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Supplement Article

XIV. The pathology of transformation of indolent B cell lymphomas

Randy D. Gascoyne*

Hematopathologist, British Columbia Cancer Agency and the Centre for Lymphoid Cancer, Vancouver, BC, Canada

**Correspondence to: Randy Gascoyne, MD, Department of Lymphoid Cancer Research, BCCA, BC Cancer Research Centre, 675 W 10th Avenue, Room 12-110, Vancouver, BC V5Z 1L3, Canada.*
E-mail: rgascoyn@bccancer.bc.ca

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Introduction

This paper will describe the histopathologic features of transformation that occur during the course of indolent B cell lymphomas. The clinical and molecular features that underlie this transformation event are described in detail in the two accompanying papers in this series (see Gribben and Rossi, respectively). The focus will be on follicular lymphoma (FL), although reference will be made to transformation involving other low-grade B cell lymphomas including chronic lymphocytic leukemia (CLL)/small lymphocytic lymphoma (SLL), lymphoplasmacytic lymphoma and marginal zone lymphomas, but only briefly.

FL is the most common indolent B cell malignancy, and histologic transformation (HT) defines a biologic event that leads to a high-grade, aggressive B cell neoplasm, typically either diffuse large B cell lymphoma (DLBCL) or a B cell lymphoma-unclassifiable (BCLU), with features intermediate between Burkitt lymphoma (BL) and DLBCL [1]. Rare cases resembling lymphoblastic lymphoma have also been described [2]. Most studies suggest that HT occurs at a steady rate of 2–3%/year from diagnosis of FL, while recent, but unsubstantiated studies suggest that the incidence of HT may be influenced by treatment [3,4]. HT typically heralds a change to clinically aggressive behavior more fully described by Dr. Gribben in his accompanying paper. A sizable proportion of transformed cases will harbor a *MYC* gene translocation, such that in cases with the typical genetic profile of FL (i.e. t(14;18)), these transformations lead to dual translocation status, also known as 'double-hit' lymphomas [5]. The molecular underpinnings of HT will be described in detail by Dr. Rossi in his accompanying paper.

Morphologic features of HT

Histologically confirmed transformation of FL must be distinguished from FL with increasing cytologic grade or FL with variable sized foci of DLBCL at initial biopsy, the latter typically known as composite histology. Similarly, HT does not include loss of the follicular architecture resulting in a diffuse pattern with a mix of small centrocytes and admixed centroblasts (so-called diffuse variants of FL) [1]. Moreover, documenting HT requires a biopsy and cannot be made with confidence based on a fine needle aspiration biopsy or even a needle core biopsy in most cases because of under-sampling. In some patients with significant co-morbidities and/or clinically inaccessible tumor masses, one may need to forgo a biopsy altogether and rely on clinical criteria to indicate transformation. This is not ideal, and thus a proper excisional biopsy is considered the gold standard for determining the presence of definitive HT [4]. Lastly, in cases where the biopsies of FL and transformed FL are separated in time, molecular studies that show clonal identity between the two biopsies are considered a required element to establish clonal relatedness and exclude the possibility of a second, unrelated *de novo* lymphoma.

The most frequent histology encountered in transformed FL (TFL) is DLBCL [6]. Typically the cells resemble centroblasts and form a diffuse infiltrate without underlying residual FL. Mitotic activity is usually increased and background centrocytes are not a feature. Precise classification can, on occasion, be a challenge, as the 2008 World Health Organization (WHO) scheme was established to classify *de novo* diseases and not those that arise as a result of transformation. In cases with a more prominent background of small lymphoid cells, the CD20 and CD3 stains may be very helpful to exclude a diagnosis of a diffuse variant of FL (grade 2 subtype).

The 2nd most common histology seen at HT of FL is BCLU [1]. This category is included in the 2008 WHO classification as B cell lymphoma, unclassifiable, with features intermediate between Burkitt lymphoma (BL) and diffuse large B cell lymphoma. These grey-zone lymphomas reveal morphologic features that overlap classic BL and DLBCL. The cells are typically medium-sized, but show more variability in size and shape and often show nucleolar features that are not typical of classic BL. Most cases have a proliferation rate of <95% [7]. For patients

with antecedent FL whose biopsy shows this histology at transformation, the likelihood of an acquired *MYC* translocation on a background of t(14;18) is very high. Double-hit lymphomas (and triple-hit) either arise as *de novo* disease, or are encountered at the time of HT of patients with antecedent FL. Thus, the majority of BCLU cases that follow an initial FL diagnosis will be dual or triple-translocation lymphomas. Importantly, this BCLU category can also be seen as an initial diagnosis; with many but not all cases having double-hit genetic features. In contrast to BL, these cases typically show complex karyotypes.

