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RESEARCH

Circulating tumor DNA assisting lymphoma genetic feature profiling and identification

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Abstract

Introduction Lymphoma tissue biopsies cannot fully capture genetic features due to accessibility and heterogeneity. We aimed to assess the applicability of circulating tumor DNA (ctDNA) for genomic profiling and disease surveillance in classic Hodgkin lymphoma (cHL), primary mediastinal large B-cell lymphoma (PMBCL), and diffuse large B-cell lymphoma (DLBCL).

Methods Tumor tissue and/or liquid biopsies of 49 cHLs, 32 PMBCLs, and 74 DLBCLs were subject to next-generation sequencing targeting 475 genes. The concordance of genetic aberrations in ctDNA and paired tissues was investigated, followed by elevating ctDNA-based mutational landscapes and the correlation between ctDNA dynamics and radiological response/progression.

Results ctDNA exhibited high concordance with tissue samples in cHL (78%), PMBCL (84%), and DLBCL (78%). In cHL, more unique mutations were detected in ctDNA than in tissue biopsies ($P < 0.01$), with higher variant allele frequencies ($P < 0.01$). Distinct genomic features in cHL, PMBCL, and DLBCL, including *STAT6*, *SOCS1*, *BTG2*, and *PIM1* alterations, could be captured by ctDNA alone. Prevalent PD-L1/PD-L2 amplifications were associated with more concomitant alterations in PMBCL ($P < 0.01$). Moreover, ctDNA fluctuation could reflect treatment responses and indicate relapse before imaging diagnosis.

Conclusions Lymphoma genomic profiling by ctDNA was concordant with that by tumor tissues. ctDNA might also be applied in lymphoma surveillance.

Keywords Circulating tumor DNA · Lymphoma · Classic Hodgkin lymphoma · Primary mediastinal large B-cell lymphoma · Diffuse large B-cell lymphoma · Disease surveillance

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Introduction

Lymphomas are a heterogeneous group of hematological malignancies that arise from the lymphatic system, including two main types: Hodgkin lymphoma (HL) and non-Hodgkin lymphoma (NHL) [1]. Classic Hodgkin lymphoma (cHL) is the most prevalent HL subtype, accounting for approximately 95% of HL cases; however, the age-standardized incidence rate of HL is as low as between 2 and 3 per 100,000 persons per year [2, 3]. Primary mediastinal large B-cell lymphoma (PMBCL) is a rare NHL subtype rising from thymic medullary B cells, with incidence rate of 0.04 per 100,000 persons per year [4]. PMBCL accounts for 2–3% of all NHL cases and is more frequently observed in young females [5]. By contrast, diffuse large B-cell lymphoma (DLBCL) is the most prevalent NHL subtype,

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accounting for 20.0–63.9% of NHL cases, with incidence ranging from 2.3 to 23.8 cases per 100,000 persons per year [6].

PMBCL was initially categorized as a subtype of DLBCL based on clinicopathologic features [7], whereas it is now recognized as a distinct entity and separated from DLBCL by the World Health Organization [8, 9]. Interestingly, recent studies on gene expression profiles have revealed that PMBCL displays shared molecular features with cHL, including activated JAK/STAT [10–15], and Nuclear Factor Kappa B (NF-ƙB) pathways [10, 15, 16], as well as PD-L1 and PD-L2 mediated immune evasion [11, 15, 17].

cHL is characterized by the presence of malignant lymphoid cells, called Reed-Sternberg cells, which comprise few malignant cells in HL tissue [18]. While tissue biopsy has traditionally been used to assess the genetic landscape of cHL, a study has highlighted the limited ability of this approach to capture clonal diversity and intra-tumoral heterogeneity [19]. On the other hand, due to the advantage of liquid biopsy, circulating tumor DNA (ctDNA) extract from plasma has shown promise in capturing the genetic profiles of cHL and overcoming the shortage of tissue biopsy. For example, previous studies have demonstrated high concordance between ctDNA and tumor tissue biopsy-based next-generation sequencing (NGS) approaches [20, 21] in cHL. Similarly, ctDNA has also been shown to fully capture the mutational landscape of PMBCL and DLBCL [22–24]; however, in previous studies, genetic profile comparison between tumor tissue and plasma samples were based on relatively small NGS panels. The assessment of liquid biopsy applicability in these three types of lymphomas has not been comprehensively investigated using a large NGS panel.

In this study, we retrospectively investigated the mutational profiles of cHL, PMBCL, and DLBCL in a Chinese population using an NGS panel covering 475 hematopoietic and lymphoid neoplasm related genes. By utilizing both tissue and liquid biopsies, we aimed to provide a more comprehensive and accurate picture of the genomic landscape of these lymphomas and facilitate disease diagnosis and classification.

Materials and methods

Patients and study design

Patients diagnosed with lymphomas between March 2018 and May 2022 were retrospectively enrolled at Cancer Hospital of Shantou University Medical College. The inclusion criteria were as follows: (1) initially diagnosed with cHL, PMBCL or DLBCL; (2) having treatment-naïve/post-treatment tumor tissue and/or plasma samples meeting quality control standards for genomic DNA or ctDNA extraction; (3) undergoing genomic profiling using an NGS panel targeting 475 hematopoietic and lymphoid neoplasm related genes (Supplementary Table 1); (4) with at least one detectable somatic alteration in tumor tissue and/or ctDNA. This study was approved by Ethics Committee of the Cancer Hospital of Shantou University Medical College (No. 2,023,059). All patients signed informed consent forms prior to enrollment and sample collection.

