that HRS cells were HTLV-1-negative by HBZ-ISH, and the 2 cases for which clonality analysis was available had a polyclonal pattern for TRG rearrangement. Gene expression analysis using nCounter (NanoString Technologies, Seattle, WA, USA) and the PanCancer Immune Profiling panel (770 genes) was performed for FFPE samples as described previously.[5] The clustering analysis and analysis of differentially expressed genes were carried out using nSolver Analysis Software (https://nanostring.com/). Gene enrichment analysis was performed using Enrichr.[6] For immunohistochemical evaluation of TBET and IDO1, all pairwise comparisons were carried out using the Mann-Whitney U test in EZR.[7] This study was approved by the Institutional Review Board of Nagoya University (2022-0406).

The clustering analysis (Figure 1A) demonstrated that ATLL-HH had analogous gene expression profiles and was grouped within the same cluster as CHL, regardless of the Epstein-Barr virus status of the CHL. In contrast, typical ATLL formed a distinct cluster. *Online Supplementary Table S2* shows the top 30 genes up-regulated in ATLL-HH and CHL compared to typical ATLL. Gene Ontology biological process pathway enrichment analysis (Figure 1B) demonstrated that 8 of the 10 pathways in ATLL-HH, compared to typical ATLL, were identical to those in CHL. The transcription factor protein-protein interaction (PPI) analysis identified involvement of the JAK/STAT pathway in both ATLL-HH and CHL (Figure 1C). Based on these findings, we focused on CCL17, STAT6, TBET, and IDO1, all of which had significantly elevated expression in ATLL-HH and CHL compared to typical ATLL (Figure 2A). The localization of these proteins was analyzed by immunohistochemistry in the 7 ATLL-HH cases, 5 CHL cases, and 5 typical ATLL cases for which additional materials were available (Figure 3A-D). We analyzed phosphorylated STAT6 (pSTAT6) instead of total STAT6 to more accurately assess the activation of the JAK-STAT6 pathway. HRS(-like) cells were positive for CCL17 in 5 of 7 ATLL-HH cases and in all 5 CHL cases. Similarly, pSTAT6 expression was observed in HRS(-like) cells in all 7 ATLL-HH cases and in all 4 evaluable CHL cases; one case was not evaluable. CCL17 expression was largely restricted to HRS(-like) cells, whereas pSTAT6 positivity was also observed in a subset of lymphocyte-like and endovascular cells, consistent with previous reports.[8] In contrast, none of the typical ATLL cases exhibited pSTAT6- or CCL17-positive tumor cells. The lower expression levels of CCL17 and STAT6 in typical ATLL than in ATLL-HH and CHL, as demonstrated by gene expression profiling, may reflect the absence of HRS(-like) cells in typical ATLL. TBET expression was observed mainly in lymphocytes, but also

in HRS(-like) cells (30-80%) in all cases of ATLL-HH and CHL. Morphologically, IDO1-positive cells were considered to be macrophages/dendritic cells, and no IDO1-positive HRS(-like) cells were observed. The numbers of TBET-pos-