Much less commonly, HT shows a histology and immunophenotype reminiscent of lymphoblastic lymphoma (LBL) [2]. Similar to BCLU, these cases can present *de novo*, or at the time of FL transformation. *De novo* cases of adult, surface immunoglobulin-positive acute lymphoblastic leukemia have also been rarely described that harbor both *BCL2* and *MYC* translocations. This uncommon form of HT in FL is characterized by blastic morphology, with medium sized cells showing fine chromatin, indistinct nucleoli and scant cytoplasm. Cases may reveal a leukemic pattern of infiltration, crush artifact and frequent mitoses, but typically do not have a starry-sky pattern. The proliferation rate is usually >95%. All cases show nuclear terminal deoxynucleotidyl transferase (Tdt) positivity, and virtually all are double-hit based on cytogenetics. Representative images of these three main types of TFL are shown in Figure 1.

Finally, a rare form of HT in FL is described as 'blastoid', with similar features as described above for the lymphoblastic transformation variant of FL, but these cases by definition lack nuclear Tdt [8,9]. The mitotic rate is typically not as high as the lymphoblastic cases, and they typically lack double-hit cytogenetics. Table 1 highlights the morphologic features, associated immunophenotype and characteristic genetic profiles of these four patterns of HT of FL.

Low-grade B cell lymphomas other than FL

HT in other subtypes of low-grade B cell lymphomas appears to be less common based on currently available literature [1]. This includes marginal zone lymphomas of splenic, nodal and extranodal types (SMZL, NMZL and MALT); lymphoplasmacytic lymphoma (LPL) and small lymphocytic lymphoma (SLL)/CLL. The latter is of course well described and is otherwise known as 'Richter's syndrome' or 'Richter's transformation' [10]. Transformation of SMZL, NMZL, MALT lymphomas and LPL is much less well described and often only in case reports or small patient series. Moreover, there are many uncertainties regarding the threshold for the diagnosis of HT in these entities, as a variable proportion of large cells does not preclude a diagnosis of a low-grade marginal zone lymphoma. Most series recommend that cases with <20% large cells be classified as low-grade histology [1]. It remains controversial though at what threshold of the percentage of large B cell blasts HT should be considered, even in cases with a clear admixture of large and small cells and no cohesive clusters of large cells. It is likely that cases with an increasing number of large cells denote those with more aggressive clinical behavior.

In 2–12% of cases of CLL or SLL, transformation occurs during the course of disease and is typically referred to as Richter's syndrome (RS). RS must be distinguished from cases with increasing growth centers, which represents progression or so-called accelerated phase, but not frank transformation. In this setting, the number of prolymphocytes and paraimmunoblasts increase within growth centers and the growth centers themselves become more numerous and confluent, but the findings fall short of definitive RS. Prolymphocytoid transformation of CLL that manifests in the peripheral blood is also not considered RS.

The incidence of RS is in part accounted for by practice patterns and is higher in jurisdictions with a low threshold for biopsy in patients with established CLL/SLL suspected of transformation [10]. The diagnosis is restricted to cases with biopsy proven transformation. Overall, RS is characterized in 90% of cases by DLBCL. In most cases the characteristic cytology is similar to centroblastic morphology and thus mimics typical DLBCL. However, in 20–40% of cases the cells show immunoblastic features with single,

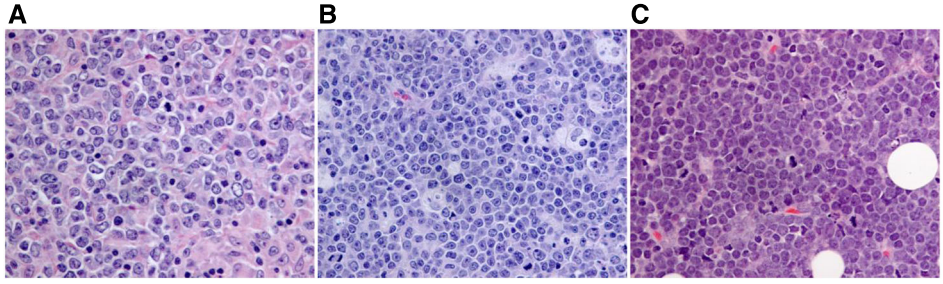

Figure 1. Representative images of transformation biopsies in FL Figure 1A represents a transformed follicular lymphoma with DLBCL morphology and a GCB phenotype; Figure 1B shows a BCLU morphology (this case showed double-hit cytogenetics with *BCL2* and *MYC* translocations) and Figure 1C shows a lymphoblastic morphology (cells in this case expressed nuclear Tdt and harbored dual translocations of *BCL2* and *MYC* oncogenes). All of these cases had antecedent FL