Among patient having both tumor tissue and plasma samples, the tissue sample was defined as a paired sample if it was collected within three months before/after collecting the plasma sample and systemic therapy was not provided during the three-month period. To assess the applicability of ctDNA testing in lymphomas, we compared the performance of ctDNA with the paired tissue biopsies, including the concordance of detected genetic alterations, the number of unique alterations defined as the alterations exclusively identified in ctDNA and tumor tissue, and the difference of variant allele frequency (VAF). Additionally, the genomic profiles were studied to assess the utility of ctDNA for lymphoma classification, and three publicly available external datasets of cHL [20], PMBCL [25], and DLBCL [26] were used to validate our findings.

DNA extraction, library preparation, and NGS

Tumor genomic DNA from formalin-fixed, paraffin-embedded (FFPE) samples was extracted using QIAamp DNA FFPE Tissue Kit (QIAGEN, Dusseldorf, Germany). 10 mL peripheral blood was collected and centrifuged (1800× g, 10 min, at room temperature) within two hours to separate plasma, from which ctDNA was extracted using QIAamp Circulating Nucleic Acid Kit (QIAGEN, Dusseldorf, Germany). Oral swab DNA was prepared by QIAamp DNA Mini Kit (QIAGEN, Dusseldorf, Germany) as control for germline mutations. Sequencing libraries were prepared using the KAPA Hyper Prep Kit (KAPA Biosystems, Wilmington, MA, USA). Briefly, fragment genomic DNA underwent end-repairing, A-tailing, adapter ligation, size selection, polymerase chain reaction amplification, and purification, sequentially. Target enrichment was performed using customized xGen lockdown probes panel targeting 475 hematopoietic and lymphoid neoplasm related genes (Hemasalus™, Nanjing Geneseeq Inc., Nanjing, China). All procedures were conducted following the manufacturers' instructions. Enriched libraries were sequenced on Illumina Hiseq4000 NGS platforms (Illumina, San Diego, CA, USA). The average sequencing depth of tissue and liquid biopsies were 1203X and 4554X, respectively.

Sequencing data processing and mutation retaining

FASTQ file quality control was performed using Trimmomatic, and leading/trailing low quality (reading < 15) or N bases were removed [27]. Sequencing data were aligned to the reference human genome (build hg19) and then processed using the Picard suite and the Genome Analysis Toolkit (GATK) [28, 29]. Germline mutations were filtered out by comparing to the oral swab controls. One somatic mutation was retained if it had VAF ≥ 0.5% and at least three unique reads on different strands with good quality scores, after filtering for common single nucleotide polymorphisms. Gene fusions and copy number variations (CNV) were analyzed using FACTERA and ADTEx [30, 31], respectively, followed by manual review in Integrative Genomics Viewer Software (IGV, Broad Institute, Cambridge, MA, USA). CNV with a fold change ≥ 1.6 and ≤ 0.6 was identified as CNV amplification and deletion, respectively.

Variables and statistical analysis

Patient age was categorized into four subgroups, including < 18 years, 18–45 years, > 45 years, and unknown. Frequencies of independent subgroups were compared using Fisher's exact test. Median and mean comparison were performed using Wilcoxon signed-rank test and two-sample t test, respectively. All quoted *P*-values were two-tailed, and *P*-values < 0.05 were considered to be statistically significant. Data were analyzed using R software (version 4.0.3).

Results

Patient characteristics

A total of 155 patients with lymphoma having tumor tissue and/or plasma samples were enrolled, including 49 cHLs, 32 PMBCLs, and 74 DLBCLs. Within our cohort, 110 patients had treatment-naive samples, 33 had post-treatment samples with active disease at the time of sampling and a median of 108 days since the start of treatment (range: 1 to 829 days), and for 12 patients, the treatment status of the samples was unknown. Details on tumor content of tissue samples and ctDNA concentration of plasma samples are summarized in Supplementary Fig. 1. One hundred and eleven patients with paired tissue and plasma samples were included in the paired analyses (22 cHLs, 15 PMBCLs, and 74 DLBCLs, Fig. 1A). The clinical and demographic characteristics of the patients were summarized in Supplementary Table 2. Patients with cHL appeared to have a relatively high proportion of males in comparison to the PMBCL or DLBCL group (63.3% vs. 53.8%, $P = 0.30$). In contrast, patient age varied significantly across three groups ($P < 0.01$). 26.5% of cHLs were < 18 years, while most of PMBCLs and DLBCLs were adults at least 18 years old. Patients aged 18–45 years accounted for 13.5% of DLBCLs, which was significantly lower than the proportion in PMBCLs (87.5%, $P < 0.01$) and cHLs (46.9%, $P < 0.01$). Disease stage at diagnosis, lactate dehydrogenase level, and bulky site size, were also presented in Supplementary Table 2 despite a relatively large proportion of patients with missing values.

