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Gene Expression Signature Distinguishes Hodgkin's Lymphoma from Related Lymphoproliferative Disorders

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Abstract

Despite the infrequent misdiagnosis of lymphoproliferative diseases such as Hodgkin's lymphoma, the introduction of novel agents directed against specific targets such as CD30, PD1, or PDL1 has underscored the need for nearly 100% accurate diagnosis. The presence of mixed cellular populations around the tumor cells complicates microscopic diagnosis of Hodgkin's lymphoma. However, the current meta-analysis focused on Hodgkin's lymphoma shows that the normal microenvironment surrounding the rare tumor cells can itself aid in accurate diagnosis due to its typical expression profile. Thus, the gene expression signature of the whole biopsy specimen, which reflects the expression of both the tumor cells and especially their rich normal microenvironment cells, can support or challenge the pathological diagnosis. The specificity of the expression signature of each lymphoproliferative disease seen in the current analysis is related to the inclusion of thousands of probes for each tumor-specific expression signature.

Keywords: Lymphoproliferative Diseases; Hodgkin's Lymphoma; Tumor Cells; Microenvironment Cells; Gene Expression

Key Points

Gene expression signatures can support or challenge the histological diagnosis of Hodgkin's lymphoma and related lymphoproliferative disorders.

The inclusion of many thousands of probes in the analysis, in contrast to the common use of a limited number of probes (only those that show >2-fold changes), can explain the very high specificity of each lymphoma-related expression signature used in the current analysis.

Introduction

Recently, it was shown that a reference panel comprising a typical gene expression profile (GEP) of 43 normal and tumor brain tissues was highly concordant with the histological diagnosis. The application of the same method in hematological biopsies would be highly valuable in the diagnosis of certain lymphoproliferative disorders, especially those where the tumor tissue includes mixed cellular populations, such as in Hodgkin's and T-cell lymphomas.

Regarding Hodgkin's lymphoma, classical Hodgkin lymphoma (cHL) is a B-cell-derived lymphoma characterized by a distinctive immunophenotype and relatively few malignant cells within a non-neoplastic inflammatory background. The malignant Reed-Sternberg Cells (HRS Cells) are large, multinucleated cells that are the hallmark of cHL. They express CD30 and CD15. However, cells with a similar morphology and immunophenotype, so--called Reed-Sternberg-like cells, are occasionally seen in both B cell and T cell non-Hodgkin Lymphomas (NHLs) [1]. The background surrounding HRS consists of non-malignant inflammatory cells, including small lymphocytes, granulocytes, and macrophages, associated with varying degrees of fibrosis [2].

Over the past years, the spectrum of gray zones and mimickers of cHL has become better recognized, leading to a refinement of the diagnostic category of true cHL. T-cell lymphomas with admixed Hodgkin-like B cells, especially those with follicular T-helper phenotype such as angioimmunoblastic T-cell lymphoma (AITL) and peripheral T-cell lymphoma - T follicular helper (PTCL-TFH) cells, are increasingly recognized as diagnostic pitfalls.

Several studies investigated the misdiagnosis of lymphomas. In 2003, the North Central London Lymphoma network was established to provide a centralized expert review service for general histopathologists based in peripheral nonspecialist hospitals [2]. A total of 1,949 patient samples were subject to expert review between 2003 and 2008. Diagnostic discordance rates were identified after expert review, and the impact on patient management was assessed by a hematologic oncology specialist. An overall discordance rate of 27.3% was identified. Among the 10 most commonly referred lymphoid malignancies, the discordance rate varied between 3.6% and 34.1%, being 3.8% for Hodgkin's lymphoma. A small but significant number of reactive (n = 17) and malignant (n = 5) discordant diagnoses were also identified.

