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Supplement Article

XIII. Molecular pathogenesis of transformed lymphomas

Davide Rossi*

Division of Hematology, Department of Translational Medicine, Amedeo Avogadro University of Eastern Piedmont, Novara, Italy

**Correspondence to: Davide Rossi, MD, PhD, Division of Hematology, Department of Translational Medicine, Amedeo Avogadro University of Eastern Piedmont, Via Solaroli 17, Novara 28100, Italy. E-mail: rossidav@med.unipmn.it*

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Introduction

Histologic transformation to an aggressive lymphoma may complicate the course of almost all subtypes of indolent B-cell tumours. However, understanding of the molecular bases of transformed lymphoma is currently limited to cases arising in follicular lymphoma (FL) and chronic lymphocytic leukemia (CLL) patients [1].

According to the World Health Organization Classification of Hematopoietic Tumours, transformed follicular lymphoma (TFL) is the progression of FL into a high-grade tumour, usually diffuse large B-cell lymphoma (DLBCL) [1]. The World Health Organization Classification defines Richter syndrome (RS) as the development of an aggressive lymphoma in patients with a previous or concomitant diagnosis of CLL and recognizes two distinct pathologic variants of RS, namely the DLBCL and the Hodgkin lymphoma (HL) variants [1]. Based on the analysis of the rearrangement of the immunoglobulin genes, virtually all the TFL are clonally related to the preceding FL phase, thus representing true transformations [1]. Although most of the DLBCL (~80%) and HL (~50%) that arise in CLL patients are clonally related to the preceding indolent phase, a fraction of cases (~20% showing a DLBCL morphology and ~50% showing a classical HL morphology) harbor distinct immunoglobulin gene rearrangements, thus representing *de novo* lymphomas arising in a CLL patient rather than true transformations [2,3].

Among TFL and RS, no single lesion or combination of genetic lesions or cellular pathways seem to be responsible for transformation, with various transformed cases differing in both the number and the type of aberrations (Figure 1). Moreover, molecular lesions of transformed DLBCL do not involve primarily specific signaling pathways or B-cell differentiation programs that are otherwise commonly targeted in *de novo* DLBCL (Figure 1). The fact that most of the TFL and RS cases display combinations of lesions of *TP53*, *MYC*, and *CDKN2A/B* suggests that transformation into DLBCL involves general regulators of tumour suppression, cell cycle control, and cell proliferation and may account for the aggressive clinical phenotype observed in this disease because of the combined effect of chemoresistance and rapid disease kinetics [2,4–8].

Molecular pathogenesis of transformed follicular lymphoma

The t(14;18)(q32;q21) translocation, resulting in overexpression of the anti-apoptotic gene *BCL2*, and mutations of the histone modifying gene *KMT2D* (also known as *MLL2*) represent the most frequent genetic lesions in FL, both occurring at a rate >80% of cases [9]. These lesions are present in nearly all tumour cells (clonal) in the indolent FL phase of the disease, suggesting that they are early driver events. Upon transformation, *BCL2* translocation and *KMT2D* mutations are stable and remain clonally dominant, thus indicating that these mutations represent key events in the founding common progenitor cell of FL that repopulates the tumour at transformation [7,8].

The most common genomic aberration specifically acquired during progression to TFL is the loss of *CDKN2A/B* in ~45% TFL (Figure 2) [8]. *CDKN2A/B* encodes two tumour suppressor genes whose protein products (p14-ARF, p16-INK4A, and p15-INK4B) play major roles as negative regulators of cell-cycle progression and as stabilizers of the tumour suppressor TP53. *CDKN2A/B* lesions and *TP53* genetic abnormalities, which are also frequently acquired at transformation in ~20% of cases, are mutually exclusive in TFL [8], suggesting that *CDKN2A/B* loss may contribute to FL transformation by affecting both cell-cycle regulation and TP53-dependent DNA damage responses, thus promoting genomic instability.

Genetic lesions deregulating *MYC*, including chromosomal translocations, gains and/or amplifications, and point mutations, are common (~40% of cases) lesions acquired during progression to TFL (Figure 2) [8]. Deregulated *MYC* oncogenic activity may provide multiple advantages to the transformed clone through its pleiotropic function in cell growth, metabolism, and genetic instability.