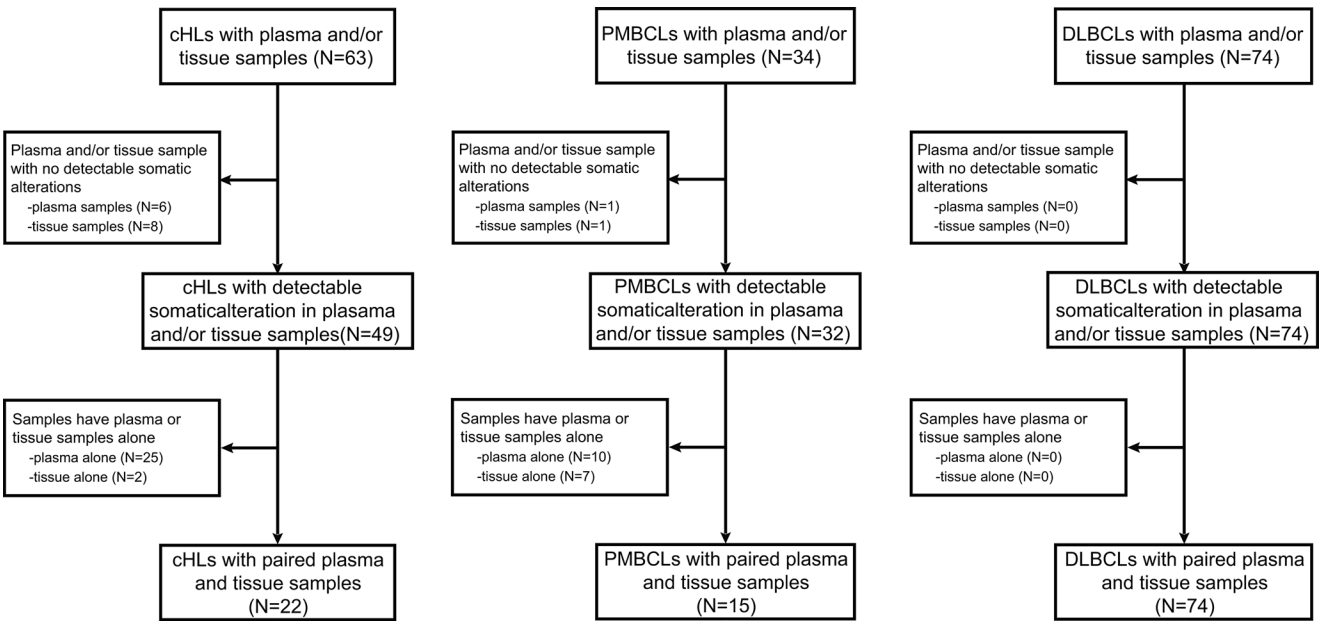

Fig. 1 Patient inclusion and demographics. The flowchart outlining the patient inclusion criteria. A total of 111 patients with paired tissue and plasma samples were enrolled in this study, including 22 cHLs, 15 PMBCLs, and 74 DLBCLs

Genomic features comprehensively captured by ctDNA

The concordance of detected genetic alterations between tumor tissue and plasma samples was investigated in cHL, PMBCL, and DLBCL separately. Among 22 cHLs with paired samples, 78% of tissue-based genetic aberrations were captured by ctDNA, with relatively frequently detected *SOCS1*, *STAT6*, and *TNFAIP3* alterations in ctDNA (Fig. 2A). Notably, *PD-L1* and *PD-L2* CNV amplifications were exclusively identified by ctDNA. *B2M* mutations, which were the most prevalent alterations observed, had similar detection rates in two biopsy types, with four and two mutations exclusively identified in plasma and tissue samples respectively (Fig. 2A). Moreover, unique mutations were more common in cHL plasma samples than in paired tissue samples ($P < 0.01$, Fig. 2B), with higher VAFs observed in ctDNA ($P < 0.01$, Fig. 2C). Increased VAFs of shared mutations were also observed in ctDNA compared to paired tissue samples ($P = 0.06$, Fig. 2C). When assessing the applicability of liquid biopsies in 15 PMBCL and 74 DLBCL patients, we observed a high concordance of detectable alterations between ctDNA and paired tissue samples (agreement: PMBCL, 84%; DLBCL, 78%, Fig. 2D, E). Our data also demonstrated that the number of unique mutations appeared to be comparable between ctDNA and tumor