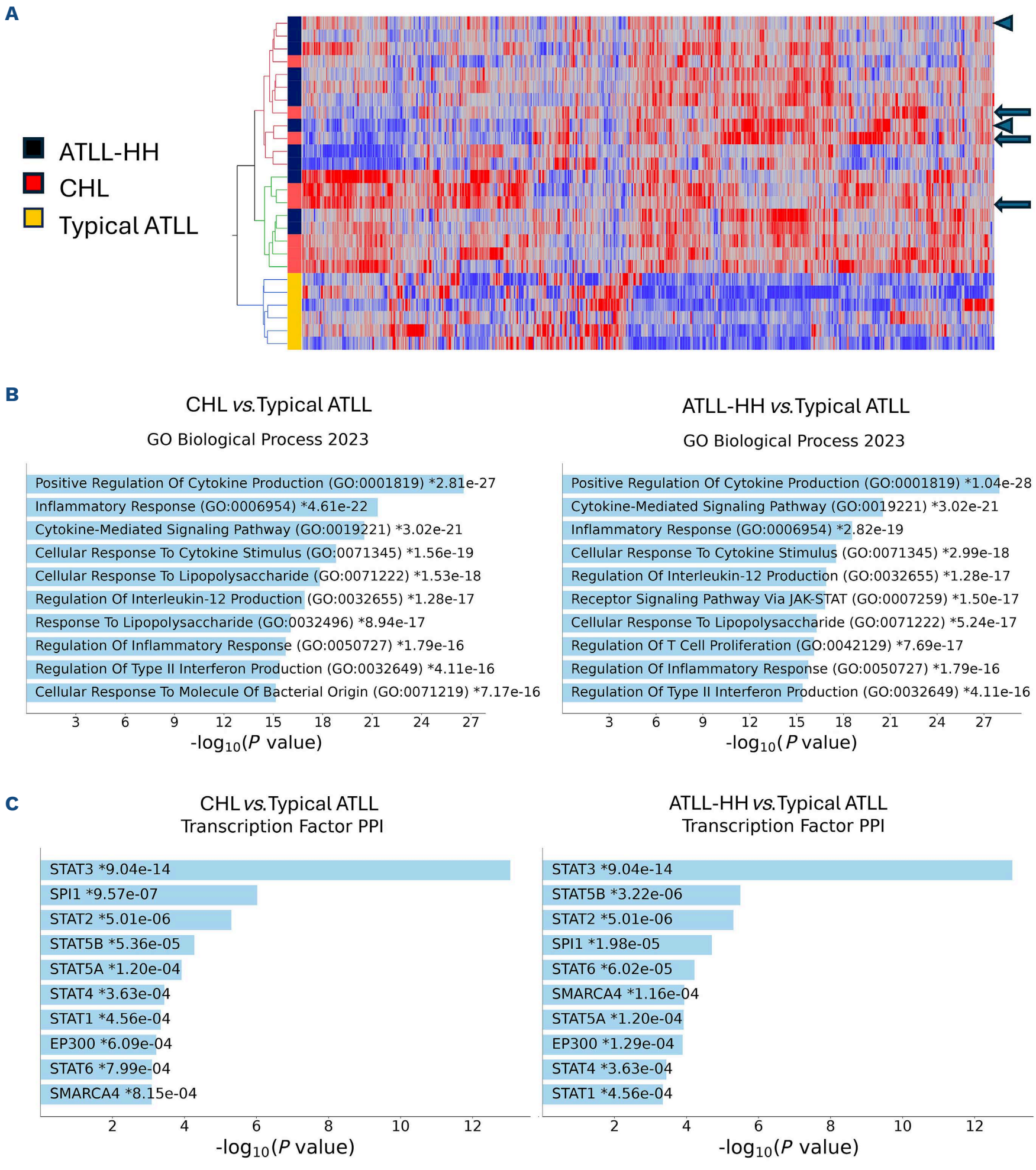


Figure 1. Comprehensive RNA expression analysis by nCounter. (A) Cluster analysis of the gene expression profiles of adult T-cell leukemia/lymphoma (ATLL) with human T-cell leukemia virus type 1 (HTLV-1)-infected Hodgkin and Reed-Sternberg (HRS)-like cells (ATLL-HH), classic Hodgkin lymphoma (CHL), and typical adult T-cell leukemia/lymphoma (ATLL). Arrows indicate Epstein-Barr virus negative CHL. Arrowheads indicate the primary and relapsed lesions of the same case. (B) Gene enrichment analysis of ATLL-HH, CHL, and typical ATLL by Enrichr using Gene Ontology (GO) Biological Process 2023. (C) Gene enrichment analysis of ATLL-HH, CHL, and typical ATLL by Enrichr using transcription factor protein-protein interactions (PPI).

itive and IDO1-positive TME cells were significantly higher in ATLL-HH and CHL than in typical ATLL (Figures 2B, 3A-D). CCL17 is a chemokine produced by HRS cells in CHL and contributes to the T-cell-rich TME.[9] HRS cells reportedly express CCL17 in 85% of CHL cases.[10] In our study, HRS-like cells in ATLL-HH were positive for CCL17, which suggests that they had similar characteristics to HRS cells in CHL. STAT6 is a representative transcription factor in the JAK/STAT pathway. In 87% of CHL cases, HRS cells have been shown to have genetic dysregulation in the JAK/STAT pathway.[11] Immunohistochemical pSTAT6 positivity in HRS cells was useful in distinguishing CHL from other histological mimickers.[12] Our immunohistochemical analysis revealed that HRS-like cells in ATLL-HH expressed pSTAT6, sug-

