Human Pathology (2019) **84**, 309–320

ELSEVIER

Human PATHOLOGY

www.elsevier.com/locate/humpath

Original contribution

The histologic, immunohistochemical, and genetic features of classical Hodgkin lymphoma and anaplastic large cell lymphoma with aberrant T-cell/B-cell antigen expression☆,☆☆

Ke Liang MD[1], Jun Wang MD[1], Yan Wang MD, Zhiqiang Zhou MD, Shuang Ge MD, Shuyu Mei MD, Hailing Li MD, Xiaotong Jing MD, Cuijuan Zhang MD, PhD*

Institute of Pathology and Pathophysiology, Shandong University School of Medicine, Jinan 250012, China

Received 13 August 2018; revised 27 September 2018; accepted 4 October 2018

Keywords:
Classical Hodgkin lymphoma;
Anaplastic large cell lymphoma;
Aberrant expression;
Immunohistochemistry;
Whole-exome sequencing

Summary Classical Hodgkin lymphoma (cHL) and ALK− anaplastic large cell lymphoma (ALCL) share many morphologic and immunohistochemical features, causing difficulties in differential diagnosis. Aberrant T-cell/B-cell antigen (TCA/BCA) expression in cHL/ALCL has previously been reported, but differences in the broader morphologic and genetic features still remain unclear. We first explored the histologic and immunohistochemical characteristics of cHL and ALCL with or without aberrant expression. Of 68 cHL cases, 10 (14.71%) were found to express 1 or more TCAs, and the frequency was as follows: CD4 > CD2 > CD3 > CD5 = CD7. Only 1 (3.33%) of 30 ALCL cases expressed BCA. Histologically, the main subtypes of cHL with aberrant TCA expression were LD and NS2. These aberrant TCA-expressing cHL tumor cells exhibited some ALCL features, and the aberrant BCA-expressing ALCL tumor cells displayed cHL characteristics. We also performed whole-exome sequencing analysis on cHL and ALCL samples with aberrant expression and compared them with those without aberrant expression. The results of this analysis showed that *GNE* and *CACNB2* mutations, involved in the MAPK signaling pathway, may play an important role in cHL. In addition, 135 mutation sites involved in multiple signaling pathways were identified in ALCL. In the aberrant-expression cases, genetic features were similar between cHL and ALCL, consistent with their morphologic features. Our results broaden the understanding of the histologic and immunohistochemical characteristics of cHL and ALCL with aberrant expression and, for the first time, compare genetic features between cHL and ALCL with and without aberrant expression.

1. Introduction

Classical Hodgkin lymphoma (cHL) and ALK− anaplastic large cell lymphoma (ALCL) share many morphologic and immunophenotypic features. Histologically, ALCL is characterized by hallmark cells, but these cells can also be present

☆ Competing interests: The authors have declared that no competing interests exist.

☆☆ Funding/Support: This work was supported by the Science and Technology Program of Shandong, China (No. 2016GSF301031), and the China Postdoctoral Science Foundation (No. 2013 M540554).

* Corresponding author at: Institute of Pathology and Pathophysiology, Shandong University School of Medicine, Wenhuaxi Rd 44#, Jinan 250012, PR China.
E-mail address: cuijuanzhang@sdu.edu.cn (C. Zhang).

[1] These 2 authors contributed equally to this work.

https://doi.org/10.1016/j.humpath.2018.10.003
0046-8177/

in cHL; in addition, some ALCL tumor cells are binucleated and are similar to RS cells. Both cHL and ALCL cells express CD30, and although cHL is considered to be a B-cell lymphoma, the tumor cells typically show loss of B-cell antigens (up to 60% of cHL cases do not express CD20; more cases do not express CD79a) [1,2]. ALCL is a T-cell lymphoma, but the tumor cells are often negative for T-cell markers [3,4], causing the differentiation between these 2 diseases to be very challenging for diagnoses. CD15 expression is relatively specific to cHL (some peripheral T-cell lymphoma cases can also express CD15), and some studies have reported that it was positive in more than 70% of cHL cases [5,6]; however, its sensitivity is very limited in many hospitals (at least in China). In recent years, the B-cell transcription factor Pax5 has been found to be weakly expressed in the RS cells of most cases of HL [7,8], whereas there are still some cases that do not express Pax5. Because cHL and ALCL have distinct prognosis and treatment options, cHL can be cured in more than 80% of patients with doxorubicin, bleomycin, vinblastine, and dacarbazine (ABVD) therapy [9,10], whereas ALCL often shows poor outcome and requires a different type of treatment [11,12]. Because of this phenomenon, it is very important to distinguish these 2 entities. Therefore, the original aim of our study was to identify a set of characteristic immunomarkers that can be applied to the differential diagnosis of cHL and ALK− ALCL.

We examined the immunophenotypic characteristics of cHL and ALCL by using an extensive immunohistochemical panel. Several immunohistochemical markers were found that may be helpful for distinguishing cHL from ALCL, and unexpectedly, we found that there were 10 (14.71%) in 68 cases of cHL that expressed 1 or more T-cell antigens (TCAs); one ALCL case expressed more than 1 B-cell antigen (BCA). To better define the genetic landscape of these aberrant expression cases, to identify genes with a statistically significant rate of mutation in cHL and ALCL, and to improve our comprehension of pathways altered in these 2 diseases, we next performed whole-exome sequencing (WES) to investigate the genetic features of these cases with aberrant expression compared with those without aberrant expression. We also followed up with these patients' treatment and clinical processes to better understand their chemotherapy response and prognosis.

Previous studies had shown that cHL cells can express TCA [13-15], particularly Venkataraman et al [16], who reported that aberrant TCA expression in cHL is associated with decreased event-free survival and overall survival, revealing the relationship between aberrant expression and disease prognosis. There are also several case reports showing Pax5 positivity in ALCL [17-19]. These studies increased our understanding of the aberrant expression of cHL and ALCL; however, the morphologic and immunohistochemical characteristics of these cases still need to be broadened, and their genetic features still remain unclear.

In our study, we summarized the morphologic characteristics of these aberrant-expression cases of cHL and ALCL and found that these tumor cells have some common features. Some cHL cases with aberrant TCA expression had worse reactivity to the ABVD treatment and poor prognosis. In addition, we also provided the genetic features of these cases compared with cHL and ALCL, allowing us to better understand the underlying relationship of cHL and ALK− ALCL.

2. Materials and methods

2.1. Histologic examination

Sixty-eight cases of cHL and 30 cases of ALCL were obtained from Shandong University, Qilu Hospital, between 2013 and 2016. Histologic sections (4 μm) obtained from 10% formalin-fixed, paraffin-embedded (FFPE) blocks were stained with hematoxylin and eosin for microscopic examination. All cases were reviewed independently by 2 pathologists to confirm the diagnoses. Diagnoses were classified according to the World Health Organization Classification 2017, and nodular sclerosis (NS) subtype cases were further graded as grade 1 (NS1) or grade 2 (NS2) as per the criteria of the British National Lymphoma Investigation.

2.2. Ethical approval statement

The study was approved by the Shandong University Ethics Committee and in accordance with the Declaration of Helsinki.

2.3. Immunohistochemistry

Immunohistochemistry was performed on 5-μm FFPE tissue sections according to standard methods. Tumor cells were considered positive for CD2, CD3, CD4, CD5, CD7, CD8, CD10, CD20, CD43, CD45, and CD79a by cell membrane staining and were considered positive for CD15 and CD30 by cell membrane and Golgi staining. Positive bcl6, Mum1, Pax5, Pax8, p53, and p63 cells exhibited a distinct nuclear staining, and the cells were considered positive for EMA and TIA-1 when the cytoplasm was stained. bcl2 was considered positive with cell membrane or cytoplasm staining. The cells that were ALK positive had strong cytoplasm/membrane/nuclear or both cytoplasm and nuclear staining. p16INK4a was indicated if either the cytoplasm or the nucleus or both were stained. The cells were positive for granzyme B if the cytoplasm had granular staining.