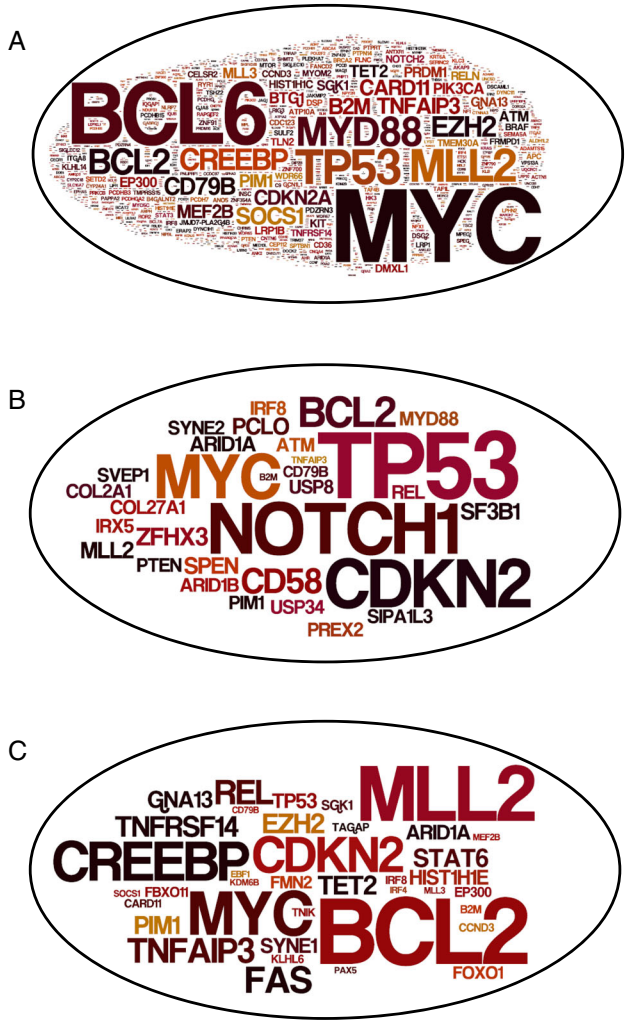

Figure 1. Mutated genes in *de novo* and transformed diffuse large B-cell lymphoma. Word clouds representing genes that are affected by molecular lesions in *de novo* diffuse large B-cell lymphoma (panel A), Richter syndrome (panel B), and transformed follicular lymphoma (panel C). The size of the font is proportional to the prevalence of gene lesions

Molecular pathogenesis of Richter syndrome

While the molecular mechanisms involved in transformation of CLL into a clonally related DLBCL have been characterized to some extent, the pathogenesis of clonally unrelated DLBCL and of the HL variant of RS is not yet determined [10].

TP53 abnormalities, including mutations or deletions of the locus, can be identified in ~60% of cases, thus representing the most frequent genetic lesion in clonally related DLBCL, and are generally acquired at the time of transformation (Figure 2) [2,4–6]. The tumour suppressor gene *TP53* codes for a central regulator of the DNA-damage-response pathway, and its activation leads to cell-cycle arrest and apoptosis, thus mediating the anti-proliferative action of several chemotherapeutic agents. Consistently, *TP53* disruption is a major determinant of the chemorefractory phenotype that characterizes transformation to clonally related DLBCL.

MYC deregulation characterizes a significant fraction (~40%) of cases of clonally related DLBCL transformation and may be sustained by genetic lesions affecting the *MYC* network. The *MYC* gene is activated by somatic structural lesions in ~30% of clonally related DLBCL transformations, including translocations juxtaposing *MYC* to immunoglobulin loci, gain/amplification at 8q24, and point mutations (Figure 2) [2,5,6]. Similarly to *TP53* lesions, also *MYC* activating events are acquired at the time of transformation in a relevant fraction of cases. *MYC* activation is also sustained by mutations affecting *MYC trans*-regulatory factors, as exemplified by mutations of *NOTCH1* that occur in ~30% clonally related DLBCL transformation, are mutually exclusive with *MYC* genetic lesions, and result in the removal of the C-terminal PEST domain of the protein (Figure 2) [4–6]. Mutations of the *NOTCH1* PEST domain cause impaired degradation and accumulation of an active *NOTCH1* isoform, which in turn sustains the deregulated transcription of multiple target genes, including *MYC*.