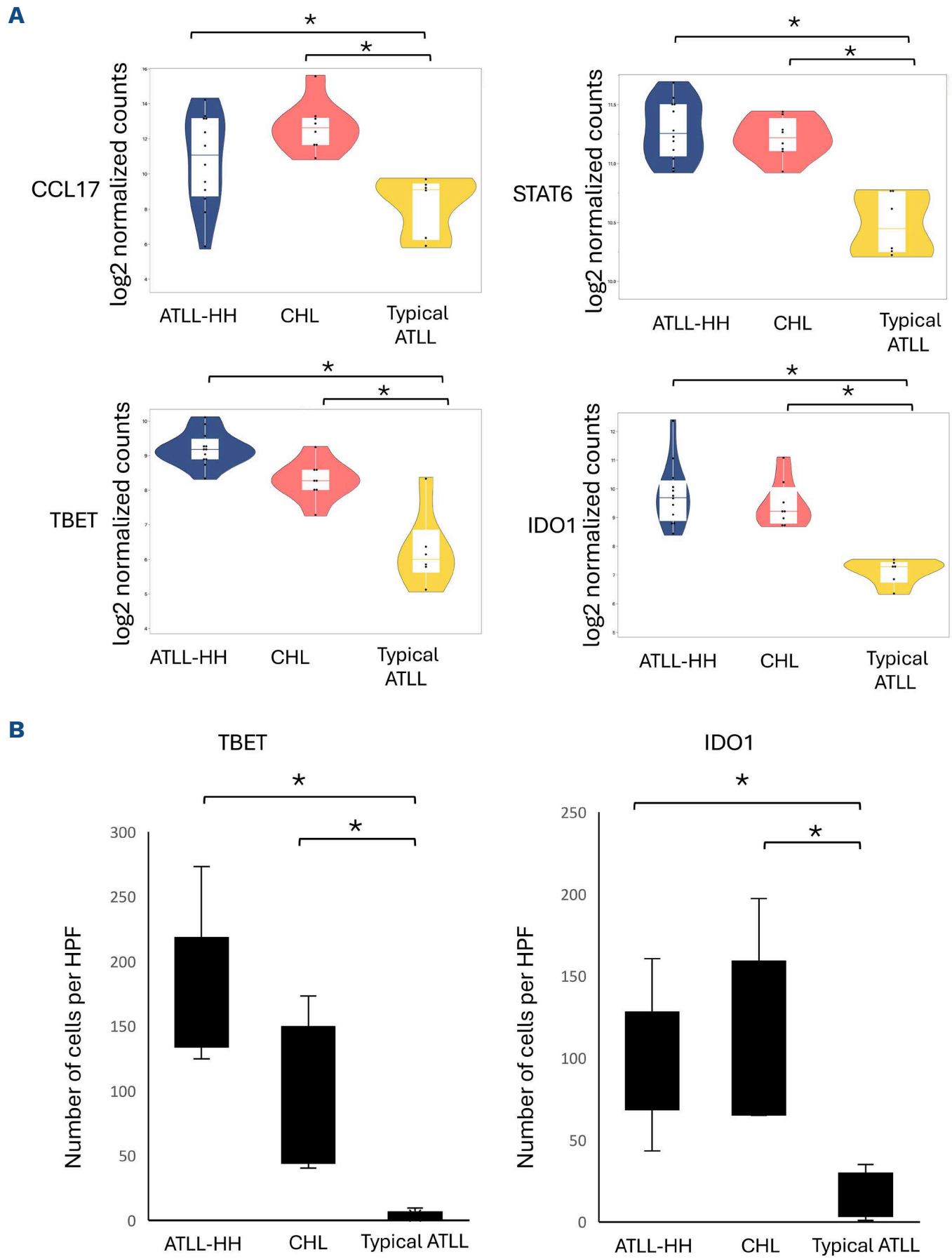


Figure 2. Expression analysis of CCL17, STAT6, TBET and IDO1. (A) Boxplot of CCL17, STAT6, TBET, and IDO1 RNA expression. (B) Number of TBET-positive and IDO1-positive cells by immunohistochemistry. The average number of positive cells in three high-power fields (HPF) (x400) was calculated. TBET-positive Hodgkin and Reed-Sternberg (HRS)-like cells were excluded. ATLL: adult T-cell leukemia/lymphoma; ATLL-HH: ATLL with human T-cell leukemia virus type 1-infected Hodgkin and Reed-Sternberg-like cells; CHL: classic Hodgkin lymphoma. *P<0.05.