2.4. In situ hybridization

In situ hybridization (ISH) for Epstein-Barr virus (EBV)–encoded RNAs (EBERs) was performed using a commercially available EBV Probe ISH Kit (ISH7001; ZSGB-BIO, Beijing, China) according to the manufacturer's protocols. Tumor cell nuclei with strong signals were considered EBV positive.

2.5. WES analysis

Genomic DNA from 18 samples of FFPE was extracted using a DNA kit (QIAamp; Qiagen, Chatsworth, CA) following the manufacturer's instructions. Genomic DNA was captured from samples using the Agilent SureSelect Human All Exon V6 kit (Agilent Technologies, Santa Clara, CA). Briefly, genomic DNA was fragmented to a size range of 150 to 220 bp using a sonicator and enriched for exome sequences using the SureSelect Human All Exon Kit, version 2 (Agilent Technologies, Santa Clara, CA), and sequencing was performed at a minimal average coverage of 100× on an Illumina HiSeq xten platform (Illumina, San Diego, CA).

2.6. Statistics

The data were analyzed using the Statistical Package for Social Sciences (SPSS; version 20) statistical program (SPSS, Chicago, IL). The frequencies between the groups were compared using the χ^2 test and Fisher exact method. A 1-way analysis of variance was applied for comparison between the groups of the sample. The results were considered statistically significant when $P < .05$.

3. Results

3.1. Histologic features of cHL and ALCL

Among 68 cHL cases, there were 31 cases of NS, with 5 NS2, 24 mixed cellularity (MC), 9 lymphocyte-rich (LR), and 4 lymphocyte-depleted (LD) cases. Three cases showed numerous tumor cells and high cohesiveness with sinusoidal growth patterns, and 2 of them were originally misdiagnosed as ALCL. Four cases showed multiple focal tumor necrosis. The male-to-female ratio was 1.27:1, and the age distribution was 3 to 77 years (mean, 38.1 years).

The 30 ALCL cases included 15 ALK+ cases and 15 ALK − cases. The male-to-female ratio was 1:1, and the age range was 4 to 86 years (mean, 37 years). Massive tumor necrosis was found in 2 cases. One case with tumor necrosis was originally misdiagnosed as ALCL according to strong CD30-positive staining and was corrected to NK/T-cell lymphoma after EBER ISH detection (this case was excluded from the ALCL group).

In our data, multiple focal tumor necrosis seems to be an important characteristic for cHL but is very rare in ALCL (occasionally, massive tumor necrosis can be seen). In addition to fibrosis, epithelioid macrophages, and neutrophils, we found that thick-walled vessels are also much more common in cHL than in ALCL. The histologic features of TCA-positive cHL cases are summarized as follows: (1) the main subtypes are LD and NS2; (2) these aberrant TCA expression tumor cells have some morphologic features of ALCL cells: numerous tumor cells, high cohesiveness, more pleomorphic with a horseshoe nucleus, and sinusoidal growth pattern (Fig. 1); and (3) multiple focal tumor necrosis is more common than those cases without aberrant expression (40% versus 5.17%, $P < .01$). Interestingly, in the BCA-positive ALCL case, most of the tumor cells expressed TCA markers, and these cells were medium sized and looked similar to classical ALCL cells; however, the size of the tumor cells with aberrant BCA markers became much larger than that of the other tumor cells, with wreath-like nuclei and eosinophilic cytoplasm, and exhibited morphologic characteristics of cHL cells (Fig. 2), and these cells were also EBER positive.

3.2. Immunohistochemical features of cHL and ALCL

The results of the expression profiles of all immunohistochemical markers in cHL and ALCL are summarized in the Table.

3.2.1. Expression of BCAs (Pax5, Pax8, CD20, and CD79a) in cHL and ALCL

The expression of CD20 was seen in 44.12% (30/68) of cHL cases, and CD79a was seen in 29.41% (20/68) of cases. When positive, CD20 and CD79a generally were expressed variably in the RS cells with a weak to moderate intensity. The extent and intensity of CD79a expression were often more focal and weaker than that of CD20.

Pax5 was reported to express in 95% of cHL cells, but in our cases, it was only expressed in 83.82% (57/68) of cHLs, and the intensity of Pax5 was always weak on the RS cells. We also detected Pax8 in cHL cells because we found by accident that Pax8 can express in RS cells. The results showed that the expression pattern of Pax8 is similar to Pax5, and in 94.11% (64-68) of cases, Pax8 was positive; among these positive cases, 7 cases were Pax5 negative or very weakly positive (Fig. 3).

For the ALCL cases, we only found 1 case that expressed BCAs. In this case, only a minority of tumor cells expressed Pax5, Pax8, CD20, and CD79a, and these cells were also EBER positive but lost the expression of the TCAs (Fig. 4). The morphology of these tumor cells became much more pleomorphic, with wreath-like nuclei and eosinophilic cytoplasm, and looked more similar to cHL tumor cells.

3.2.2. Expression of TCAs (CD2, CD3, CD5, CD7, CD4, and CD8) and cytotoxic markers (TIA-1 and granzyme B) in cHL and ALCL

In 68 cHL cases, we found 10 (14.71%) cases that expressed 1 or more TCAs, and the frequency of TCA expression was as follows: CD4 (8 cases) > CD2 (6 cases) > CD3 (3 cases) > CD5 (2 cases) = CD7 (2 cases). Among these 10 cases, 2 cases coexpressed CD2, CD3, CD4, CD5, and CD7; 1 case coexpressed CD2, CD3, and CD4; 1 case coexpressed CD2 and CD4; 2 cases only expressed CD2; and 4 cases only expressed CD4. The frequency of TCA expression according to the subtype of cHL was as follows: LD (3/4; 75%) > NS2

