Clonal Evolution in t(14;18)-Positive Follicular Lymphoma, Evidence for Multiple Common Pathways, and Frequent Parallel Clonal Evolution

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Abstract **Purpose:** Follicular lymphoma typically has acquired a t(14;18) translocation, but subsequent additional cytogenetic abnormalities contribute to disease progression. The main aims of the study are to (*a*) identify the frequency and temporal sequence of cytogenetic events in t(14;18)-positive follicular lymphoma, (*b*) determine if there are specific pathways in the evolution of follicular lymphoma, (*c*) determine the clonal divergence in cases with sequential biopsies or multiple clones from a single biopsy, and (*d*) determine the association of genetic imbalances with clinical outcome.

Experimental Design: All cases with a histologically confirmed diagnosis of follicular lymphoma and cytogenetic analysis showing t(14;18)(q32;q21) were included. The karyotypes were reviewed and cytogenetic data were entered into a relational database for further computational analysis; 418 biopsies from 360 follicular lymphoma patients including 43 sequential biopsies were analyzed.

Results: Of the cases with only one or two genomic imbalances, the most frequent chromosomal imbalances were +7, del(6q), +der(18)t(14;18), +18, and +X. These abnormalities were also among the most frequent ones encountered when all karyotypes were analyzed. Cytogenetically abnormal clones in the same (26%) and sequential biopsies (63%) often showed divergence of genetic alterations. Balanced translocations other than the t(14;18) were uncommon events, but chromosomal breaks involving 14q32, 18q21, 1p36, 1q21, 10q22, 10q24, and a large cluster at 6q occurred relatively frequently. del(6q), +5, +19, and +20 were associated with poorer overall survival, and del(17p) was associated with poorer event-free survival. Lower-grade tumors (1 and 2) were associated with fewer imbalances.

Conclusion: Our analysis suggested that +der(18)t(14;18) may be an entry point to a distinct pathway of genetic evolution in follicular lymphoma. The other common early events appeared to provide multiple entry points, and they might cooperate in the pathogenesis and progression of the follicular lymphoma. Cytogenetically abnormal clones from same patients often showed divergence of genetic alterations, suggesting that parallel evolution from precursor clones are frequent events. This study provides the framework for further analysis of genetic pathways of tumor progression.

Follicular lymphoma is the second most common histologic subtype of non-Hodgkin's lymphomas in North America, accounting for more than one-fourth of all newly diagnosed adult cases (1). Although follicular lymphoma is a well-defined entity histopathologically, it is a heterogeneous disease with regard to clinical behavior, ranging from indolent cases not requiring treatment for several years to cases rapidly progressing into high-grade histology with an aggressive

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Translational Relevance

Follicular lymphoma, similar to other lymphomas, progresses through a series of genetic alterations. Our studies examined a large series of follicular lymphoma with cytogenetics assays and constructed a framework of the genetic evolution of follicular lymphoma. It also showed the frequency of parallel evolution of neoplastic clones and identified several genetic alterations associated with poorer outcome. Understanding the major pathways of oncogenesis in a tumor is important, as many of these steps may be potential targets of therapeutic intervention. Identifying genetic changes that have a major effect on prognosis is also important in management decisions.

clinical course (2, 3). The tumor cell in follicular lymphoma arises from a germinal center B-cell that, in 85% to 90% of cases, has acquired a t(14;18) translocation (4). Although crucial in the initiation of the malignant process, the t(14;18) translocation alone is not sufficient for the establishment of follicular lymphoma. In fact, the classic t(14;18)(q32;q21) has been detected and followed up in healthy individuals without any apparent sign of disease (5, 6). Clearly, additional genetic alterations are necessary to establish a follicular lymphoma, and once established, a follicular lymphoma will continue to acquire additional genetic abnormalities with consequent changes in biological and clinical behavior of the tumor. Clonal evolution leading to histologic transformation occurs in 20% to 60% of cases and several cytogenetic abnormalities associated with the transformation have been described (7 – 10). When analyzing primary clones with single additional abnormality in t(14;18)-positive follicular lymphoma cases, we have identified numeric and structural abnormality of chromosomes 18 and 7, specifically +der(18)t(14;18), +18, and +7, as the early events (11). In a cytogenetic model proposed by Hoglund et al. (12), four specific cytogenetic abnormalities [del(6q), add(1p), +der(18)t(14;18), and +7] have been identified as early events in the clonal evolution of t(14;18)-positive follicular lymphoma. These then lead to specific downstream pathways of clonal progression in a distinct temporal sequence of chromosomal aberrations (12). We have examined 418 biopsies from 360 cytogenetically characterized follicular lymphoma cases with abnormal karyotypes containing the t(14;18). Forty-three cases contained two or more sequential biopsies, and in 106 cases, there were two or more abnormal clones. In this study, we present a comprehensive analysis of this large set of karyotypic data to (*a*) identify the frequency and temporal sequence of cytogenetic events in t(14;18)-positive follicular lymphoma, (*b*) determine if there are specific pathways in the evolution of follicular lymphoma, (*c*) determine the clonal divergence in cases with sequential biopsies or multiple clones from a single biopsy, and (*d*) determine the association of genetic imbalances with clinical outcome.