CDKN2A/B is recurrently affected by focal and homozygous losses in ~30% of clonally related DLBCL transformations (Figure 2) [5,6]. The notion that *CDKN2A/B* deletions are rare in unselected CLL and may be acquired at the time of transformation points to a direct implication of this lesion in the development of RS.

Transformation to clonally related DLBCL shows a biased usage of the B-cell receptor (BCR) in the subset 8 configuration, suggesting that it has been selected to bind a restricted set of antigenic epitopes and supporting a role of BCR signaling in transformation [11]. The particular aggressiveness of BCR subset 8 and its increased propensity to transform into RS may be explained by the strong and unlimited capacity of CLL harboring this BCR configuration to respond to multiple auto-antigens and immune/inflammatory stimuli present in the microenvironment [12]. Among stereotyped subsets, this high reactivity is specific for subset 8 and may elicit unabated stimulation throughout the natural history of these patients, leading to progressive selection of the more aggressive clonal variants.

Epstein–Barr virus (EBV) infection has been suggested by some studies as a potentially relevant factor for RS pathogenesis. The observation that the overwhelming majority (85–100%) of DLBCL transformed from CLL does not carry EBV infection in the malignant cells, however, does not favor this hypothesis. In contrast, EBV infection conceivably has a role in the HL variant of RS, that is EBV positive in ~70% of cases, as documented by staining for latent membrane protein 1 on immunohistochemistry or by *in situ* hybridization of EBV-encoded RNA transcripts [10].

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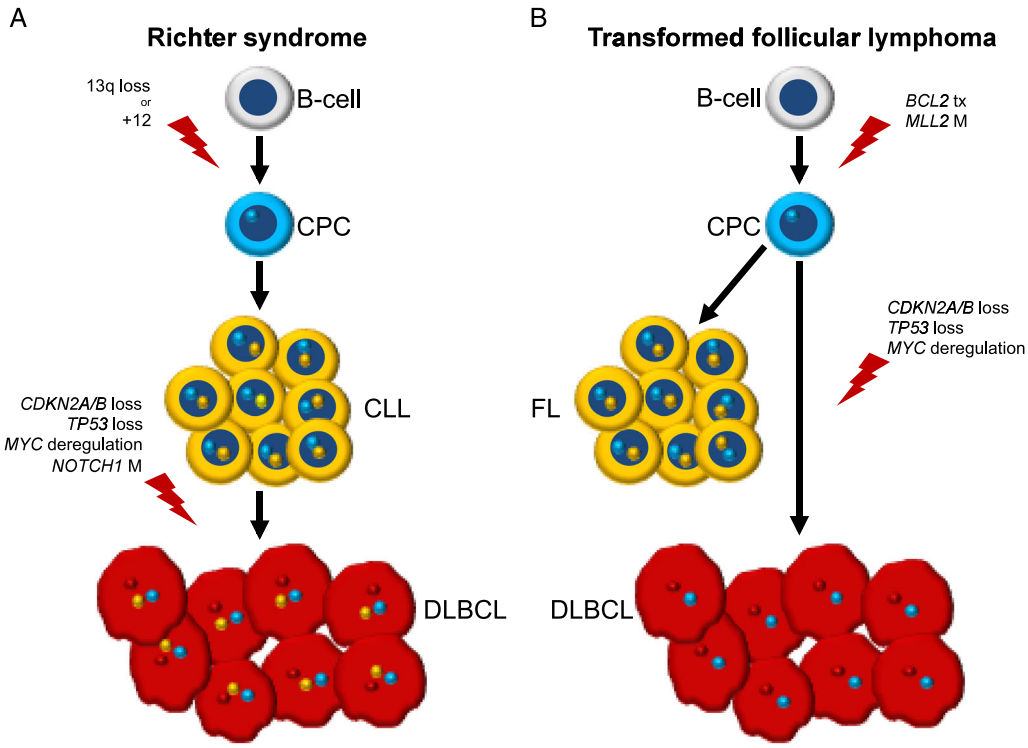

Figure 2. Clonal evolution models of Richter syndrome and transformed follicular lymphoma (FL). Most Richter syndrome cases transform through linear evolution, where diffuse large B-cell lymphoma (DLBCL) represents the final stage of evolution of the original chronic lymphocytic leukemia (CLL) clone (panel A). Virtually all FL transform through branching evolution, where the DLBCL clone arises from an ancestral common progenitor cell (CPC) through the acquisition of independent genetic events that are only partially shared by the indolent FL phase of the disease (panel B). M, mutation; tx, translocation