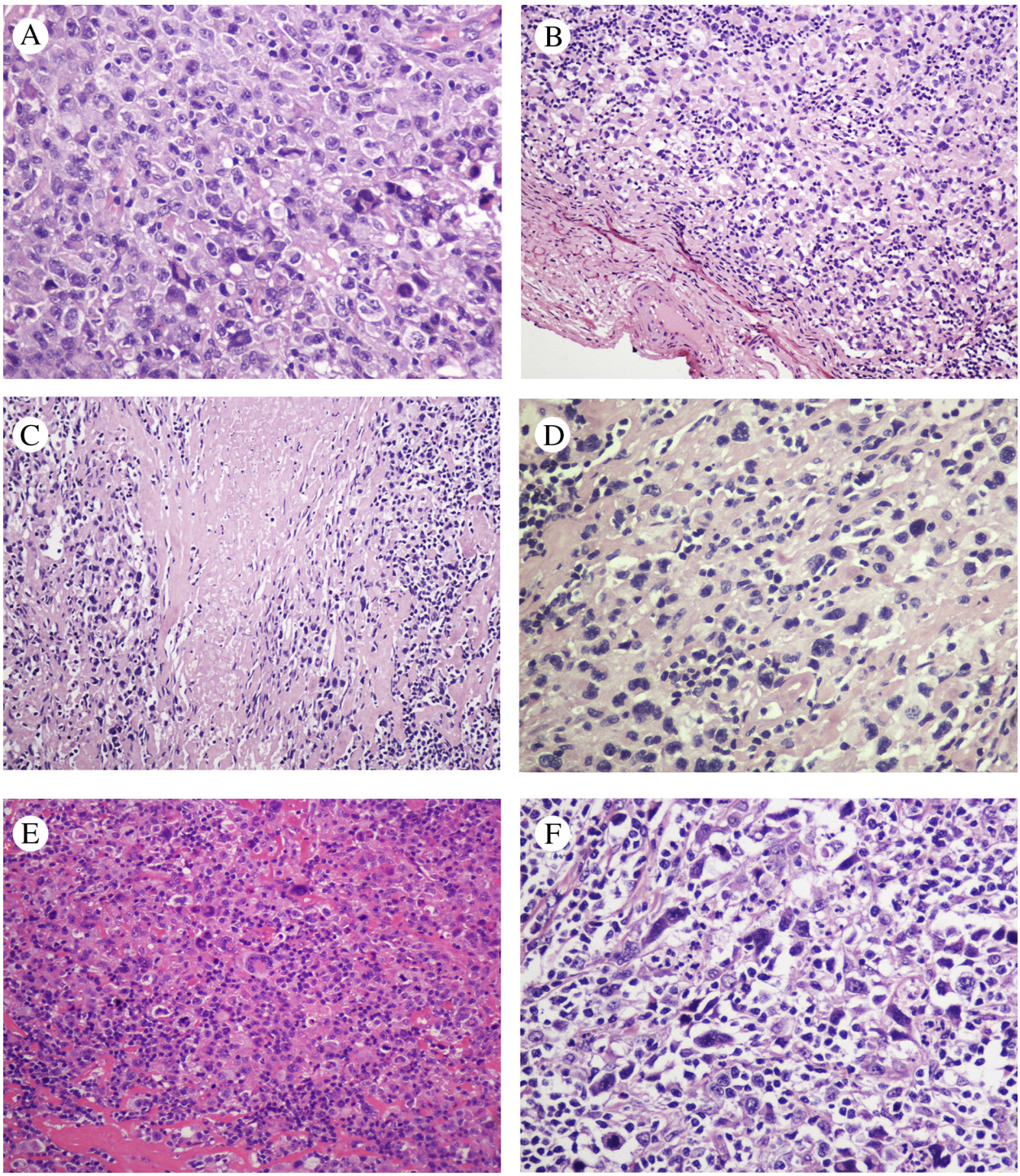

Fig. 1 The morphologic features of TCA-positive cHL cases. A, Numerous tumor cells and high cohesiveness (original magnification ×400). B, Sinusoidal growth pattern of tumor cells (×200). C, Focal tumor necrosis (×200). D-F, Pleomorphic cells, more eosinophilic cytoplasm, and horseshoe nucleus (×400 [D], ×200 [E], ×400 [F]).

(3/5; 60%) > MC (2/24; 8.33%) > NS1 (1/31; 3.23%) > LR (0/9; 0%). The TCA expression intensity in these cases was variable (weak to moderate) and not diffuse (10%-50% positive) on RS cells. Generally, CD4 often showed strong membrane positive staining in RS cells and displayed a greater proportion of cells than the other TCAs. CD5 and CD7 showed weak membrane-positive staining in RS cells but strong membrane-positive staining in mature T cells (Fig. 5). There was no CD8-positive case in our samples. There were several cases (5/68; 7.35%) that expressed TIA-1, whereas many more cases 22 (22/68; 32.35%) expressed granzyme B in RS cells (30%-50%).

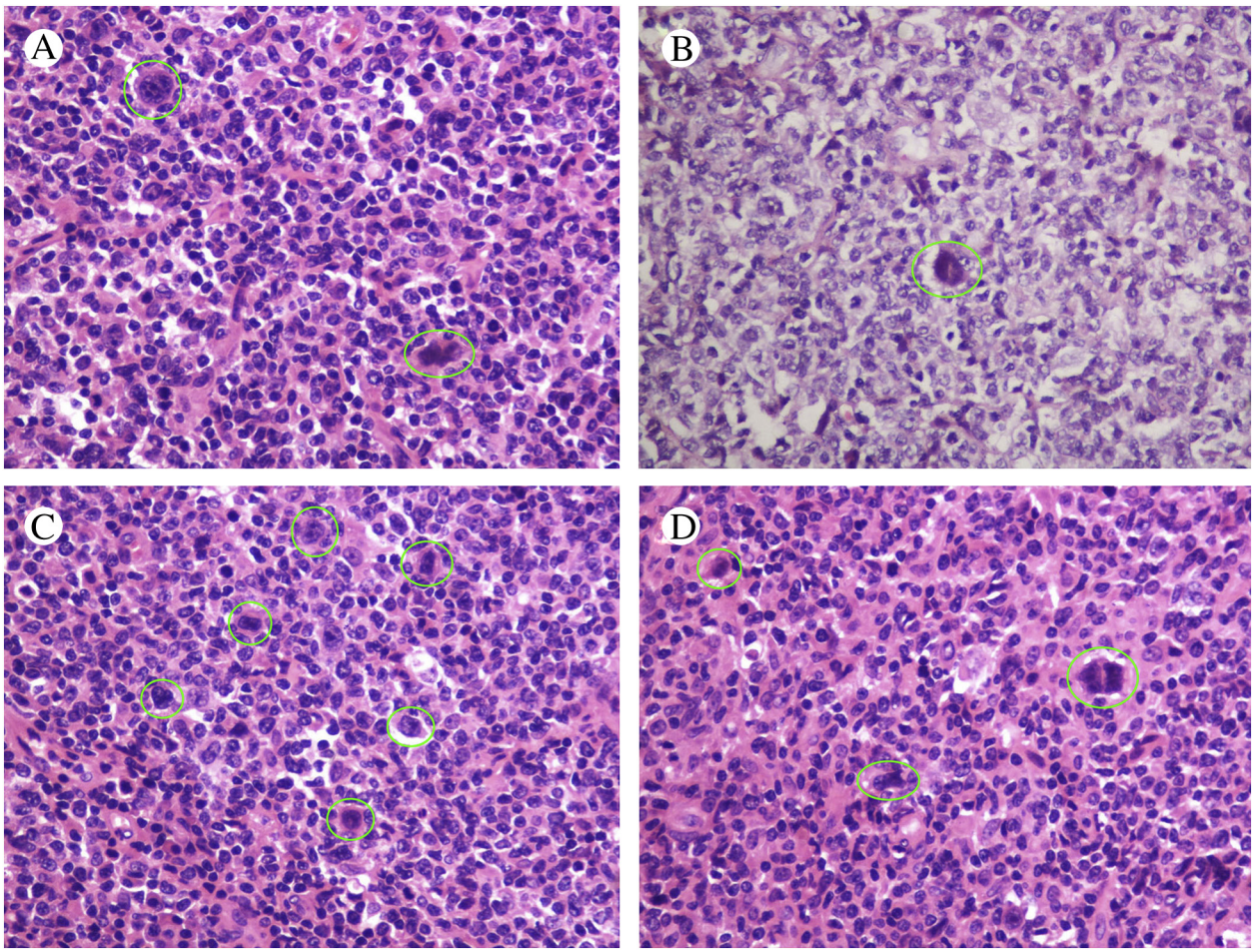

Fig. 2 The morphologic features of a BCA-positive ALCL case (tonsil). A-D, On the background of medium-sized T tumor cells mixed with minimal mature lymphocytic cells, there are much larger cells, with wreath-like nuclei and eosinophilic cytoplasm, that resemble cHL cells (original magnification ×400).