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Table 1. Morphologic patterns of histologic transformation in FL with associated phenotypic and genetic alterations

Histologic subtype	Morphologic features	Immunophenotype and cytogenetics
DLBCL	Sheets of centroblasts or immunoblasts; loss of follicular architecture, diffuse infiltrate	Most show a GCB immunophenotype, $CD10^+$, $BCL6^+$; $BCL2^+$, MYC^+; *MYC* translocations may be present; *BCL2* translocations expected, some cases now recognized as ABC or unclassified type
BCLU	Diffuse infiltrate of cells with medium-sized nuclei, some centroblasts; +/− starry-sky pattern and variable mitotic rate	Most are GCB with $BCL2^+$; proliferation typically <95%, MYC protein often positive and frequent double/triple-hit cytogenetic profile
Lymphoblastic	Diffuse infiltrate, leukemic pattern, medium-sized cells with fine chromatin and inconspicuous nucleoli, frequent mitoses	Tdt^+; often lose BCL6, but are $PAX5^+$, $CD10^+$ and $BCL2^+$, CD34-negative. High proliferation rate and cytogenetics characteristically reveal dual translocations.
Blastoid	Residual follicular structures may be seen, medium-sized cells with fine chromatin, increased mitoses	GCB phenotype with $CD10^+$, $BCL6^+$, $BCL2^+$ and Tdt-negative. Dual translocation not present. Cytogenetic profiles largely unknown.

DLBCL = diffuse large B cell lymphoma, BCLU = unclassifiable B cell lymphoma, GCB = germinal center B cell subtype and ABC = activated B cell subtype

central, prominent nucleoli, vesicular chromatin and moderate amounts of amphophilic cytoplasm. In the vast majority (90%) of RS the disease is clonally related to the original CLL/SLL, but in 10% a clonal relationship cannot be established and thus, may represent a *de novo* disease. In 10% of cases RS is characterized by histologic findings more akin to classical Hodgkin lymphoma (HL), so-called Hodgkin variant of RS [11]. The large Hodgkin Reed–Sternberg (HRS) cells typically express CD15 and CD30, lack CD20 and often harbor latent EBV. In these cases a background of inflammation characteristic of classical HL is required in order to distinguish these cases from typical CLL/SLL with rare, scattered HRS cells. In the latter setting, the HRS cells express CD20 and CD30, but fail to express CD15 and have the typical background of SLL/CLL with monotonous sheets of small, mature lymphocytes and characteristic growth centers. In RS of the usual DLBC-type, the large B cells often fail to express CD5 and CD23. The cell-of-origin subtype is ABC (based on gene expression) or non-GCB (defined by immunohistochemistry) in most cases.

Immunophenotype of HT following FL

The immunophenotype in most cases of HT of FL typically preserves the germinal center pattern of antigen expression with both CD10 and BCL6 positivity. In most cases the malignant B cells express BCL2; as loss of BCL2 protein expression upon transformation is a rare event in the author's experience. These cases also typically express GCET1 and LMO2 [12]. The proliferation rate increases in comparison to FL, and follicular dendritic cell meshworks are no longer evident or are only loosely formed. CD20 stains would be expected to show only few small B cells.

Previously published work and accepted dogma indicated that FL cases undergoing HT maintained germinal center fidelity and thus all were of GCB subtype [13]. More recent studies suggest that some cases of transformed FL reveal a cell-of-origin phenotype more reminiscent of the ABC or unclassifiable phenotype [14]. This result may be linked to the specific pattern of acquired genetic alterations that underlie transformation (see accompanying paper by Rossi).

The microenvironment in FL transformation

FL is characterized by a prominent microenvironment rich in immune and stromal cells, recently referred to as showing a 're-education' pattern [15]. The cellular milieu in FL is reminiscent of normal follicles, rich in follicular dendritic cells (FDC) and T-follicular helper cells (T_{FH}). The neoplastic B cells in FL rely on the microenvironment to receive growth and survival signals and utilize ligand and receptor interactions as well as a network of chemokines and cytokines for this purpose. In addition, the neoplastic B cells promote immune escape by a number of mechanisms including cultivating an immunosuppressive microenvironment with M2 polarized macrophages and regulatory T cells as well as inducing T cell exhaustion of infiltrating cytotoxic T cells. Several changes in the microenvironment have been described that appear to accompany FL transformation. Many of these studies antedate the routine use of rituximab-containing regimens and for the most part, analyzed patient cohorts lacking uniform therapies [16]. The result is that many of these studies preclude any definitive conclusions regarding the prognostic significance of the tumor microenvironment in FL and/or TFL.