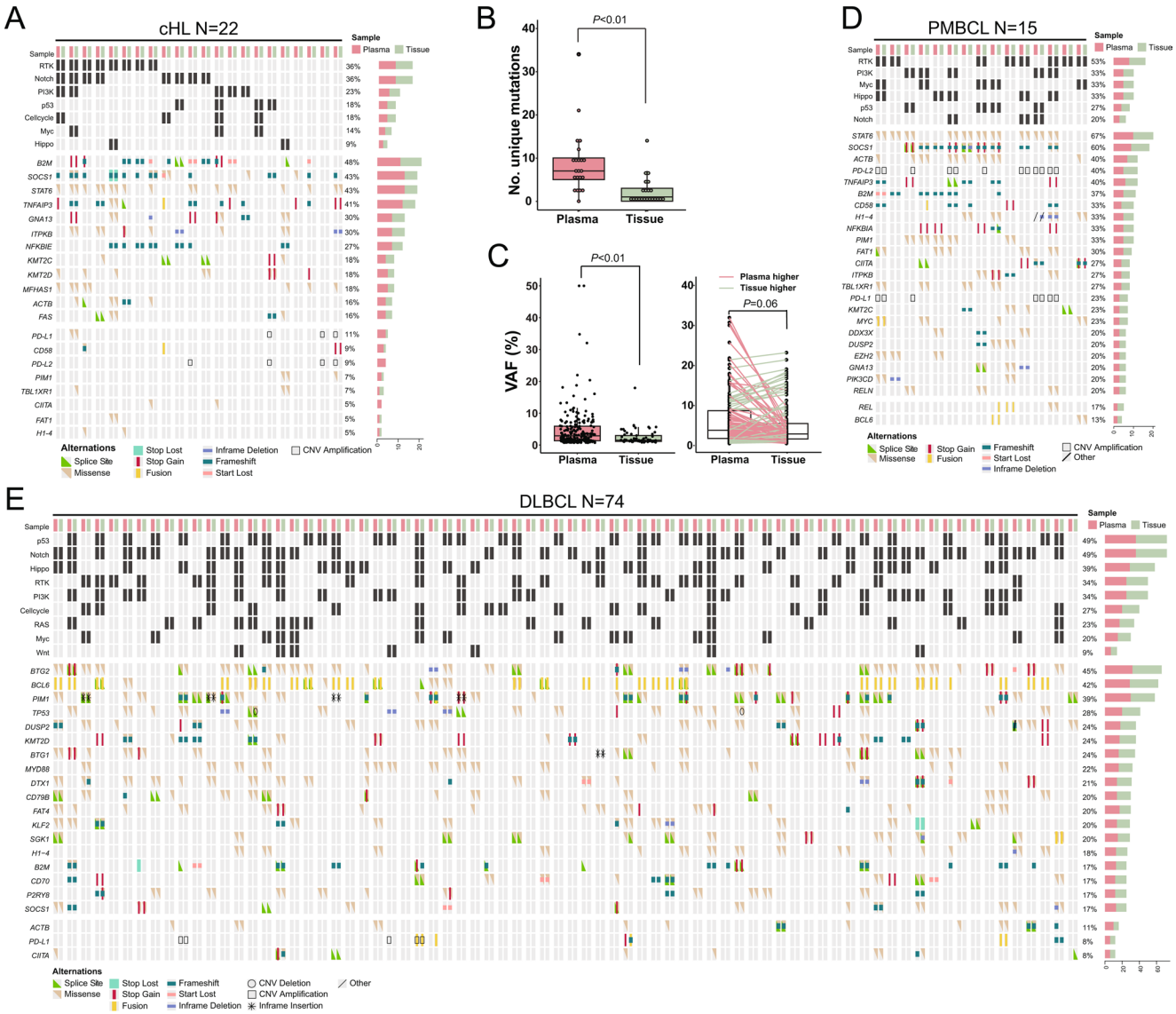

Fig. 2 ctDNA concordance in 22 cHLs, 15 PMBCLs and 74 DLBCLs with paired samples. (**A**) The oncoplot of 22 cHL patients with paired plasma and tissue samples. (**B**) The number of unique genetic alterations observed in plasma and tissue biopsies of cHL. (**C**) Boxplots comparing the unique mutation VAFs, and shared mutation VAFs in plasma and tissue sample of cHL. (**D**, **E**) The oncoplot of 15 PMBCLs and 74 DLBCLs with paired plasma and tissue samples

tissues in PMBCL (Supplementary Fig. 2A) and DLBCL (Supplementary Fig. 2B).

ctDNA assisting in lymphoma identification and classification

As considerable genetic alterations were detected in plasma samples alone, we then investigated if plasma-unique features could assist in distinguishing PMBCL from cHL and DLBCL. Thus, the mutational landscapes based on tissue and liquid biopsies were combined and summarized in Supplementary Fig. 3 for further analyses. Within 19 cHLs having paired samples, the most frequently altered gene was *SOCS1* (64%, Supplementary Fig. 3), followed by *B2M* (59%), *STAT6* (59%), and *TNFAIP3* (59%). Mutated *STAT6* genes were also commonly identified in 15 PMBCLs with paired samples ($P > 0.99$, Fig. 3A); however, *STAT6* mutations were rare in DLBCL when compared to PMBCL ($P < 0.01$, Fig. 3A). Similar to *STAT6*, the prevalence of *SOCS1* mutation was comparable between cHL and PMBCL ($P > 0.99$, Fig. 3B), whereas *SOCS1* mutations were less common in DLBCL ($P < 0.01$, Fig. 3B). *SOCS1* mutation subtypes exhibited a huge diversity, with considerable truncating variants (black dots, Fig. 3C). Both cHL and PMBCL were rarely detected with *BTG2* mutations compared to DLBCL ($P = 0.01$, Fig. 3D). In contrast, similar proportions of PMBCL and DLBCL patients were detected with *PIM1* mutations ($P = 0.57$, Fig. 3E), whereas few cHL patients carried *PIM1* mutations ($P = 0.10$, Fig. 3E), suggesting the possible similarity between PMBCL and DLBCL in molecular features. Moreover, *PD-L1* and *PD-L2* CNV amplifications were observed in patients with PMBCL and cHL (Supplementary Fig. 3), suggesting potentially high PD-L1 and PD-L2 expression. When compared to patients with wild-type *PD-L1* and *PD-L2*, the number of concomitant alterations was significantly higher in patients harboring *PD-L1* and/or *PD-L2* CNV amplification ($P < 0.01$, Fig. 3F), and no obvious differences were observed between plasma and tissue samples. In DLCBL, *BCL6* fusions were detected in 36.5% of patients with paired samples. Although most of these fusions involved immunoglobulin (Ig) genes (Supplementary Fig. 3), multiple non-Ig chromosomal loci were