The Lymphopath Network is a national hematopathology network established in France in 2010 which provides an expert pathologic review of every newly diagnosed lymphoma before therapy is started [3]. From January 2010 to December 2013, 42,145 samples from patients with newly diagnosed or suspected lymphomas were reviewed, according to the 2008 WHO classification, in real time by experts through the Lymphopath Network. Changes in diagnosis between referral and expert review were classified as major or minor according to their potential impact on patient care. A diagnostic change between referral and expert review occurred in 19.7% of patients, with an estimated impact on patient care for 17.4% of patients. The most frequent discrepancies were misclassifications in lymphoma subtype (41.3%), with 12.3% being misclassifications among small B-cell lymphoma entities. Fewer than 2% of changes were between benign and malignant lymphoid conditions. Minor changes (2.3%) mostly consisted of follicular lymphoma misgrading and diffuse large B-cell lymphoma subtype misclassification. Most cHLs (93.3%) were accurately referred, in contrast to an accuracy of only 62.8% for the NLPHL diagnosis. Indeed, 266 (6.7%) of 4,010 cHLs were initially diagnosed as unclassified lymphomas (n = 138), PTCLs (n = 44), B-cell lymphomas (n = 40), NLPHLs (n = 27), myeloid neoplasms (n = 2), or other benign lymphoid conditions (n = 15), whereas 150 (37.2%)of 404 NLPHLs were initially diagnosed as unclassified lymphomas (n = 55), cHLs (n = 47), B-NHLs (n = 28), PTCLs (n = 1), or benign lymphoid conditions (n = 19).

In an expert review of 1010 cases referred to the Nebraska Lymphoma Study Group (NLSG) during a 2-year period (2009–2010), revised diagnoses were grouped into major and minor discrepancies and all major discrepancies were reviewed by a haematologist to determine the effect the diagnostic change would have on therapy [4]. There was no change in diagnosis in 861 (85.2%) cases. In 149 (14.8%) cases, second review resulted in major diagnostic change, of which 131 (12.9%) would have resulted in a therapeutic

change. The highest rates of revision were for follicular, high-grade B-cell, and T-cell lymphomas. 6 NHL cases revised to HL while 8 HL revised to NHL.

In a Dutch study published in October 2022, based on an in-depth pathology review of a nationwide cohort of patients diagnosed with cHL in the Netherlands (2006-2013), from among 2,669 patients with biopsy-proven cHL, 54 were registered with secondary NHL [5]. On review, cHL was confirmed in 25/54 patients. In six of these, the subsequent lymphoma was a primary mediastinal B-cell lymphoma/mediastinal gray zone lymphoma, biologically related to cHL and 19/25 were apparently unrelated B--cell NHL. In 29/54 patients, cHL was reclassified as NHL, including T-cell lymphomas with secondary Hodgkin-like B-blasts (n=15), Epstein Barr virus-positive diffuse large B-cell lymphoma (n=8), CD30 T-cell lymphoma (n=3) and indolent B-cell proliferations (n=3).

In reviewing all second-opinion pathology of lymphoma at the National Cancer Institute-designated Comprehensive Cancer Center (NCI-CCC) from January to June 2001 and from January to June 2006, discrepancies between submitted and second-opinion diagnoses were scored based upon an a priori grading scheme [6]. Major diagnostic revision was rendered in 65 of 365 cases (17.8%) in 2001 and 58 of 354 (16.4%) in 2006 (P=NS). Including cases reviewed and revised beforehand at another NCI-CCC, rates of major diagnostic revision were 21.4% and 18.6%, respectively (P=NS). Discrepancy rates varied by diagnosis, from Hodgkin lymphoma (10%) to Burkitt's lymphoma (75%).

Finally, a retrospective Chinese analysis was performed on 2291 cases of haematolymphoid diseases evaluated by the Department of Pathology of our hospital from 1 July 2019 to 30 June 2021. All 2291 cases were reviewed by two hematopathologist experts and classified according to the 2017 revised WHO classification criteria [7]. In total, 912 cases did not conform to the expert diagnoses among all the 2291 cases, with a total misdiagnosis rate of 39.8%. Among them, misdiagnosis between benign and malignant lesions accounted for 24.3% (222/912), misdiagnosis between haematolymphoid neoplasms and non-haematolymphoid neoplasms accounted for 3.3% (30/912), misdiagnosis among lineages accounted for 9.3% (85/912), misclassification in lymphoma subtypes accounted for 60.8% (554/912), and other misdiagnoses among benign lesions accounted for 2.3% (21/912) of cases, among which misclassification of lymphoma subtypes was the most common.