For ALCL cases, the frequency of CD2, CD3, CD5, CD7, CD4, TIA-1, and granzyme B expression was 14 (46.67%), 12 (40%), 2 (6.67%), 13 (43.33%), 12 (40%), 10 (33.33%), and 13 (43.33%) of 30 cases, respectively. There was no case that expressed CD8 in our ALCL cases. It should be noted that in the 2 cases that were originally misdiagnosed as ALCL (1 is cHL, 1 is NK/T-cell lymphoma), both of them expressed CD8, reminding us that if a case is suspected of ALCL but expresses CD8, cHL or NK/T cell lymphoma should not be ruled out. The intensity of CD2, CD3, and CD4 expression generally was moderate to strong, whereas CD5 expression was weak. For most cases of ALCL, the expression pattern of CD7 was weak in tumor cells but strong in mature T cells, but in several cases, its expression pattern was strong both in tumor cells and mature T cells.

3.2.3. p16INK4a expression in cHL and ALCL

p16INK4a expression was considered positive when more than 30% of tumor cells were stained. p16INK4a expression was found in 55 (80.88%) of 68 cHLs and 13 (43.33%) of 30 ALCLs. Although we cannot make a differential diagnosis of cHL and ALCL solely on p16INK4a staining, it should be mentioned that in some cHLs, the tumor cells are very difficult to be found in hematoxylin and eosin sections, and p16INK4a staining can help us easily find these tumor cells (Fig. 6).

To better understand the diagnostic value of p16INK4a for cHL, we next detect its expression in 30 cases of reactive hyperplasia of the lymph node. In 24 (80%) of 30 cases, p16INK4a was negative, and 6 (20%) of 30 cases showed that p16INK4a can express in immunoblasts, resulting in a diagnostic dilemma. For this situation, we need to carefully combine morphologic and immunohistochemical features to make a final diagnosis.

3.2.4. Routine immunohistochemistry (CD30, CD15, ALK, CD45, CD43, and EMA) and EBER ISH in cHL and ALCL

In all cHLs and ALCLs, neoplastic cells were positive for CD30. All ALCLs were CD15−, and 18 8 (26.47%) of 6 cHLs were CD15+. ALK was positive in 15 (50%) of 30 ALCLs and negative in all cHLs. As reported, CD45 and CD43 are very helpful for the differential diagnosis of cHL and ALCL. In

Table A panel of immunohistochemical markers and EBER expression in cHL and ALCL

Immunohistochemical markers and EBER	cHL (n = 68)		ALCL (n = 30)	
	Positive case no.	Positive rate (%)	Positive case no.	Positive rate (%)
Pax5	57/68	83.82	1/30	3.33
CD20	30/68	44.12	1/30	3.33
CD79a	20/68	29.41	1/30	3.33
Pax8	64/68	94.11	1/30	3.33
CD2	6/68	8.82	14/30	46.67
CD3	3/68	4.41	12/30	40
CD5	2/68	2.94	2/30	6.67
CD7	2/68	2.94	13/30	43.33
CD4	8/68	11.76	12/30	40
CD8	0/68	0	0/30	0
TIA-1	5/68	7.35	10/30	33.33
Granzyme B	22/68	32.35	13/30	43.33
p16	55/68	80.88	13/30	43.33
CD30	68/68	100	30/30	100
CD15	18/68	26.47	0/30	0
ALK	0/68	0	15/30	50
EMA	13/68	19.12	17/30	56.67
CD45	4/68	5.88	14/30	46.67
CD43	14/68	20.59	22/30	73.33
bcl2	49/68	72.06	6/30	20
bcl6	5/68	7.35	0/30	0
mum1	26/68	38.24	3/30	10
p53	41/68	60.29	8/30	26.67
p63	3/68	4.41	4/30	13.33
CD10	0/68	0	0/30	0
EBER	19/68	27.94	1/30	3.33

our cases, CD45 was negative in most of the cHLs (64/68; 94.12%), but its expression was also lost in 16 (53.33%) of 30 ALCLs. CD43 was negative in 54 (79.41%) of 68 cHLs and was also negative in 8 (26.67%) of 30 ALCLs. EMA was considered positive in most ALCL cases and a few of the cHL cases. Our data showed that EMA was positive in 17 (56.67%) of 30 ALCLs and 13 (19.12%) of 68 cHLs.

EBER was positive in 19 (27.94%) of 68 cHLs, and the frequency in the different subtypes was as follows: 15.58% (4/26) NS, 50% (8/16) MC, 29.41% (5/17) LR, and 50% (2/4)

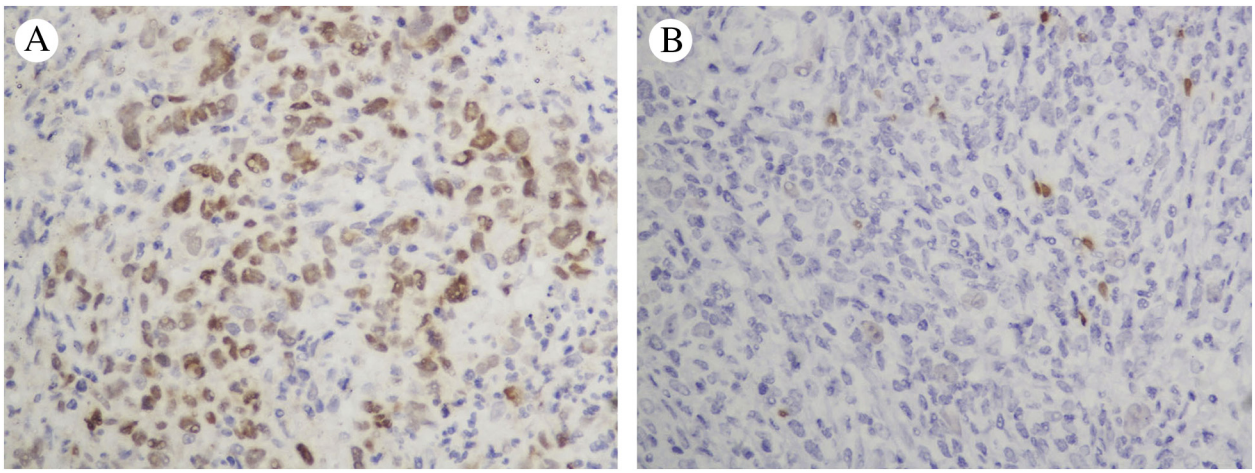

Fig. 3 Pax8 and Pax5 expression in the same cHL case. A, Pax8 was positive in the tumor cells of cHL (original magnification ×400). B, Pax5 was negative in the tumor cells of cHL (×400).