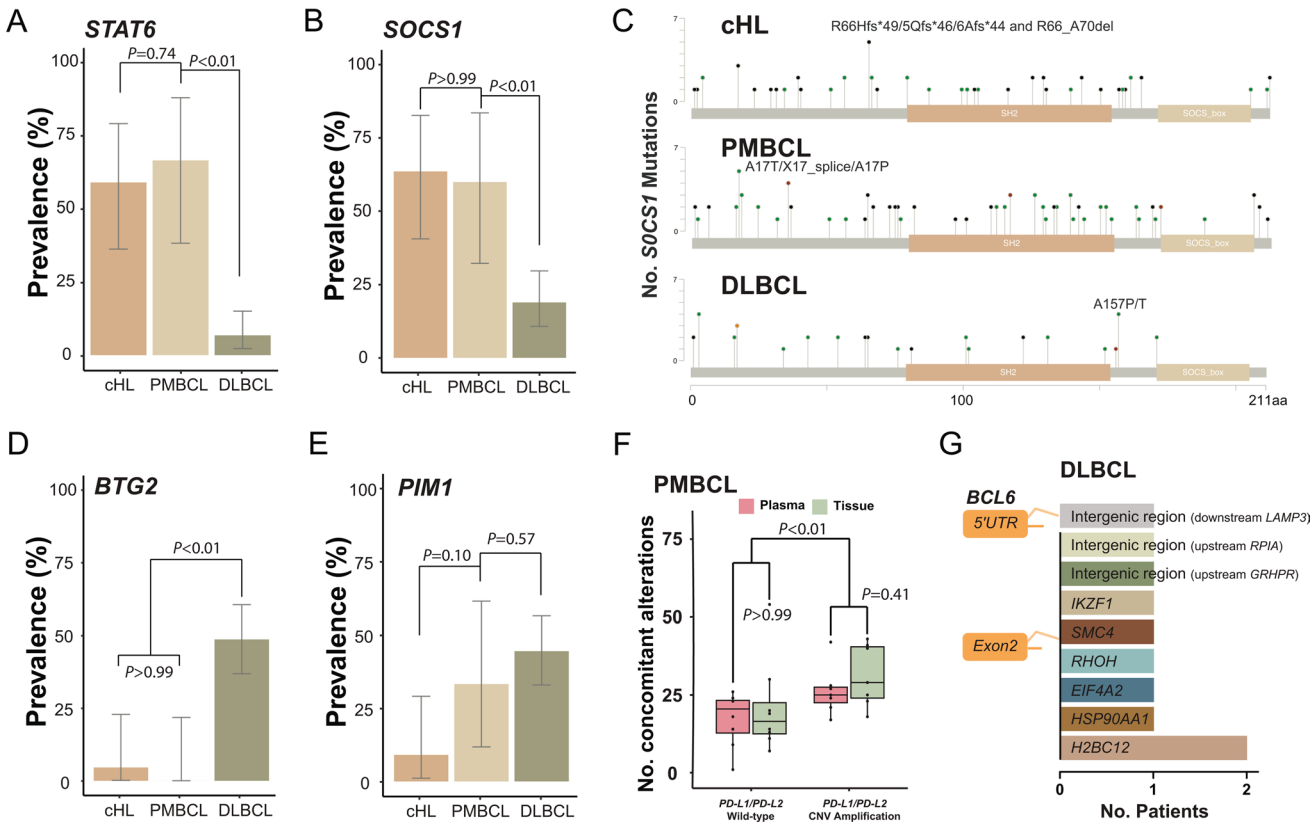

Fig. 3 Shared and unique genomic features in three kinds of lymphomas (**A**) Comparison of *STAT6* mutations prevalence in cHL, PMBCL, and DLBCL. (**B**) Comparison of *SOCS1* mutations prevalence in DLBCL, PMBCL, and cHL. (**C**) Lollipop plots revealing that no obvious hotspots *SOCS1* mutations. (**D**) Comparison of *BTG2* mutations prevalence in cHL PMBCL, and DLBCL, with cHL exhibiting significantly lower *BTG2* mutation rates than DLBCL. (**E**) Comparison of *PIM1* mutations prevalence in cHL PMBCL, and DLBCL, with cHL exhibiting potentially lower *PIM1* mutation rates than PMBCL and DLBCL. (**F**) The boxplot comparing the number of concomitant alterations for carriers and non-carriers of *PD-L1* and/or *PD-L2* CNV amplification. (**G**) *BCL6* fusions detected in DLBCL

identified as partners, including *LAMP3*, *RPIA*, *GRHPR*, *IKZF1*, *SMC4*, *RHOH*, *EIF4A2*, *HSP90AA1*, and *H2BC12* (Fig. 3G).