Materials and Methods

Case materials. From a total of 655 biopsies from 570 patients with histologically confirmed follicular lymphoma in the database at the Human Genetics Laboratory of the University of Nebraska Medical Center, 533 of 655 (81%) biopsies yielded cytogenetic results. Of these, all the biopsies showing t(14;18)(q32;q21) were identified for analysis. The karyotypes were reviewed and the updated cytogenetic data were

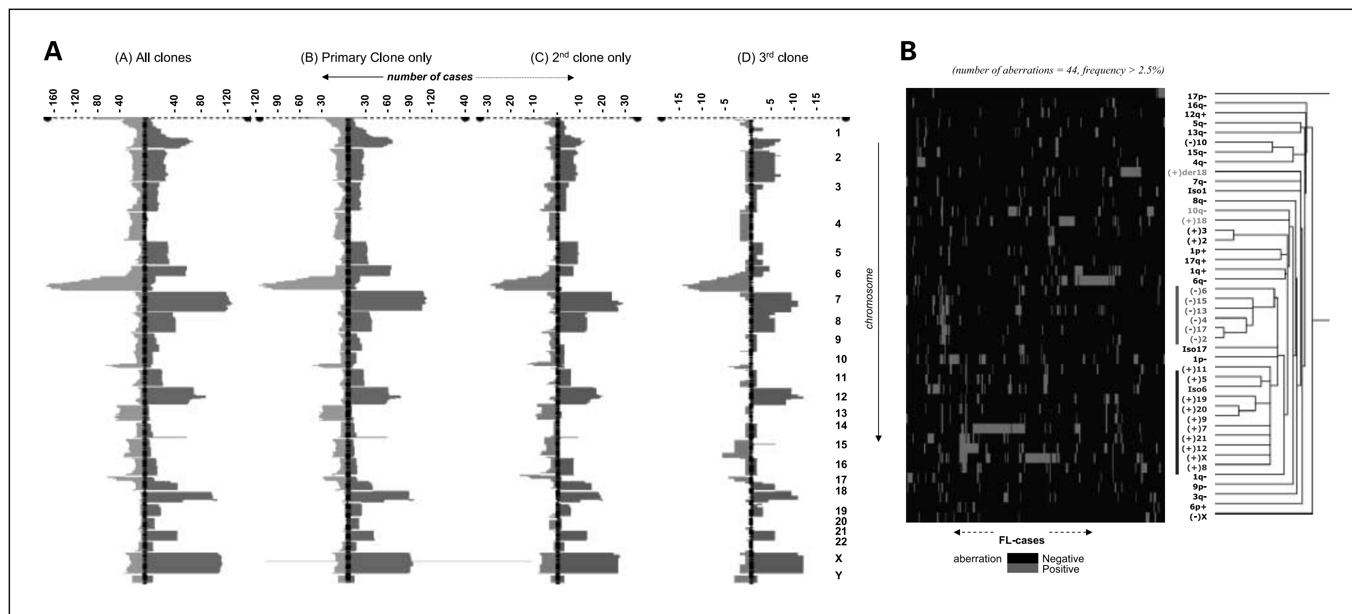

Fig. 1. *A,* gains and losses in neoplastic clones. The frequency of gains and losses is illustrated for the abnormal clones found in the biopsy specimens. *Left,* losses; *right,* gains. The profiles of gains and losses are very similar among primary, second, and third clones. *B,* hierarchical clustering of the most frequent aberrations. Aberrations that occur at a frequency of >2.5% of the abnormal primary clones are represented (n = 44). The cluster stretching from +11 to +8 contains many of the most common abnormalities detected and the members of the cluster consist of gains of the whole chromosome, except i(6p). The adjacent cluster from -6 to -2 contains exclusively whole chromosomal losses. +der 18, +18, and 10q- do not cluster with other common events.