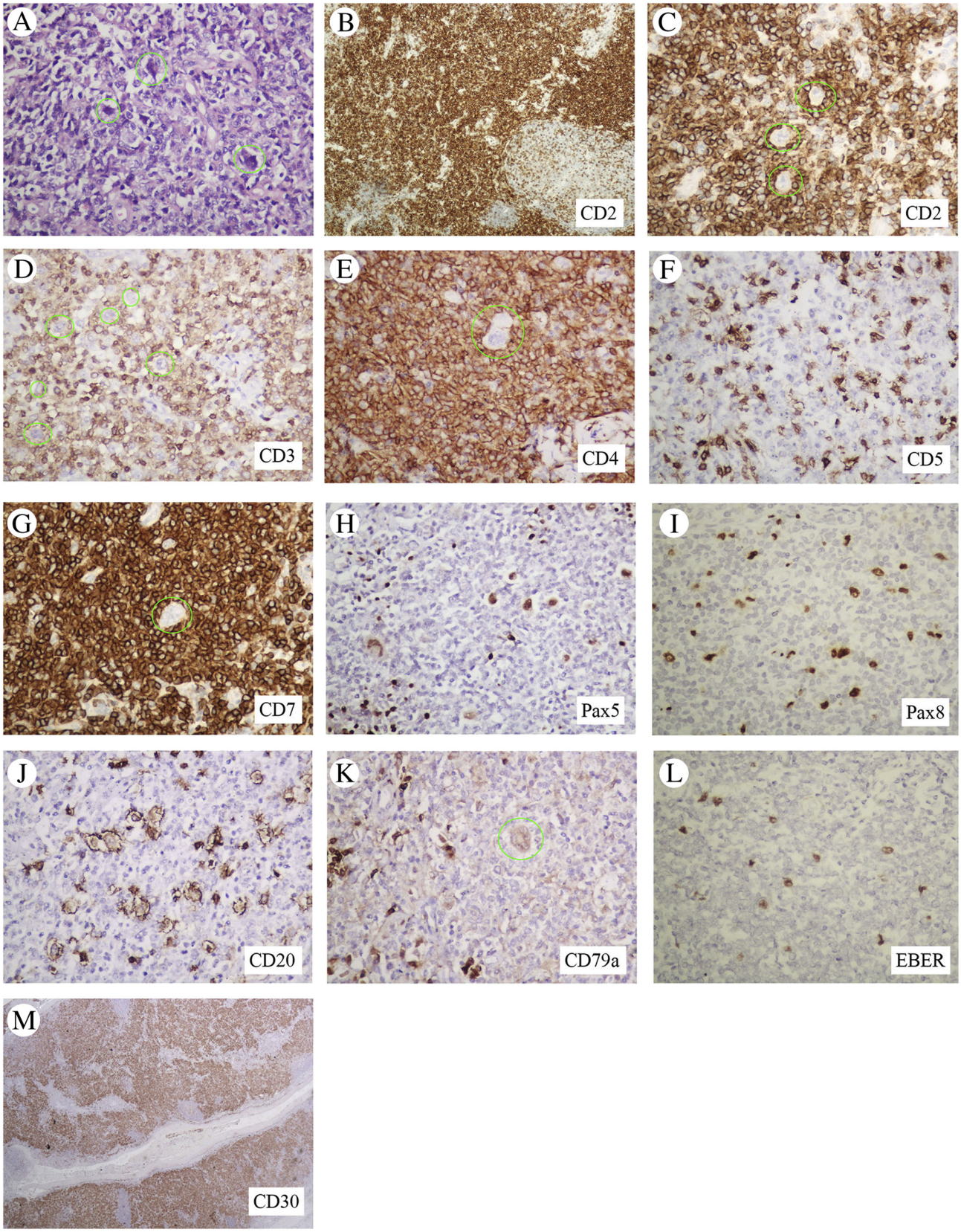

Fig. 4 TCA/BCA expression in ALCL (tonsil). A, Most tumor cells are medium sized, and there are a small subset of cells that are much larger, polymorphic and with highly eosinophilic cytoplasm (original magnification ×400; indicated with green oval). B, CD2 was positive in most of the tumor cells (×200). C-G, The larger tumor cells lost CD2, CD3, CD4, CD5, and CD7 expression (×400; indicated with green oval). H-K, The larger cells were Pax5, Pax8, CD20, and CD79 positive (×400). L, The larger cells are EBER positive (×400). M, Tumor cells are CD30 diffuse positive (×100).

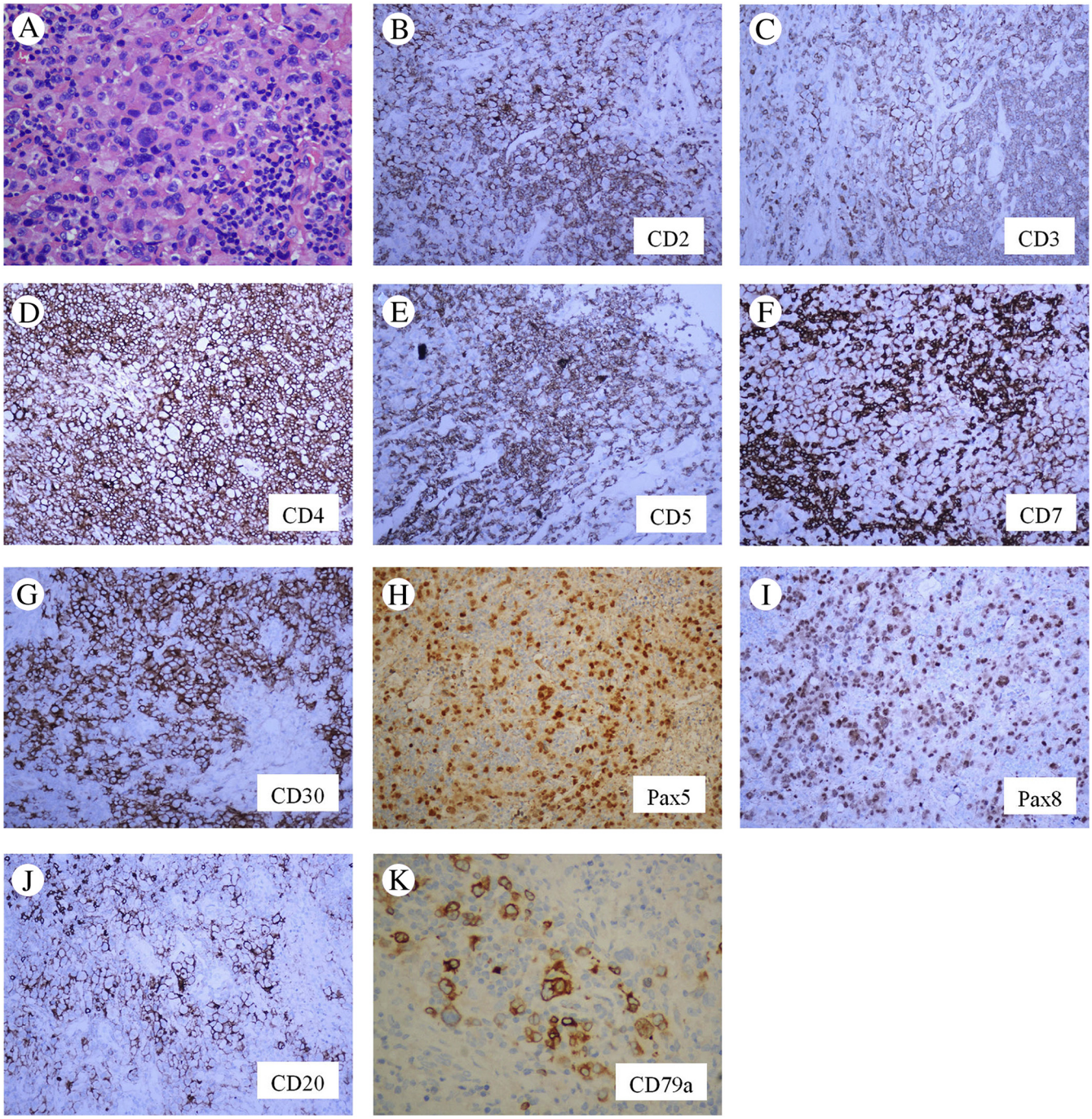

Fig. 5 TCA/BCA expression in cHL. A, Numerous tumor cells were polymorphic and highly cohesive (original magnification ×400). B, C, E, and F, CD2, CD3, CD5, and CD7 showed variable membrane positivity in cHL tumor cells (×200). D, CD4 was strong positive in most cHL cells (×200). G, CD30 expression in tumor cells (×200). H and I, Pax5 and Pax8 staining was weakly positive in tumor cells (×200). J, CD20 was variably positive in tumor cells (×200). K, CD79 was variable positive in fewer tumor cells (×400).

LD. All ALCLs are EBER−, except for 1 case with aberrant BCA expression.