Next, we evaluated the utility of ctDNA for lymphoma classification by identifying typical genetic alterations that were discovered in paired samples. *STAT6* mutations was more common in PMBCL than in cHL, and patients with DLBCL exhibited the lowest *STAT6* mutation prevalence ($P < 0.01$, Fig. 4A). Comparable prevalence of *STAT6* mutations was observed in ctDNA when compared to tissue biopsies. Similar results in the prevalence of *SOCS1* mutations were also observed, even though ctDNA appeared to capture more *SOCS1* mutations in comparison to tissue samples (Fig. 4B). The characteristics of mutated *BTG2* (Supplementary Fig. 4A) and *PIM1* (Supplementary Fig. 4B) profiled using ctDNA alone did not show obvious differences in comparison to those using paired samples. Based on plasma samples alone, PMBCL patients harboring *PD-L1* and/or *PD-L2* CNV amplifications still had higher concomitant mutation numbers than *PD-L1* and *PD-L2* wild-type patients ($P = 0.07$, Fig. 4C).

ctDNA performance validation by comparing to external datasets

To further confirm the performance of ctDNA and the NGS panel applied in this study, the prevalence of multiple genetic alterations in our lymphoma patients with plasma samples were compared to that in external datasets. The prevalence of cHL related genes in our study was similar to the external cHL dataset including 106 tissue/plasma samples; however, some genes were not covered by the NGS panel used in the external cHL cohort, such as *SOCS1*, *ACTB*, *KMT2C*, *BTG2* (Supplementary Table 3). The detection rates were also comparable between our study cohorts and external cohorts in PMBCL and DLBCL; however, intriguingly, *BTG2* mutations were significantly more frequently identified in our DLBCL cohort than in the external dataset consisting of 79 DLBCL plasma samples (43.2% vs. 6.3%, $P < 0.01$, Supplementary Table 3).

Lymphoma disease surveillance by serial ctDNA

Finally, we explored the utility of ctDNA for lymphoma disease surveillance by analyzing the correlation between ctDNA positivity and responses to treatment or disease progression. Herein, we presented a patient receiving multiple liquid biopsies since the initial diagnosis of cHL in February 2020 (Fig. 5). When the patient was pathologically diagnosed with stage IIA cHL, several genetic alterations were detected in ctDNA, with VAF ranging approximately 1.5–5%. The patient underwent chemotherapy (ABVD: adriamycin, bleomycin, vinblastine, plus dacarbazine) for six cycles. At the cycle 3 (Month 3), complete response was achieved, with no detectable mutations observed by ctDNA test, which suggested that ctDNA positivity could reflect the chemotherapy treatment efficacy. Also, ctDNA status remained negative in Month 6 and 9. However, considerable mutations were identified by the ctDNA test in Month 19, suggesting the possible disease progression which was further confirmed as relapsed stage IV cHL. After immune-based therapy of which the best of response was complete response, ctDNA clearance was observed in Month 21.

Discussion

In this study, we assessed the applicability of ctDNA in lymphomas, and our data demonstrated a high concordance of genetic alterations identified in paired tumor tissue and plasma samples. More alterations were detected exclusively in cHL plasma than in tissue samples, highlighting liquid biopsies as a valuable supplementary approach for genomic profiling in lymphomas. Compared to DLBCL, the genomic characteristics of PMBCL were more similar to cHL, even though these three lymphomas had their unique features. Our data also confirmed the feasibility of serial ctDNA for disease surveillance during chemotherapy and immune-based therapy.

The good concordance between tumor tissue and liquid biopsies in our cohort revealed the promise of ctDNA in

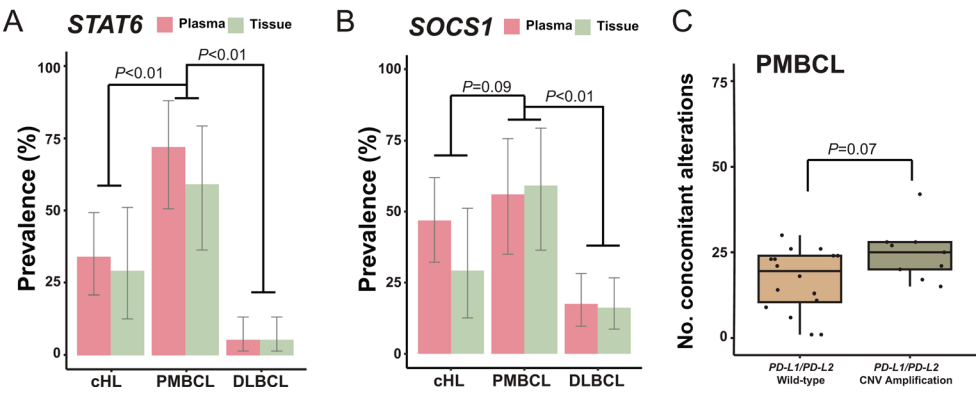

Fig. 4 Genetic features of lymphomas based on ctDNA alone. (**A**) The prevalence of mutated *STAT6* genes in three lymphomas. (**B**) The prevalence of mutated *SOCS1* genes in three lymphomas. (**C**) The boxplot comparing the number of concomitant alterations for carriers and non-carriers of *PD-L1* and/or *PD-L2* CNV amplification in PMBCL