Clonal evolution models of chronic lymphocytic leukemia and follicular lymphoma transformation

The clonal evolution of any given tumour type occurs either through a linear model, in which the predominant clone acquires novel genetic lesions leading to progression, or through a branching model, in which a common ancestor evolves to the initial tumour and to the progressed stage through distinct genetic pathways. Paired analysis of the indolent and transformed clones represents an ideal setting for the clarification of clonal evolution in FL and CLL.

The FL–TFL transition almost always occurs through a branching rather than linear evolution (Figure 2) [7,8]. The dominant TFL clone arises in most patients from an ancestral mutated common progenitor cell through the acquisition of independent genetic events that are only partially shared by the indolent FL phase of the disease. Conversely, CLL–DLBCL transition occurs mainly through a linear model of evolution, where DLBCL represents the final step in the progressive and additive acquisition of genetic lesions that starts at the stage of the original founder CLL clone (Figure 2) [6].

Molecular biomarkers for the prediction of transformation in follicular lymphoma and chronic lymphocytic leukemia

Few studies have actuarially assessed the contribution of FL genetics to the risk of transformation. Beside translocation, the *BCL2* gene is also targeted by point mutations in FL conceivably as a consequence of misfire of the somatic hypermutation process. The presence of *BCL2* mutations at diagnosis, which occur in ~10% of FL, correlates with a ~3-fold increased risk of transformation accounting for a 10-year cumulative incidence of TFL of ~80% [13]. *BCL2* mutations conceivably mark the genetic instability of the FL clone promoted by such aberrant somatic hypermutation process that may represent a major driver of transformation. Acquired uniparental disomy involving chromosome 16p has also been shown to predispose to transformation in FL, although the genes involved have not yet been identified [14].

In CLL, the risk of RS development is strongly affected by the presence of *NOTCH1* mutations and by the configuration of the BCR harbored by the leukemic clone [4,11]. CLL harboring *NOTCH1* mutations has a significantly higher

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cumulative probability of developing RS (45%) compared with CLL without *NOTCH1* mutations (4%) [4]. Also, CLL utilizing a subset 8 BCR has a very high risk of RS development, translating into a cumulative incidence of transformation of nearly 80% at 10 years [11].

Molecular biomarkers for the prediction of post-transformation survival

The prognosis of the DLBCL transformation is generally unfavorable. However, prognosis is not uniformly poor and may be predicted on clinical and biological grounds. In addition to the clinical risk factors, survival post-transformation may also be predicted by the tumour genotype. In clonally related RS, patients lacking *TP53* mutations and deletion display a better outcome than *TP53* disrupted cases, conceivably because they maintain a chemosensitive disease [2]. The most important prognostic factor in RS is the clonal relationship between the CLL and the DLBCL clones. Despite similar clinical features at presentation, clonally unrelated DLBCL is characterized by a significantly longer survival (~5 years), which is in the range of that of *de novo* DLBCL, compared with clonally related cases (8–16 months) [2]. Such differences in clinical outcome between clonally related and clonally unrelated DLBCL reflect differences in the genetics of the disease. Indeed, the prevalence of *TP53* disruption in clonally unrelated DLBCL is low (~20%) and overall similar to that of *de novo* DLBCL. Also, stereotyped heavy variable complementarity determining region 3, an immunogenetic feature that is frequent in clonally related DLBCL transformation (~50% of cases) but very rare in *de novo* DLBCL, is virtually absent in clonally unrelated DLBCL [2]. Overall, these observations point to the clinical relevance of investigating the clonal relationship in RS patients and suggest that clonally unrelated DLBCL should be considered, and probably managed, as a secondary DLBCL arising *de novo* in the context of CLL rather than a true RS transformation.