3.2.5. Expression of some unusual biomarkers (bcl2, mum1, bcl6, p53, p63, and CD10) in cHL and ALCL

In addition to CD45 and CD43, bcl2 also showed much higher frequency of expression in cHLs (49/68; 72.06%) than that in ALCLs (6/30; 20%); Mum1 was positive in 26 (38.24%) of 68 cHLs and 3 (10%) of 30 ALCLs. Bcl6 was detected in 5 (7.35%) of 68 cHLs and negative in all ALCLs. p53 was found in 41 (60.29%) of 68 cHLs and 8 (26.67%) of 30 ALCLs. The frequency of p63 expression was much lower in cHLs and ALCLs (3/68 [4.41%] and 4/30 [13.33%], respectively). CD10 was negative in all cHLs and ALCLs.

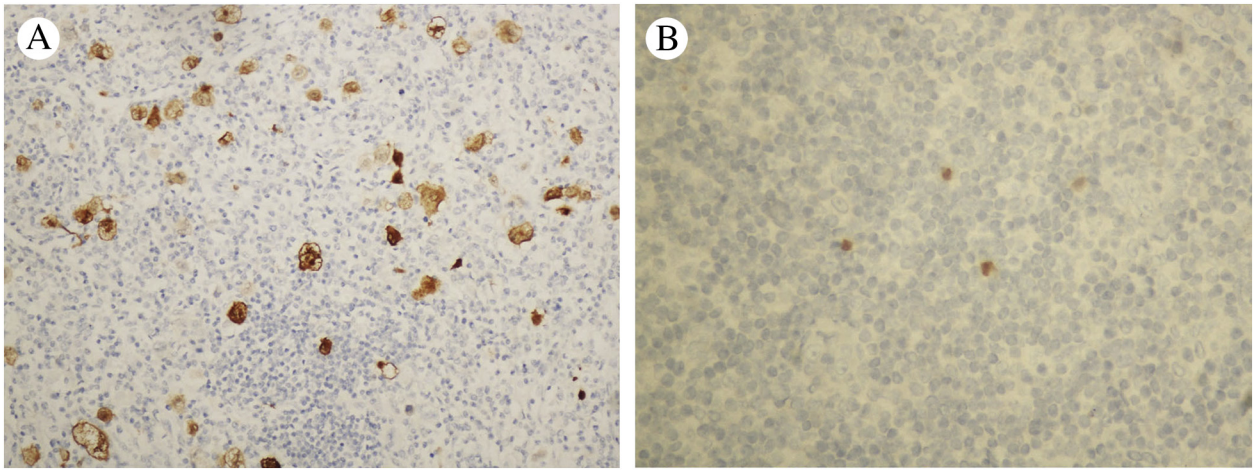

Fig. 6 p16INK4a expression in cHL and reactive hyperplasia cases. A, p16INK4a was positive and highlighted tumor cells in cHL cases. B, p16INK4a was negative in reactive hyperplasia cases (400×). A and B, Original magnification ×400.

3.3. WES of cHLs and ALCLs

We performed WES for 18 samples. Four were cHL samples that exhibited aberrant expression in more than 1 TCA (namely, S1, S2, S3, and S4), 1 was an ALCL sample with aberrant BCA-expression (named as S5), 5 were cHL cases without aberrant TCA expression (cHL group); and the following cases were EBER−: 5 were ALK-ALCL cases without aberrant BCA expression (ALCL group) and 3 were reactive hyperplasia of lymph node cases (control group). The data were intersected for the cHL group and the ALCL group. The data were merged for the control group. Each group was compared with the control group before comparison between the 2 groups.

3.3.1. Single-nucleotide polymorphism and insertion or deletions

The results showed that only 2 mutation sites (GNE and CACNB2) were identified in the cHL group compared with the control group. In the ALCL group, 135 mutation sites (112 genes involved) were identified compared with the control group, suggesting more complex molecular changes in ALCL than those in cHL, and the mutation sites in cHLs and ALCLs did not overlap. The genes with more frequent mutation sites (≥3) were MUC3A, EXOC3L4, METRNL, RPL23AP87, LOC100129697, ZNF283, MAU2, GVINP1, and GPBP1L1. Five aberrant-expression cases (S1-S5) were also checked and had high-confidence somatic mutations. Interestingly, the genetic signature of each TCA-positive cHL case overlapped with that of ALCLs. In S1 to S4, there were 64, 75, 50, and 68 mutation sites that occurred in all ALCLs (135 mutation sites), respectively. In the BCA-positive ALCL case (S5), there was also the *CACNB2* mutation that was seen in all cHLs but not in BCA-negative ALCLs.

3.3.2. Copy number variation

Copy number variation (CNV) analysis was performed using the CNV kit software (v0.8.2) and the data were filtered by log2 ≥ 0.6 or log2 ≤ −1 and depth > 10. Finally, the CNV scatterplot was made, and the involved genes were labeled. The results indicated that compared with the reactive hyperplasia cases, there was no CNV alteration in the cHL group, whereas the amplification of 13 genes was found in the ALCL group compared with the control group. Among these 13 gene alterations, 12 gene amplifications were found in S5,

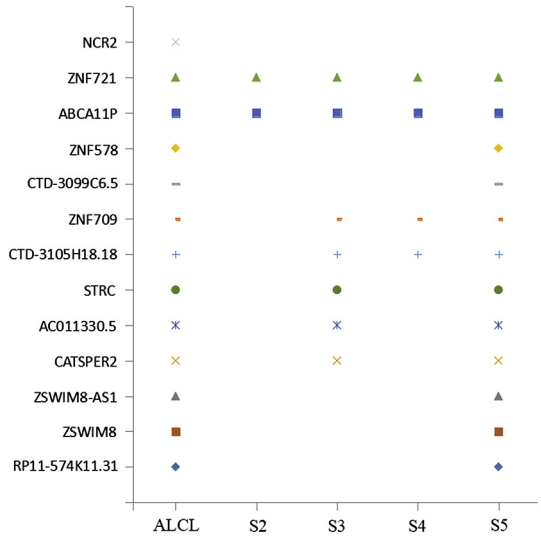

Fig. 7 CNV in ALCL and cases with aberrant expression. There are 13 genes amplified in the ALCL group compared with the control group. Twelve of the 13 genes that were amplified were found in S5, which is an ALCL case that exhibits aberrant BCA expression. However, there are also 2 gene (ABCA11P and ZNF721) amplifications found in S2, 7 gene amplifications in S3, and 4 gene amplifications in S4, which are cHLs cases that exhibit aberrant TCA expression.

and NCR2 alteration was lost. Interestingly, 2 to 7 gene amplifications were also found in S2 to S4, which are cHL samples with aberrant TCA expression (Fig. 7). Four cases (S2-S4) showed ABCA11P and ZNF721 gene amplification, and 3 cases (S3-S5) showed ZNF709 and CTD-3105H18.18 gene amplification.

3.3.3. Involved major signaling pathways

To identify associated biological pathways, we performed a functional enrichment analysis and identified the pathway involved in the extracellular matrix–receptor interaction, protein digestion and absorption, focal adhesion, and glycerophospholipid metabolism. The single-nucleotide polymorphism/insertion or deletions KEGG result showed that the MAPK signaling pathway was involved in the cHL group. Renal cell carcinoma pathways in cancer and Rap1 signaling pathways were mostly involved in the ALCL group, and less than 3 gene mutations were involved in HIF-1. Leukocyte transendothelial migration, PI3K-Akt, and mTOR signaling pathways were also found. The CNV KEGG results revealed no signaling pathway for the cHL group. Several signaling pathways involved in chemical carcinogenesis, drug metabolism, starch and sucrose metabolism, and steroid hormone biosynthesis were found in the ALCL group (Supplementary Figure). Less than 3 gene amplifications involved in Jak-STAT or MAPK signaling pathway were also found.