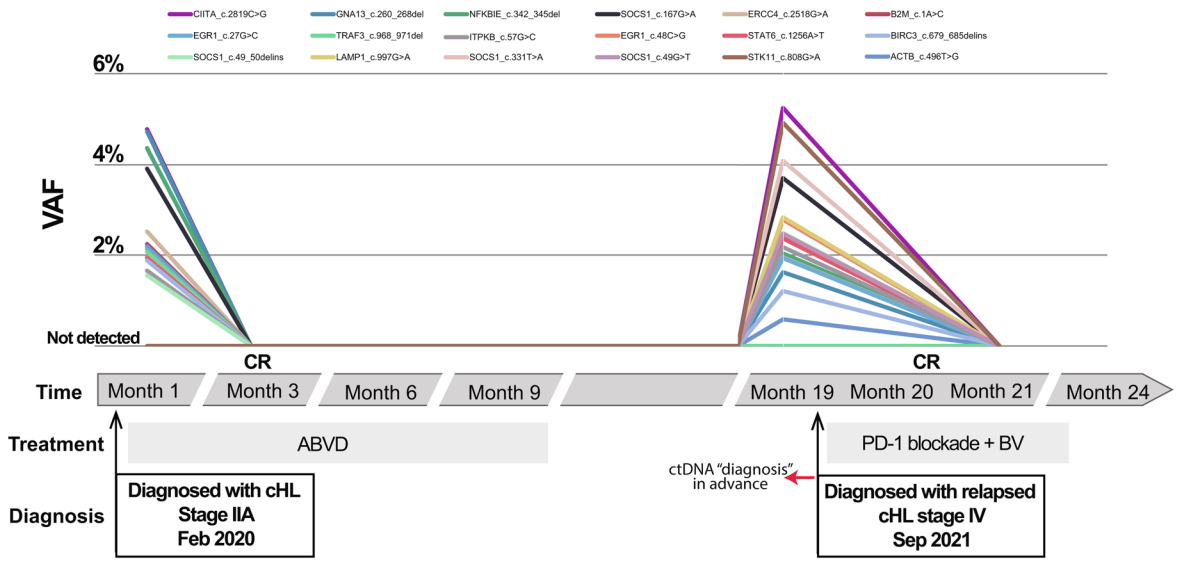

Fig. 5 ctDNA surveillance for disease progression and responses to chemotherapy or immune-based therapy. CR: complete response, MAF: mutant allele frequency, ABVD: adriamycin, bleomycin, vinblastine, plus dacarbazine, BV: brentuximab vedotin

lymphoma research. *STAT6*, *SOCS1* and *TNFAIP3*, which were frequently mutated genes, were more likely to be identified in cHL plasma samples than in tissue samples, suggesting that potential driver mutations of cHL could be captured more sensitively by ctDNA. A recent study has highlighted that DNASE1L3, which is an enzyme breaking down multinucleosomal DNA molecules, shows elevated activity in cHL compared to large B cell lymphomas. The increased activity potentially leading to greater ctDNA release by HRS cells, suggesting a more comprehensive method for lymphoma genomic profiling through plasma ctDNA [32]. A previous study by Spina et al. also demonstrated the utility of ctDNA in profiling genomic features, investigating clonal evolution, and detecting residual disease in cHL [20]. However, a relatively small panel covering 77 genes was applied by Spina et al., and several critical genes, such as *SOCS1*, *PD-L1* and *PD-L2*, were not included. Consistent with our results, $STAT6^{N417Y}$ and $STAT6^{N419N}$ were identified as potential hotspot mutations by both NGS and allele-specific polymerase chain reaction; however, $STAT6^{N419H}$, which was commonly observed in our cHL patients, was not identified by Spina et al. A study by Tiacci et al. defined $STAT6^{N419H}$ as a potential hotspot mutation of cHL by whole-exome sequencing, and, interestingly, they also successfully detected $STAT6^{N421S}$ in cHL patients harboring $STAT6^{N417Y}$ simultaneously [33]. For PMBCL and DLBCL, although previous studies tried assessing the applicability of ctDNA, comprehensive analyses based on paired tissue and liquid biopsies, as well as large NGS panels have not been well performed. For instance, mutational landscape of liquid biopsy was highly consistent with that of tissue biopsy, with sensitivity of 69% (95% confidence interval: 60–78%) in a customized 112-gene panel [25]. In DLBCL, measured mutant molecules per mL, which was calculated using ctDNA and an approximately 320Kb panel including recurrently mutated DLBCL genes, was used to stratify patients with inferior progression-free survival and overall survival [34]. Therefore, based on previous studies and our study, findings on both HL and NHL systemically demonstrated that liquid biopsy would be a promising approach to genomic profiling in lymphomas.

Our data showed that mutated *SOCS1* and *STAT6* genes were common in both cHL and PMBCL. Previous studies revealed that *SOCS1* inactivating mutations, CNV deletions, and inactivating fusions could negatively regulate the JAK-STAT signaling pathway, and further leaded to the overexpression of STAT proteins [35]. Moreover, it is believed that *STAT6* mutations could also cooperated with SOCS1 disruption [33]. Consistent with these previous findings, our data demonstrated that *STAT6* might be an oncogene with hotspot mutations related to gain-of-function, and *SOCS1* truncating mutations were observed. Although we did not observe a co-occurrence pattern in alterations between these two genes, considerable cHL and PMBCL patients harbored them simultaneously. Noerenberg et al.'s study revealed no significant co-occurrence ($q = 0.18$) between *STAT6* and *SOCS1* mutations and their minimal impact on progression-free survival and overall survival in PMBCL patients [36]. However, it might be interesting to compare the prognosis of cHL patients with both *SOCS1* and *STAT6* mutations and prognosis of patients with only one of them. Noerenberg et al. also highlighted the prognostic significance of *DUSP2* and *CD58* mutations in PMBCL. We observed high alignment for *DUSP2* (100%, 3 of 3) and substantial agreement

for *CD58* (67%, 4 of 6) mutations between ctDNA and tissue biopsies, underscoring ctDNA's utility in predicting patients' outcomes [36]. *GNA13*, a tumor suppressor inhibiting AKT phosphorylation, was also a frequently mutated gene in our patients with cHL, which was consistent with previous studies [37, 38]. *GNA13* mutations related to loss of function were considered to be associated with *STAT6* aberrations [35]; however, our data did not show a statistically significant co-occurrence pattern between mutated *GNA13* and *STAT6* genes, which might be rationalized by the relatively small sample size.

