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Article

Genetics of Follicular Lymphoma Transformation

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SUMMARY

Follicular lymphoma (FL) is an indolent disease, but 30%–40% of cases undergo histologic transformation to an aggressive malignancy, typically represented by diffuse large B cell lymphoma (DLBCL). The pathogenesis of this process remains largely unknown. Using whole-exome sequencing and copy-number analysis, we show here that the dominant clone of FL and transformed FL (tFL) arise by divergent evolution from a common mutated precursor through the acquisition of distinct genetic events. Mutations in epigenetic modifiers and antiapoptotic genes are introduced early in the common precursor, whereas tFL is specifically associated with alterations deregulating cell-cycle progression and DNA damage responses (*CDKN2A/B*, *MYC*, and *TP53*) as well as aberrant somatic hypermutation. The genomic profile of tFL shares similarities with that of germinal center B cell-type de novo DLBCL but also displays unique combinations of altered genes with diagnostic and therapeutic implications.

INTRODUCTION

Follicular lymphoma (FL) is the second most common type of B cell non-Hodgkin lymphoma, comprising ~25% of all new diagnoses (Swerdlow et al., 2008) (http://seer.cancer.gov/statistics/). Although initially indolent and responsive to a variety of treatments, this disease remains largely incurable (Kridel et al., 2012). One particularly compelling problem in the clinical history of FL is its histologic transformation to a more aggressive malignancy, typically represented by a diffuse large B cell lymphoma (DLBCL) (Montoto and Fitzgibbon, 2011). FL transformation has been reported to occur in 16% to 70% of patients over time, with a consensus rate of 3% per year, and is associated with a mean survival posttransformation of less than 2 years (Montoto and Fitzgibbon, 2011). Thus, there is a strong need for an increased understanding of both the dynamics of tumor clonal evolution and the mechanisms that are responsible for transformation, which may in turn be translated into more effective therapies.

Although the process of transformation to DLBCL was originally described several decades ago, few studies have specifically addressed this question in longitudinal series with documented clonal relationship between the two phases (Lossos and Gascoyne, 2011). Current knowledge of the biology of transformation suggests the involvement of heterogeneous genetic, epigenetic, and microenvironment-dependent factors, most notably mutations of *TP53* (Lo Coco et al., 1993; Sander et al., 1993), genetic and/or epigenetic inactivation of the *CDKN2A*/p16 tumor suppressor gene (Pinyol et al., 1998), translocations deregulating the *BCL6* proto-oncogene (Akasaka et al., 2003), alterations involving chromosome 1p36 (Martinez-Climent et al., 2003), and changes in *MYC* expression (Lossos et al., 2002). Additionally, analysis of selected genes in few cases revealed an association between progression to DLBCL and aberrant somatic hypermutation (ASHM) (Rossi et al., 2006), a mechanism of genetic instability resulting from the abnormal functioning of the physiologic somatic hypermutation (SHM) process that operates in germinal center (GC) B cells (Pasqualucci et al., 2001). However, these findings were based on small number of cases and a candidate gene approach as opposed to an