Included among factors associated with transformation of FL are FDC loss or immaturity, increased intrafollicular

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$CD4^+$ T cells, changes in the numbers and pattern of distribution of regulatory T cells, decreased PD-1^+ T cells and increased microvessel density, all of which have been linked to an increased risk of transformation [16–18]. Furthermore, neoplastic B cells in FL alter gene expression derived from benign tumor infiltrating $CD4^+$ and $CD8^+$ T cells (TILs), with the expression of specific genes, notably *PMCH* and *ETV1*, having been linked to time to progression [19]. A cause and effect relationship is difficult to establish, and thus these observations may represent only epiphenomena. Despite these drawbacks, these data do implicate FL-microenvironmental crosstalk as relevant biology impacting HT. A clearer picture of the role of the microenvironment in HT of FL must await a re-evaluation of these data in the current era of rituximab; most appropriately done in the context of uniform therapy and in the setting of randomized controlled clinical trials. Functional data will also add texture to an improved understanding of the role of the tumor microenvironment in TFL.

Gene expression patterns that underlie HT

A number of gene expression profiling (GEP) studies using paired biopsy specimens of FL and subsequent DLBCL have been published [13,20–22]. Most studies have included only small numbers of paired samples, as this clinical material can be difficult to ascertain. In 2002, Lossos and colleagues examined 12 pairs of matched, frozen biopsies of FL and subsequent DLBCL [20]. They established two different patterns of gene expression profiles associated with HT; one showing an increase in *MYC* and its target genes and the other associated with a decrease in *MYC* and downstream targets. They compared the GEP of TFL to *de novo* DLBCL and were able to establish significant differences in gene expression. The author's postulated that multiple different pathways underlie HT and that at the level of GEP; one subgroup appears to lose proliferation control (increased MYC and targets) and the other loss of apoptotic signaling (decreased MYC and its targets). In 2003, Elenitoba-Johnson and colleagues implicated increased p38 MAP kinase as a distinguishing pathway acquired during HT of FL [21]. Davies *et al.* in 2007 analyzed 20 paired samples of FL and TFL and, similar to Lossos, could show that one altered pathway common to TFL was increased proliferation [13]. They were also able to show that *TP53* mutations, *TP53* loss, *CDKN2A* loss and *c-REL* amplification tended to be associated with the subgroup defined by increased proliferation. The second subgroup was defined by a lack of a proliferation signature, but a specific oncogenic pathway perturbation was not found. In 2009, Gentles and coworkers analyzed cases of TFL and were able to establish that a 'pluripotency signature' related to embryonic stem cells was over-expressed in cases following HT, but was not increased in the antecedent FL [23]. More recently, Brodtkorb *et al.* used an integrative analysis strategy merging high-resolution copy number data with GEP in FL and then looking for cis-correlated genes (i.e. genes whose expression was increased coordinate with an increase in gene dosage) that correlated with HT [22]. They discovered a group of 14 genes linked to the NF-κB pathway where six genes were significantly associated with HT, including *BTK*, *IGBP1*, *IRAK1*, *ROCK1*, *TMED7-TICAM2* and *TRIM37*. The expression levels of two of these genes (*IRAK1* and *TRIM37*) were highly correlated with an increased risk of HT, suggesting that predictive assays might be constructed using these genes and possibly their downstream targets.

Summary

In summary, two major histologies account for the vast majority of HT from an initial diagnosis of FL. The commonest histology encountered at the time of transformation is DLBCL, but BCLU bases are also commonly seen. Both may show evidence of acquiring a *MYC* translocation on a background of t(14;18), leading to double-hit lymphomas, but these are enriched within the BCLU category. In most cases the immunophenotype remains consistent with a GCB pattern of gene expression, but some cases are now recognized that harbor mutational spectra that deregulate gene expression resulting in some TFL with unclassified or ABC profiles.

HT of FL cannot be accounted for by perturbation of a single pathway. Transformation is likely the result of a number of different alterations, and a detailed understanding of the molecular underpinnings is further complicated by clonal heterogeneity and variable clonal architecture that characterize most FL. A common progenitor cell (CPC) may be the culprit that leads to both chemotherapy resistance and HT. Because the size of the CPC clone is variably represented in diagnostic and/or relapse biopsies, a comprehensive understanding of HT will require careful attention to the role of evolutionary genetics in FL.

Conflict of interest

The author has no conflicts to disclose related to this work.

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