In our PMBCL cohort, considerable patients were identified with CNV amplification of *PD-L1* and *PD-L2* genes, and these patients also carried more concomitant alterations than those without *PD-L1* or *PD-L2* amplifications. In the phase IB KEYNOTE-013 and the phase II KEYNOTE-170 trials, relapsed or refractory PMBCLs with high PD-L1 scores could achieve better responses and efficacy under pembrolizumab treatment than those with low PD-L1 scores [39]. Similarly, our data suggested that immunotherapy might be a potentially good option to PMBCL detected with *PD-L1* or *PD-L2* amplifications. Notably, we were not able to identify prevalent *PD-L1* or *PD-L2* amplifications in our cHL cohort, whereas *PD-L1* and/or *PD-L2* amplifications were considered as a defining feature of cHL. A fluorescent in situ hybridization (FISH) assay was used to characterize 9p24.1 *PD-L1* and/or *PD-L2* alterations in 108 cHL patients, and 56% and 36% patients were classified as copy gain and amplification, respectively [40]. Moreover, 97 of 108 patients had concordant alterations of *PD-L1* and *PD-L2*. By contrast, Vranic et al.'s study including 11 cHL patients revealed that all three cHL patients with positive FISH results were not NGS-positive under a panel covering 592 genes [41], which resembles our findings that the prevalence of *PD-L1* and *PD-L2* CNV amplification was not as high as expected. Vranic et al. believed that there might be unknown mechanism involved in cHL PD-L1 upregulation and overexpression when given the fact that a fraction of immunohistochemically positive cHLs were NGS and/or FISH negative; however, we supposed that our relatively low *PD-L1* and *PD-L2* CNV amplification prevalence could be partially rationalized by clinical stage, as the FISH amplification detection rate increased when clinical stage getting advanced [40]. Owing to a lack of clinical stage data in our cHL cohort, further studies are required.

This study has limitations. As a retrospective study, there were considerable missing values for patients' clinical information, such as clinical stage, bulky disease, lactate dehydrogenase level, treatment history, survival data, etc., resulting in the difficulty in defining whether our study cohort was comparable to the cohorts of other studies. Also, the sample size of each lymphoma cohort was limited, especially when paired tissue and plasma samples were analysed. Moreover, the sample size of our study cohort might limit the ability to draw definitive conclusion regarding the sensitivity of ctDNA genotyping, and studies with larger study populations are required. Finally, samples included in this study were not completely treatment-naïve, which means that there might be secondary mutations acquired due to resistance.

In conclusion, liquid biopsy was a promising approach to genomic profiling and disease surveillance in cHL, PMBCL, and DLBCL, with highly concordant alterations identified by tissue biopsies and good agreement with response to treatment. Despite some distinctive molecular features, PMBCL had more genetic abnormalities like cHL than DLBCL. Further analyses using large cohorts for the independent use of liquid biopsies in disease surveillance are warranted.

Abbreviations

cHL	classic Hodgkin lymphoma
CNV	copy number variations
ctDNA	circulating tumor DNA
DLBCL	diffuse large B-cell lymphoma
FFPE	formalin-fixed paraffin-embedded
HL	Hodgkin lymphoma
Ig	immunoglobulin
VAF	variant allele frequency
NGS	next-generation sequencing
NHL	non-Hodgkin lymphoma
PMBCL	primary mediastinal large B-cell lymphoma

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s00277-024-05782-0.

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Author contributions Z-M Li and H Yang conceived and design the study. H Wang, Z Wang, S Zhu, Z Li, H Yang, P Sun, L Shen, M Zhu, X Zhao, and Q Ou analyzed data and interpreted results. M Zhu, L Shen, and X Zhao were responsible for data visualization. All authors wrote, revised, and reviewed the manuscript. Z-M Li and H Yang supervised the whole project. All authors read and approved the final manuscript.

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Data availability The lists of mutations identified in 155 tumor tissue

and/or liquid biopsy samples are provided as supplementary tables. Other datasets used and/or analyzed in the current study are available from the corresponding author on reasonable request.

Declarations

Competing interests M Zhu, X Zhao, L Shen, and Q Ou are employees of Nanjing Geneseeq Technology Inc., China. The remaining authors have nothing to disclose.

Ethical approval and consent to participate This study was approved by Ethics Committee of the Cancer Hospital of Shantou University Medical College (No. 2023059). All patients signed informed consent forms prior to enrollment and sample collection.

Conflict of interest M Zhu, X Zhao, L Shen, and Q Ou are employees of Nanjing Geneseeq Technology Inc., China. The remaining authors have nothing to disclose.

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