4. Discussion

In this study, we investigated the histologic, immunohistochemical, and genetic features of cHL and ALCL. It should be mentioned that our samples are Chinese, and the types of cHL in our data were different from those in Europe and America. In Europe and America, the NS subtype accounts for approximately 70% to 80% of cHL, whereas in China, it is less than 50% in most of hospitals. For the expression rates of some immunohistochemical markers in cHL and ALCL, such as CD15, mum1, and CD43, there are also significant differences between Eastern and Western populations.

Our study indicated that there are a certain number of cHLs with aberrant TCA expression, especially in the LD and NS2 subtypes. The frequency of aberrant BCA expression of ALCL was much lower than that of cHL. In the 10 cHL cases with aberrant TCA expression, 3 cases had worse reactivity to ABVD treatment and the prognosis was poor (7-25 months). In fact, previous studies had noted TCA expressing in RS cells [13,14,20,21]. One of the first large experiments was undertaken by Tzankov et al [14], who identified 12 cases (5%) of cHL (including 9 NS subtypes) that expressed 1 or more TCA among 259 cases using a tissue microarray–based approach. In addition, Tzankov et al noted that one-fourth of cHLs with TCA expression in their study died compared with 9 deaths among 105 patients in the control group of cHLs without TCA expression, suggesting a marginal association between TCA expression and adverse outcomes. Venkataraman et al [16] further reported that aberrant TCA expression in cHL is associated with decreased event-free survival and overall survival and pointed out that routine testing for TCAs in cHL may aid in the identification of cHLs with inferior survival. In their cases, most of the TCA-positive cHL cases were of the NS2 subtype. In our study, the frequency of TCA expression in cHLs was much higher than that reported by Tzankov et al (14.71% versus 5%), and there was also a slight difference in the rank order of the frequency of TCA expression (CD4 > CD2 > CD3 > CD5 = CD7) from previous studies. Besides the NS2 subtype, the LD subtype was also a common cHL subtype that exhibited aberrant TCA expression in our cases. Indeed, we also noted that in the data of Venkataraman et al, there were 2 cases of LD subtype, and one of them had aberrant TCA expression; this outcome is consistent with our results. These results may provide a possible explanation for why patients with the LD and NS2 cHL subtypes have poor prognosis. In the study of Venkataraman et al, most of the T-cell/cytotoxic marker–positive cases were also present in the NS/NS2 subgroup of patients. However, in our cases, most of the cHLs with aberrant TCA expression were TIA-1 or granzyme B negative, showing the difference between the Westerners and the Chinese. Considering that in many hospitals, a panel of TCAs (other than CD3) is not frequently performed in routine diagnosis of cHL, we recommend routine testing of a TCA panel (at least CD4, CD2, and CD3) in cHLs (especially in NS2 and LD subtype), and this testing may indicate that these patients could respond poorly to ABVD and have a poor prognosis.

RS cells are characterized by weak expression of Pax5 [7,22], but we observed a small subset of cases that were totally negative for Pax5, possibly providing a mechanism for upregulation of TCA. Cobaleda et al [23] confirmed that repression of Pax5 can convert mature B cells into functional T lymphocytes in a mouse model. In our 10 cHL cases with aberrant TCA expression, there were 1 case that was totally negative for Pax5 and 3 cases that showed very weak Pax5 expression, supporting the idea that Pax5 downregulation may be involved in the aberrant expression of TCA in cHL. For Pax5 and the other B/T-cell markers, in the negative cases, Pax8 seemed to be very helpful for diagnosis. Although there is an article that reported that an N-terminal Pax8 polyclonal antibody shows cross-reactivity with the N-terminal region of Pax5 and is responsible for Pax8 positivity in malignant lymphomas [7], in our study, there were several cases that showed Pax8 positivity but Pax5 negativity in cHL tumor cells. We tested these 2 antibodies in ovarian serous carcinoma cells and found that the tumor cells are Pax8 positive, whereas Pax5 was negative, showing the heterologous characteristics of Pax8 and Pax5 antibodies. We suggest that Pax8 at least can replace Pax5 for the diagnosis of cHL. In addition to Pax8 and Pax5, CD45 and CD43 are also useful for distinguishing cHL from ALCL. Generally, CD45 and CD43 were negative in most cHL cases and positive in most ALCL cases [24-27]. However, in our cases, CD45 and CD43 expressions

were lost in 53.33% and 26.67% of ALCL cases, respectively, showing the need for other immunomarkers. In our immunohistochemistry panel, bcl2 and p53 showed much higher frequency expression in cHL than that in ALCL (72.06% versus 20% and 60.29% versus 26.67%, respectively), providing some insight for the differential diagnosis of cHL and ALCL.

Kim et al [28] reported that expression of p16INK4A in RS cells was found in 49.2% cases, and Al-Salam et al [29] found that p16INK4A was positive in 62% of cHL cases and was strongly associated with the expression of EBV, showing the variant involvement of p16 in the pathogenesis of cHL in different samples. A study conducted by Caliò et al [30] demonstrated that patients with cHL exhibiting an absence or low expression of p16INK4a in RS cells tended to relapse or did not respond to therapy. In our study, an association between p16INK4a expression and the presence of EBV was not found, but we recommend that p16INK4a can be used to highlight the tumor cells in the diagnosis of cHL, especially in cases with few tumor cells.

We also investigated the genetic features of cHL and ALK− ALCL. As expected, the genetic analysis indicated much more complex molecular changes in ALCL than those in cHL. *GNE* and *CACNB2* mutations may be important for cHL pathogenesis, whereas CNV alteration seems to have a minor impact. Despite the clinical and morphologic heterogeneity of cHL, only 2 signaling pathways, JAK–STAT and NF-κB, have been reported to drive pathogenesis [31]. In our data, mutations in the genes mutation involved in MAPK signaling pathways were found, extending our understanding of cHL.

For ALCL, ALK+ cases have been related to the translocation of the ALK gene, and in a fraction of ALK− cases, translocations involving DUSP22 have been described in a fraction of cases [32,33]. In our results, no DUSP22 alterations were detected, but 135 mutation sites (112 genes) were found in all ALK− ALCL cases compared with the control group. We assessed the mutation and CNV distribution of the major pathways associated with the pathogenesis of cHL and ALCL, and several major signaling pathways including renal cell carcinoma, pathways in cancer, Rap1, HIF-1, leukocyte transendothelial migration, PI3K-Akt, and mTOR were found in ALCL. CNV distribution results showed that the case of ALCL with aberrant BCA expression kept most features of the ALCL group but NCR2 gene amplification. Combining all the genetic information, in the cHL cases with aberrant TCA expression, they seemed to lose some of their cHL genetic features and gain some ALCL genetic features. In accordance with this outcome, the ALCL case with aberrant BCA expression also lost some of the ALCL genetic features and acquired some cHL genetic features; this outcome is consistent with the morphologic findings. Although most studies support a B-cell derivation of the tumor cells in HL [34,35], the finding that RS cells can express BCAs and TCAs and may have the possibility of transforming into ALCL raises questions about the origin of cHL.

In conclusion, we explored the histologic, immunohistochemical, and genetic features of cHL and ALK− ALCL. We found that cHL with aberrant TCA expression accounts for a certain proportion of all cHLs, and these cases, including the ALCL case with aberrant BCA expression, have unique morphologic and genetic features. Although these results must be validated in a larger number of samples, our results provide important information for a better understanding of cHL and ALCL and may be useful in informing the development of therapeutic strategies for cHL and ALCL with aberrant expression.

Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.humpath.2018.10.003.