In considering the introduction of novel targeted therapy to the standard care of lymphoma during the last years (i.e., anti-CD30, anti-CD20, PD1-PDL1, etc.), a correct diagnosis becomes even more crucial. One potential method to improve diagnostic precision is the use of GEP to identify specific signatures characterizing each lymphoma subtype. Discrimination of different tissues has been shown possible using a subset of several dozen probes selected due to their relative selectivity, which can classify the tissue of origin of tumors. For example, a 154-gene expression signature could discriminate the origin of tumor tissue with an overall leave-one-out cross-validation accuracy of 96.5% [9]. Another study used microarray to profile 229 primary and metastatic tumors representing 14 tumor types and multiple histologic subtypes [10,11]. This data set was subsequently used for training and validation of a support vector machine (SVM) classifier, demonstrating 89% accuracy using a 13-class model.

Discrimination between tumors originating from the same tissue, like bone marrow or lymph nodes, seems more difficult due to the sharing of many similar features. One example is the discrimination of germinal center (GCB) vs. activated B-cell-like cell of origin in DLBCL [8]. In relation to Hodgkin's lymphoma, Tiacci et al. [12] used microarrays interrogating ≅47,000 transcripts to generate expression profiles of HRS cells isolated from lymph node biopsies of 16 cHL patients and compared them with the profiles similarly obtained from the main cHL cell lines, GC/post-GC B-cell lymphomas, and normal mature B-cell subsets. Overall, the clustering analysis indicates that HRS cells define a lymphoma entity characterized by a specific gene expression profile, which is distinct from other B-cell neoplasms and surprisingly globally closer to nLPHL than to PMBL, as also confirmed by the high coclustering confidence (80% on bootstrap resampling) of cHLs with nLPHLs and of PMBLs with the non-HLs in the two respective sub--branches of the primary tumors. The discrimination power of the 235 cHL-specific probe sets was tested by subjecting the primary tumor dataset to class prediction (cHL vs. non-

cHL) using κ-nearest neighbors leave-one-out cross-validation (with κ = 10). Notably, such an approach, which was undertaken to validate this large-sized cHL-specific signature through an independent bioinformatic method (rather than to develop a small-sized diagnostic classifier for cHL), assigned 44 of 46 lymphomas to the correct class with high statistical significance while leaving unpredicted the remaining 2 cases (both TCRBLs).

In a proof-of-principle study, gene expression microarray was evaluated by Loi et al. as a single platform test in the differential diagnosis of common lymphoma subtypes and reactive lymphadenopathy in lymph node biopsies [13]. The binary classifications provided prediction accuracies, between a subtype of interest and the remaining samples, of 88.5%, 82.8%, 82.8%, and 80.0% for FL, cHL, DLBCL, and RL, respectively. The optimal number of probes comprising each signature in that study was 10 to 130 in most comparisons.

In fact, a similar number of probes comprising gene expression signatures have been always used to characterize hematological and non-hematological tumors due to the limited number of probes showing the required >2-3 fold change variations among the tissues compared.

However, as seen above, the prediction of expression signatures comprised of dozens of probes is limited even when tested in a binary fashion between a subtype of interest and the remaining samples (i.e., Hodgkin's vs. all other lymphoma subtypes).

In contrast, in a recent meta-analysis, I introduced an expression panel, which includes 43 normal and tumor reference brain signatures, allowing prediction of the histology of brain biopsy very effectively [14]. The high concordance between the reference signatures and the 43 different brain histology's could be explained by the inclusion of thousands of probes within each signature, which reflect their expression rank, so even tiny differences in fold-change intensities gather to a significant difference among different histological groups.

Methods

In the current meta-analysis, a similar expression signature panel as described (14) was applied to lymphoproliferative disorders, with a major focus on distinguishing between Hodgkin's lymphoma and NHL. This distinction is crucial to minimize incorrect treatment.

Constructing the Reference Lymphoma-Specific Expression Intensity Signature Panel

I systematically gathered GEP series of all public datasets that included lymphoproliferative disorders. Relevant keywords (e.g., lymphoma, Hodgkin, lymphoproliferative, etc.) were used, sourcing these datasets from the Gene Expression Omnibus (GEO) repository (across any platform).

For each individual histological lymphoproliferative category (such as DLBCL, MZL, and Hodgkin's lymphoma), I generated representative GEPs by calculating the average expression intensity of each probe within the selected dataset. The various reference signatures were then correlated with each test biopsy specimen to identify the best match.