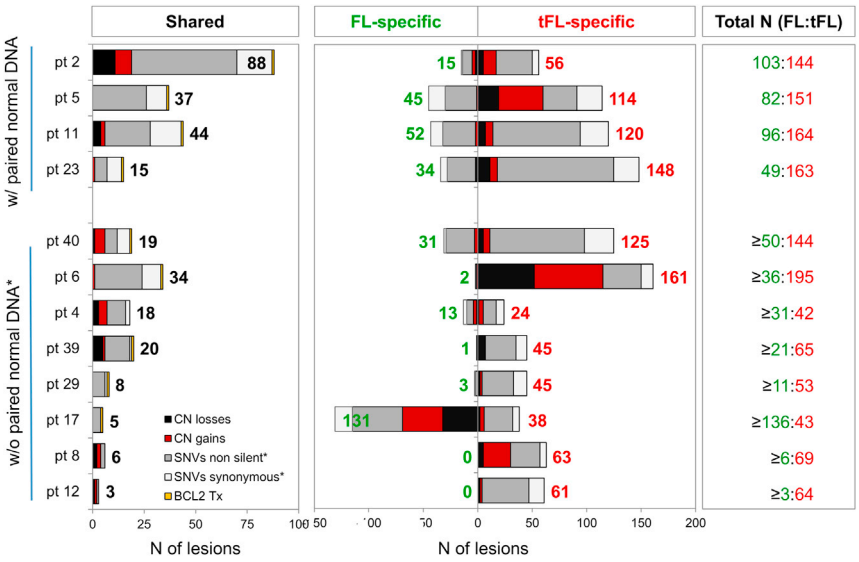

Figure 1. FL and tFL Display Shared and Unique Genomic Aberrations

Overall load of genetic lesions identified by WES and CN analysis in the dominant clone of the 12 discovery cases. Color codes denote distinct types of aberrations (Tx, translocation). *In cases lacking matched normal DNA, shared SNVs are limited to those affecting 52 selected genes with well-established roles in lymphomagenesis (see the Experimental Procedures); thus, the total number of genetic lesions in these patients (right column) most likely represents an underestimate. FL-specific SNVs that could be due to genomic loss or cnLOH of the same region in the tFL phase were excluded.

unbiased, genome-wide analysis. Thus, the biological mechanisms that are responsible for the lethal event of FL transformation remain incompletely understood.

The present study was aimed at examining the history of clonal evolution during FL transformation to DLBCL and comprehensively identifying molecular determinants that underlie this process.

RESULTS

Divergent Evolution of FL and tFL from a Common Mutated Precursor

To investigate whether transformation of FL evolves as a linear process (i.e., through the emergence of an aggressive subclone from the initial dominant FL population) or derives from the divergent evolution of an ancestral common precursor cell (CPC) that acquired distinct mutations to become a FL or a transformed FL (tFL), we integrated massively parallel whole-exome sequencing (WES) and genome-wide high-resolution SNP array analysis in a "discovery panel" of sequential FL and tFL biopsies obtained from 12 patients, including four with available matched normal DNA (Tables S1 and S2 and Figure S1). In all cases, investigation of the rearranged immunoglobulin (Ig) genes by Sanger sequencing and/or SNP array analysis confirmed the clonal relationship between the two phases, whereas the inferred copy-number value at the segment of deletional recombination within the Ig loci was used to quantify the percentage of tumor cells in the biopsy (Bergsagel and Kuehl, 2013), allowing to normalize the data for clonal representation (Table S1). Fluorescence in situ hybridization (FISH) analysis was used to assess the presence of chromosomal translocations affecting *BCL2*, *MYC*, and *BCL6*.

We extrapolated the evolutionary history of transformation by defining genomic alterations that are present in the dominant clone of both pre- and posttransformation specimens ("shared lesions") and contrasting them to those that are present exclusively in the FL or tFL biopsy ("phase-specific lesions"). This analysis allows to discriminate between a linear, sequential model, wherein the tFL dominant clone will maintain all lesions present in the FL dominant clone, along with additional tFL-acquired alterations, and a divergent evolution model, which postulates the existence of lesions that are unique to the dominant clone of the FL or the tFL in addition to the set of shared alterations (Experimental Procedures and Figure S1).

Overall, we found 52 clonally represented, shared copy-number aberrations (CNAs; average, 4.3 per sample; range, 0 to 19 per sample) and 234 shared single-nucleotide variants (SNVs), including silent and nonsilent mutations (average, 38.5 per sample in the four patients with matched normal DNA; in the remaining eight pairs, shared SNVs were only considered if they affected 52 genes that have been previously validated as functional targets of somatic mutations in lymphoid malignancies, because of the exceedingly high number of variants that are predicted in the absence of matched normal DNA, most likely reflecting private SNPs not reported in public databases; see the Experimental Procedures). The presence of shared genetic alterations was documented in all sample pairs analyzed, confirming the original clonal relationship between the FL and tFL sample (Figure 1, left).

In addition to shared lesions, all tFL cases harbored unique mutations and CNAs that were not present in the major FL clone at diagnosis, indicating acquisition during the transformation process or selection of a minor subclone, the size of which was below the detection threshold of the methodologies used. The number of tFL-specific lesions (n = 709 SNVs and 291 CNAs, including 119 losses and 172 gains) was widely heterogeneous across different patients, ranging from 24 to 161 per case (average, 83 per sample) (Figure 1, right; see also Figure S2A and Table S3). Importantly, unique, clonally represented events were also detected in 10 of 12 baseline FL biopsies (n = 327, including 229 SNVs and 98 CNAs; Figure 1, middle, and Figure S2A). The presence of FL-specific lesions was not due to CN loss or copy-neutral loss of heterozygosity (cnLOH) affecting the same region in the sequential tFL biopsy, as documented by both SNP array and WES analysis. Thus, these events had been acquired independently by the dominant FL clone, consistent with divergent evolution.