References

[1] Schmid C, Pan L, Diss T, Isaacson PG. Expression of B-cell antigens by Hodgkin's and Reed-Sternberg cells. Am J Pathol 1991;1394:701-7.
[2] Tzankov A, Zimpfer A, Pehrs AC, et al. Expression of B-cell markers in classical Hodgkin lymphoma: a tissue microarray analysis of 330 cases. Mod Pathol 2003;1611:1141-7.
[3] Juco J, Holden JT, Mann KP, Kelley LG, Li S. Immunophenotypic analysis of anaplastic large cell lymphoma by flow cytometry. Am J Clin Pathol 2003;1192:205-12.
[4] Kesler MV, Paranjape GS, Asplund SL, et al. Anaplastic large cell lymphoma: a flow cytometric analysis of 29 cases. Am J Clin Pathol 2007; 1282:314-22.
[5] Hall PA, D'Ardenne AJ. Value of CD15 immunostaining in diagnosing Hodgkin's disease: a review of published literature. J Clin Pathol 1987; 4011:1298-304.
[6] Morente MM, Piris MA, Abraira V, et al. Adverse clinical outcome in Hodgkin's disease is associated with loss of retinoblastoma protein expression, high Ki67 proliferation index, and absence of Epstein-Barr virus–latent membrane protein 1 expression. Blood 1997;906:2429-36.
[7] Foss HD, Reusch R, Demel G, et al. Frequent expression of the B-cell–specific activator protein in Reed-Sternberg cells of classical Hodgkin's disease provides further evidence for its B-cell origin. Blood 1999;949: 3108-13.
[8] Torlakovic E, Torlakovic G, Nguyen PL, Brunning RD, Delabie J. The value of anti–pax-5 immunostaining in routinely fixed and paraffin-embedded sections: a novel pan pre–B and B-cell marker. Am J Surg Pathol 2002;2610:1343-50.
[9] Jalali A, Ha FJ, Chong G, et al. Hodgkin lymphoma: an Australian experience of ABVD chemotherapy in the modern era. Ann Hematol 2016; 955:809-16.
[10] Mottok A, Steidl C. Biology of classical Hodgkin lymphoma: implications for prognosis and novel therapies. Blood 2018;13115:1654-65.
[11] Savage KJ. Peripheral T-cell lymphomas. Blood Rev 2007;214:201-16.
[12] ten Berge RL, de Bruin PC, Oudejans JJ, et al. ALK-negative anaplastic large-cell lymphoma demonstrates similar poor prognosis to peripheral T-cell lymphoma, unspecified. Histopathology 2003;435:462-9.
[13] Kadin ME, Muramoto L, Said J. Expression of T-cell antigens on Reed-Sternberg cells in a subset of patients with nodular sclerosing and mixed cellularity Hodgkin's disease. Am J Pathol 1988;1302:345-53.
[14] Tzankov A, Bourgau C, Kaiser A, et al. Rare expression of T-cell markers in classical Hodgkin's lymphoma. Mod Pathol 2005;1812: 1542-9.

[15] Al Saati T, Galoin S, Gravel S, et al. IgH and TcR-gamma gene rearrangements identified in Hodgkin's disease by PCR demonstrate lack of correlation between genotype, phenotype, and Epstein-Barr virus status. J Pathol 1997;1814:387-93.
[16] Venkataraman G, Song JY, Tzankov A, et al. Aberrant T-cell antigen expression in classical Hodgkin lymphoma is associated with decreased event-free survival and overall survival. Blood 2013;12110:1795-804.
[17] Arun I, Roy P, Arora N, et al. PAX-5 positivity in anaplastic lymphoma kinase-negative anaplastic large cell lymphoma: a case report and review of literature. Int J Surg Pathol 2017;254:333-8.
[18] Ong DM, Cummins KD, Pham A, Grigoriadis G. PAX5-expressing ALK-negative anaplastic large cell lymphoma with extensive extranodal and nodal involvement. BMJ Case Rep 2015;2015. https://doi.org/10.1136/bcr-2015-211159.
[19] Griesser H, Feller AC, Mak TW, Lennert K. Clonal rearrangements of T-cell receptor and immunoglobulin genes and immunophenotypic antigen expression in different subclasses of Hodgkin's disease. Int J Cancer 1987;402:157-60.
[20] Falini B, Stein H, Pileri S, et al. Expression of lymphoid-associated antigens on Hodgkin's and Reed-Sternberg cells of Hodgkin's disease. An immunocytochemical study on lymph node cytospins using monoclonal antibodies. Histopathology 1987;1112:1229-42.
[21] Muschen M, Rajewsky K, Brauninger A, et al. Rare occurrence of classical Hodgkin's disease as a T cell lymphoma. J Exp Med 2000;1912:387-94.
[22] Garcia-Cosio M, Santon A, Martin P, et al. Analysis of transcription factor OCT.1, OCT.2 and BOB.1 expression using tissue arrays in classical Hodgkin's lymphoma. Mod Pathol 2004;1712:1531-8.
[23] Cobaleda C, Jochum W, Busslinger M. Conversion of mature B cells into T cells by dedifferentiation to uncommitted progenitors. Nature 2007;4497161:473-7.
[24] Arici DS, Aker H, Gungor M. Utility of CD15, CD30 & CD45 in the immunohistochemical diagnosis of Hodgkin's disease by antigen retrieval method. Indian J Med Res 1999;109:33-7.
[25] Karnik S, Srinivasan B, Nair S. Hodgkin's lymphoma: immunohistochemical features and its association with EBV LMP-1. Experience from a South Indian hospital. Pathology 2003;353:207-11.
[26] Vasef MA, Alsabeh R, Medeiros LJ, Weiss LM. Immunophenotype of Reed-Sternberg and Hodgkin's cells in sequential biopsy specimens of Hodgkin's disease: a paraffin-section immunohistochemical study using the heat-induced epitope retrieval method. Am J Clin Pathol 1997;1081:54-9.
[27] Clavio M, Rossi E, Truini M, et al. Anaplastic large cell lymphoma: a clinicopathologic study of 53 patients. Leuk Lymphoma 1996;223-4:319-27.
[28] Kim LH, Peh SC, Poppema S. Expression of retinoblastoma protein and P16 proteins in classic Hodgkin lymphoma: relationship with expression of p53 and presence of Epstein-Barr virus in the regulation of cell growth and death. Hum Pathol 2006;371:92-100.
[29] Al-Salam S, Awwad A, Sudhadevi M, et al. Epstein-Barr virus infection correlates with the expression of COX-2, p16(INK4A) and p53 in classic Hodgkin lymphoma. Int J Clin Exp Pathol 2013;612:2765-77.
[30] Caliò A, Zamo A, Ponzoni M, et al. Cellular senescence markers p16INK4a and p21CIP1/WAF are predictors of Hodgkin lymphoma outcome. Clin Cancer Res 2015;2122:5164-72.
[31] Kuppers R. The biology of Hodgkin's lymphoma. Nat Rev Cancer 2009;91:15-27.
[32] Feldman AL, Dogan A, Smith DI, et al. Discovery of recurrent t(6;7)(p25.3;q32.3) translocations in ALK-negative anaplastic large cell lymphomas by massively parallel genomic sequencing. Blood 2011;1173:915-9.
[33] King RL, Dao LN, McPhail ED, et al. Morphologic features of ALK-negative anaplastic large cell lymphomas with DUSP22 rearrangements. Am J Surg Pathol 2016;401:36-43.
[34] Hummel M, Marafioti T, Stein H. Clonality of Reed-Sternberg cells in Hodgkin's disease. N Engl J Med 1999;3405:394-5.
[35] Marafioti T, Hummel M, Foss HD, et al. Hodgkin and Reed-Sternberg cells represent an expansion of a single clone originating from a germinal center B-cell with functional immunoglobulin gene rearrangements but defective immunoglobulin transcription. Blood 2000;954:1443-50.