Due to the limited number of series, it was not possible to establish a single reference panel common to all series and platforms. This contrasts with the universal single panel obtained for 43 different brain signatures [14].

Consequently, each series retains its own reference panel, representing the average expression of each probe in the platform used.

Results and Discussion

The relative correlations between the expression intensities of all probes constructing the GEP of each biopsy specimen and the corresponding probes constructing each of the reference signatures included in the same GEO dataset are presented in Supplemental file 1 (https://f2h.io/b3opbdk8vqwx). The highest correlation, which indicates the best match between the histological diagnosis and the lymphoma-specific reference signature, is marked in red. The reference signatures of each GEO dataset are presented to the right of the same GEO dataset.

Table 1: Concordance between the reference expression signature and the histological diagnosis

Series	Specimen	Concordance	Platform (No. of Probes)
GSE120124	ABC DLBCL	40/40 (100%)	GPL17586 (67528)
	GCB DLBCL		
	Burkitt lymphoma		
	Follicular lymphoma		
	Hodgkin lymphoma		
	Mantle cell lymphoma		
	Peripheral T cell lymphoma (NOS)		
	ALK- anaplastic large cell lymphoma		
	ALK+ anaplastic large cell lymphoma		
GSE39133	Reactive lymph node	34/34 (100%)	GPL570 (54613)
	Hodgkin lymphoma		
GSE17920	classic Hodgkin lymphoma	128/130 (98.46%)	GPL570 (54613)
GSE14879	ALK- ALCL	63/64 (99%)	GPL570 (54613)
	ALK+ ALCL		
	cALCL		
	tcr-cHL		
	activated T-helper cells		
	regulatory T cells		
	resting T-helper cells		
	CD30+ T-cells		
	NK cells, isolated from tonsils by fluorescence-activated cell sorting		
	NKT cells,		
	isolated from peripheral blood by fluorescence-activated cell sorting		
	activated cytotoxic T-cells		
	resting cytotoxic T-cells		
GSE78513	ALK high	35/35 (100%)	GPL570 (54613)
	ALK low		
	Normal tissue		
GSE12453	classical Hodgkin lymphoma	64/67 (95.5%)	GPL570 (54613)
	NLPHL		
	T-cell rich B-cell lymphoma		

	follicular lymphoma		
	Burkitt lymphoma		
	diffuse large B-cell lymphoma		
	naive B-cells		
	memory B cells		
	centrocytes		
	centroblasts		
	plasma cells		
GSE7788	NLPHL	20/21 (95.2%)	GPL570 (54613)
	THRBL		
	Normal		
GSE13996	Hodgkin's Lymphoma	67/70 (95.7%)	GPL570 (54613)
	T-Cell/Histiocyte-Rich Large B-Cell		
	Lymphomas		
	Adenite		
GSE47044	NLPHL	28/35 (82.9%)	GPL571 (22215)
	TCHRBCL-like		
	NLPHL		
	TCHRLBCL		
	germinal center B cells		

As seen in Table 1, using the reference panel of each dataset to correlate with the corresponding probes of each biopsy specimen's related GEP provided a very high match between the lymphoma-specific reference signature and the histological diagnosis in most series. For example, for GSE120124 performed using the GPL17586 platform (containing 67,528 probes), the highest correlation of each histological category was virtually with the corresponding reference signature. Only for GSE47044, performed using the GPL571 platform, which includes just 22,215 probes, the match between the histological diagnosis and the corresponding reference signature was less than 95% (82.9%), suggesting that more probes give a better match.

The main limitation of the current meta-analysis is the small number of series, which precluded the ability to optimize and select the best probes to build a universal reference panel, in contrast to the 4,621 probes that were sufficient to predict the histological diagnosis in brain biopsies from all series and platforms used.

Nonetheless, the current meta-analysis provides an important proof of principle that medical centers and central laboratories can easily build their own reference panel using commercial platforms to provide an objective tool to support or question the diagnosis of Hodgkin's lymphoma, T-cell lymphoma, and related lymphoproliferative disorders in necessary cases. Until sufficient in-hospital samples are gathered to construct and validate their lymphoma-specific reference panel, laboratories can correlate their GEP results of biopsy specimens with the reference signatures supplied in Supplemental file 1.

Supplemental file 1 for download: https://f2h.io/b3opbdk8vqwx

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