gesting the importance of the JAK/STAT pathway in its pathogenesis similar to CHL, as shown in the PPI analysis based on the gene expression profile. TBET is a transcription factor important for the differentiation of CD4-positive T cells into Th1 T cells. The TME of CHL consists of various cells, including T cells, especially Th1 T cells, and CHL cases with more TBET-positive cells have been reported to have better disease-specific survival.[13] We found numerous

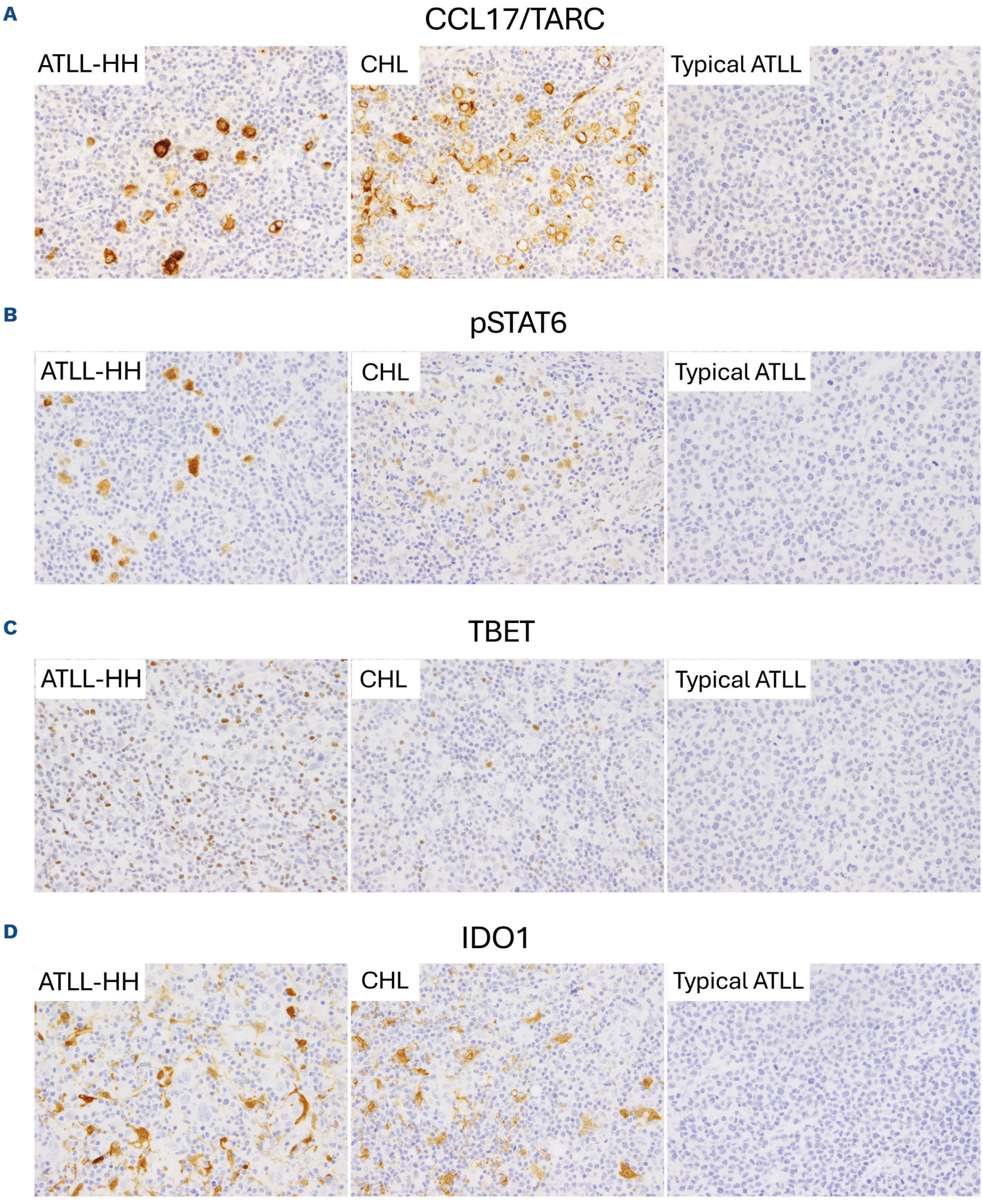


Figure 3. Representative immunostaining images. (A) CCL17, (B) pSTAT6, (C) TBET, and (D) IDO1 in adult T-cell leukemia/lymphoma (ATLL) with human T-cell leukemia virus type 1 (HTLV-1)-infected Hodgkin and Reed-Sternberg-like cells (ATLL-HH), classic Hodgkin lymphoma (CHL), and typical ATLL. Magnification ×400.