Perspectives

Changes in the treatment scenario of indolent B-cell tumours might also change the epidemiology of transformation. The non-genotoxic mechanism of action of new drugs, their activity against clones harboring high-risk mutations that emerge at the time of transformation (i.e., *TP53* mutations), and the better preserved immune function under these treatments may result in a decrease of the incidence rate of transformation. Because the selective pressure imposed by treatment may shape the genetics of the transformed clone, the molecular pathogenesis of transformation occurring in patients treated solely with novel targeted drugs may be different from that currently observed in transformed patients coming from immunochemotherapy. Future efforts to incorporate clinical factors and genetic factors (e.g., clonal relationship, *TP53* disruption, loss of *CDKN2A/B*, and *NOTCH1* mutations) into a single prognostic model are likely to further improve the accuracy of risk stratification. Drugs targeting the molecular programs that are altered in transformed lymphomas, such as the cell cycle (cyclin-dependent kinase inhibitors), the *MYC* pathway (bromodomain and extra-terminal inhibitors), BCR signaling (ibrutinib and idelalisib), *NOTCH* signaling (gamma secretase inhibitors and anti-negative regulatory region antibodies), and novel compounds entering the management of aggressive lymphoma (i.e., immune checkpoint inhibitors, XPO1 inhibitors, and ABT-199) may have promise in the management of these tumours as single agents or in combination with traditional chemoimmunotherapy approaches.

Conflict of interest

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

References

1. Swerdlow SH, Campo E, Harris NL, *et al.* (eds.) World Health Organization Classification of Tumours, Pathology and Genetics of Tumours of Haematopoietic and Lymphoid Tissues. IARC: Lyon; 2008.
2. Rossi D, Spina V, Deambrogi C, *et al*. The genetics of Richter syndrome reveals disease heterogeneity and predicts survival after transformation. *Blood* 2011; **117**: 3391–3401.
3. Ohno T, Smir BN, Weisenburger DD, Gascoyne RD, Hinrichs SD, Chan WC. Origin of the Hodgkin/Reed-Sternberg cells in chronic lymphocytic leukemia with Hodgkin's transformation. *Blood* 1998; **91**: 1757–1761.
4. Rossi D, Rasi S, Fabbri G, *et al.* Mutations of *NOTCH1* are an independent predictor of survival in chronic lymphocytic leukemia. *Blood* 2012; **119**: 521–529.
5. Chigrinova E, Rinaldi A, Kwee I, *et al.* Two main genetic pathways lead to the transformation of chronic lymphocytic leukemia to Richter syndrome. *Blood* 2013; **122**: 2673–2682.
6. Fabbri G, Khiabanian H, Holmes AB, *et al.* Genetic lesions associated with chronic lymphocytic leukemia transformation to Richter syndrome. *J Exp Med* 2013; **210**: 2273–2288.
7. Okosun J, Bödör C, Wang J, *et al.* Integrated genomic analysis identifies recurrent mutations and evolution patterns driving the initiation and progression of follicular lymphoma. *Nat Genet* 2014; **46**: 176–181.
8. Pasqualucci L, Khiabanian H, Fangazio M, *et al*. Genetics of follicular lymphoma transformation. *Cell Rep* 2014; **6**: 130–140.
9. Kridel R, Sehn LH, Gascoyne RD. Pathogenesis of follicular lymphoma. *J Clin Invest* 2012; **122**: 3424–3431.
10. Rossi D, Gaidano G. Richter syndrome. *Adv Exp Med Biol* 2013; **792**: 173–191.
11. Rossi D, Spina V, Cerri M, *et al.* Stereotyped B-cell receptor is an independent risk factor of chronic lymphocytic leukemia

DOI: 10.1002/hon

transformation to Richter syndrome. *Clin Cancer Res* 2009; **15**: 4415–4422.
12. Gounari M, Ntoufa S, Chu CC, *et al.* Promiscuous antigen reactivity may underlie clinical aggressiveness and increased risk for Richter's syndrome in chronic lymphocytic leukemia with stereotyped IGHV4-39/IGKV1(D)-39 B cell receptors. *Blood* (ASH Annual Meeting Abstracts) 2012; **120**: 561.
13. Correia C, Schneider PA, Dai H, *et al.* BCL2 mutations are associated with increased risk of transformation and shortened survival in follicular lymphoma. *Blood* 2015; **125**: 658–667.
14. O'Shea D, O'Riain C, Gupta M, *et al*. Regions of acquired uniparental disomy at diagnosis of follicular lymphoma are associated with both overall survival and risk of transformation. *Blood* 2009; **113**: 2298–2301.

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