TBET-positive cells in the TME of ATLL-HH, suggesting a Th1-dominant TME in ATLL-HH similar to CHL. IDO1 is an enzyme in the kynurenine pathway and a recently studied immunosuppressive molecule. Overexpression of IDO1 in macrophages and dendritic cells in the TME suppresses effector T-cell function and induces Tregs, resulting in immune tolerance.[14] Regarding the TME of CHL, cases with more IDO1-positive macrophages have a worse prognosis.[14] Significantly more IDO1-positive cells were found in the TME of ATLL-HH than in typical ATLL, suggesting that they play a role in the tumorigenesis of ATLL-HH through immune tolerance.

One limitation of our study is the lack of comprehensive clinical information; therefore, the prognostic significance of the gene expression profiles remains unclear. Nevertheless, ATLL-HH was associated with relatively high serum LDH and sIL-2R levels, as well as frequent extranodal involvement (*Online Supplementary Table S1A*). These findings may provide supportive information for distinguishing ATLL-HH from CHL, although their diagnostic utility requires validation in larger studies. Another unresolved issue is the cellular origin of HRS-like cells in ATLL-HH. Although 5 cases showed a clonal peak within a predominantly polyclonal background, including cases in which the HRS-like cells were positive for PAX5, these findings alone are insufficient to establish the lineage of HRS-like cells in the absence of single-cell–based analyses (*Online Supplementary Table S1A, Online Supplementary Figure S1*). A third limitation is that Hodgkin-like ATLL,[15] which has been reported to occur in HTLV-1 carriers with CHL-like morphology, was not included in this study. The morphology and number of atypical cells around HRS-like cells in Hodgkin-like ATLL were diverse (*data not shown*), and it was often difficult to clearly distinguish them from CHL or typical ATLL. Further collection of cases and integrated analysis of Hodgkin-like ATLL is in progress.

In conclusion, comprehensive gene expression profiling revealed that ATLL-HH shares a molecular signature with CHL. This similarity appears to be attributed to CCL17 expression and STAT6 activation in HRS-like cells, along with the presence of numerous TBET-positive Th1 cells and IDO1-positive cells in the TME. These shared pathological features may support common therapeutic strategies targeting both ATLL-HH and CHL.

Authors

Shohei Miyagi,[1*] Kyohei Mori,[2*] Kohta Miyawaki,[2] Masatoshi Shimo,[2] Tomoko Tamaki,[3] Shugo Sakihama,[4] Mitsuyoshi Takatori,[5] Koichi Akashi,[2] Kennosuke Karube[1] and Koji Kato[2]

[1]Department of Pathology and Laboratory Medicine, Graduate School of Medicine, Nagoya University, Nagoya; [2]Department of Medicine and Biosystemic Science, Kyushu University Graduate School of Medical Sciences, Fukuoka; [3]Department of Diagnostic Pathology, University of the Ryukyus Hospital, Okinawa; [4]Laboratory of Hematoimmunology, Graduate School of Health Sciences, University of the Ryukyus, Ginowan and [5]Department of Molecular and Cellular Physiology, Graduate School of Medicine, University of the Ryukyus, Ginowan, Japan

**SM and KMo contributed equally as first authors.*

Correspondence:
K. KARUBE - karube.kennosuke.x1@f.mail.nagoya-u.ac.jp
K. KATO - kato.koji.429@m.kyushu-u.ac.jp

https://doi.org/10.3324/haematol.2025.289270

Received: September 25, 2025.
Accepted: April 7, 2026.
Early view: April 16, 2026.

©2026 Ferrata Storti Foundation
Published under a CC BY-NC license

Disclosures

KKar has received honoraria from Janssen, Chugai, Takeda, Kyowa Kirin, Otsuka, AbbVie, Genmab, Eisai, AstraZeneca, SymBio, and Meiji-Seika Pharma, and research grants from Takeda and Eisai. All the other authors have no conflicts of interest to disclose.

Contributions

SM, KKar and KKat designed and conducted the research, reviewed all the cases, and wrote the manuscript. KMo, KMi and MS provided the genetically analyzed data. The other authors provided us with specimens, clinical information, or pathological evaluation. All authors read and approved the final version of the manuscript for publication.

Funding

This study was supported by Grants-in-Aid for Scientific Research (KAKENHI) from the Ministry of Education, Culture, Sports, Science, and Technology of Japan (25K18745 to SM and 23K27384 to KKar).

Data-sharing statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

References

1. Karube K, Cook LB, Ishitsuka K, et al. Adult T-cell leukaemia/lymphoma. In: WHO Classification of Tumours: Haematolymphoid tumours. Lyon (France), 5th ed., vol. 11. International Agency for Research on Cancer; 2024.

2. Karube K, Takatori M, Sakihama S, et al. Clinicopathological features of adult T-cell leukemia/lymphoma with HTLV-1-infected Hodgkin and Reed-Sternberg-like cells. Blood Adv. 2021;5(1):198-206.
3. Nakahata S, Enriquez-Vera D, Jahan MI, Sugata K, Satou Y. Understanding the immunopathology of HTLV-1-associated adult T-cell leukemia/lymphoma: a comprehensive review. Biomolecules. 2023;13(10):1543.
4. Duffield AS, Ascierto ML, Anders RA, et al. Th17 immune microenvironment in Epstein-Barr virus-negative Hodgkin lymphoma: implications for immunotherapy. Blood Adv. 2017;1(17):1324-1334.
5. Miyawaki K, Kato K, Sugio T, et al. A germinal center-associated microenvironmental signature reflects malignant phenotype and outcome of DLBCL. Blood Adv. 2022;6(7):2388-2402.
6. Chen EY, Tan CM, Kou Y, et al. Enrichr: interactive and collaborative HTML5 gene list enrichment analysis tool. BMC Bioinformatics. 2013;14:128.
7. Kanda Y. Investigation of the freely available easy-to-use software 'EZR' for medical statistics. Bone Marrow Transplant. 2013;48(3):452-458.
8. Skinnider BF, Elia AJ, Gascoyne RD, et al. Signal transducer and activator of transcription 6 is frequently activated in Hodgkin and Reed-Sternberg cells of Hodgkin lymphoma. Blood. 2002;99(2):618-626.
9. Kilsdonk M, Veldman C, Rosati S, Plattel W, Diepstra A. The value of thymus and activation related chemokine immunohistochemistry in classic Hodgkin lymphoma diagnostics. Histopathology. 2023;82(3):495-503.
10. Peh SC, Kim LH, Poppema S. TARC, a CC chemokine, is frequently expressed in classic Hodgkin's lymphoma but not in NLP Hodgkin's lymphoma, T-cell-rich B-cell lymphoma, and most cases of anaplastic large cell lymphoma. Am J Surg Pathol. 2001;25(7):925-929.
11. Tiacci E, Ladewig E, Schiavoni G, et al. Pervasive mutations of JAK-STAT pathway genes in classical Hodgkin lymphoma. Blood. 2018;131(22):2454-2465.
12. Satou A, Takahara T, Yamashita D, et al. Diagnostic utility of STAT6 and pSTAT6 immunohistochemistry for distinguishing classic Hodgkin lymphoma and peripheral T-cell lymphoma with Hodgkin and Reed-Sternberg-like cells. Am J Surg Pathol. 2023;47(8):897-906.
13. Greaves P, Clear A, Owen A, et al. Defining characteristics of classical Hodgkin lymphoma microenvironment T-helper cells. Blood. 2013;122(16):2856-2863.
14. Choe JY, Yun JY, Jeon YK, et al. Indoleamine 2,3-dioxygenase (IDO) is frequently expressed in stromal cells of Hodgkin lymphoma and is associated with adverse clinical features: a retrospective cohort study. BMC Cancer. 2014;14:335.
15. Ohshima K, Kikuchi M, Yoshida T, Masuda Y, Kimura N. Lymph nodes in incipient adult T-cell leukemia-lymphoma with Hodgkin's disease-like histologic features. Cancer. 1991;67(6):1622-1628.