Table 1. Most frequent genetic imbalances

Aberration	No. cases*	% (n = 360)
del(6q)	91 (30)	25
+7	76	21
+X	66	18
del(1p)	57 (11)	16
+12	40	11
dup 6p	37 (30)	10
+18	36	10
del(10q)	35	10
dup 1q	34 (11)	9
+der(18)t(14;18)	34	9
+21	28	8
-13	26	7
+8	25	7
del(17p)	21 (12)	6
del(1q)	20	6
+5	20	6
dup17q	17 (12)	5

*Numbers in parentheses are numbers contributed from isochromosomes.

entered into a relational database (see Computational Analysis of Karyotype Data). Cytogenetic data of 418 biopsies with abnormal karyotypes from 360 follicular lymphoma patients were analyzed. Of these, 312 had one, 76 had two, 24 had three, and six had four abnormal clones. Multiple sequential biopsies with cytogenetic data were available in 43 cases: 32 had two biopsies, 8 had three, 2 had four, and 1 had five biopsies.

Routine karyotyping. Routine karyotyping was carried out by standard cytogenetic methods from mechanically disaggregated tissue after 24 to 48 h of culture as described previously (13 – 15). Karyotypes of Giemsa-banded metaphase chromosomes were described according to the ISCN 1995 nomenclature (14 – 16). As a rule, cytogenetic abnormalities are defined as clonal when two or more cells have the same structural abnormality or gain of the same chromosome or if three or more cells have loss of the same chromosome. When multiple clonal abnormalities are detected in the same patient, the primary clone was operationally defined as the one with the lowest karyotypic complexity.

Computational analysis of karyotype data. The karyotype profile for 441 chromosome bands was entered in an Microsoft Excel format, similar to the one reported by Hoglund et al. with minor modifications (12, 17). Chromosomal gains and losses resulting from numeric or structural abnormalities and balanced and unbalanced translocations were translated in Excel format. Each chromosome was assessed for complete or partial gain/loss and breakpoints for structural changes were recorded in a separate column with appropriate annotations. The data were reviewed by an expert cytogeneticist (B.J.D.) after incorporation into the Excel format. For the computational or statistical analysis, a matrix file was generated, where each karyotype was scored for the presence (1) or absence (0) of each of the recurrent abnormalities.

To evaluate the temporal pattern of occurrence of imbalances, the distribution of the number of imbalances per tumor (NIPT) and the modal value of NIPT for each abnormality were determined. (12). The relationship of the different clones from the same or sequential biopsies was made directly by band-by-band comparisons. The designation of the primary (less complexity) clone versus the secondary (higher complexity) clone was based on the assumption that the primary clone should have genetic abnormalities of lower complexity than observed in the secondary clone. We applied permutation test to assess the significance of the association between paired abnormalities present in at least 2.5% of tumors. We also applied hierarchical clustering (12) to find associations among imbalances using Eisen's Cluster and TreeView software (18).

Clinical characteristics and overall survival. The Kaplan-Meier method was used to estimate overall survival (OS) and event-free survival (EFS) and the log-rank test was used to compare differences in survival among genetic abnormalities. Only recurrent abnormalities with a frequency of >2.5% were included in the analysis (n = 42); 289 cases were available for the analysis of OS data and 145 for EFS.

The NIPT was also correlated with age, tumor grade, and OS. Cox regression proportional hazards model was used for multivariate analysis. Abnormalities that were significantly related to OS from the univariate analysis were examined in the multivariate model. First, only the significant imbalances were considered; then, FLIPI (19) was added to the model to see if the imbalances were still significant after adjusting for FLIPI score. Fisher's exact test was used to determine if there is a significant association of any of the imbalances with the FLIPI risk groups. SAS software was used for the data analysis (SAS Institute).

Results

Cytogenetic analysis: frequencies and types of genetic imbalances. The frequency of chromosomal gains and losses in different abnormal clones is summarized in Fig. 1A and abnormalities observed in the primary clone with a ≥5% frequency are tabulated in Table 1. These abnormalities represented more gains (11 of 17) than losses. Five alterations that were present in >10% of all follicular lymphoma cases included del(6q), +7, +X, del(1p), and +12. A significant

Table 2. Clones with a single additional abnormality

Aberration	No. cases (n = 57)
+der(18)t(14;18)	9
+7	6
del(6q)	6 (1)*
+X	4
+18	2
del(10q)	2
del(17p)	2 (1)*
del(1p)	2 (0)*
1q-	2
-Y	1
+12	1
+15	1
+19	1
+2	1
+21	1
del(7q)	1
del(11q)	1
12q+	1
del(16p)	1
del(19q)	1
del(7p)	1
del(8p)	1
ins(12;13)	1
Inv(9)	1
t(1;10)(p12;p11.2)	1
t(1;19)(p36.1;p13.3)	1
t(1;3)(p21;q29)	1
t(1;3;7)(q31;q27;q34)	1
t(3;5)(p25;q13)	1
t(3;6)(q21;p25)	1
t(3;8)(p25;q24.1)	1

NOTE: Includes abnormalities other than imbalances.
*Numbers in parentheses are numbers contributed from isochromosomes.

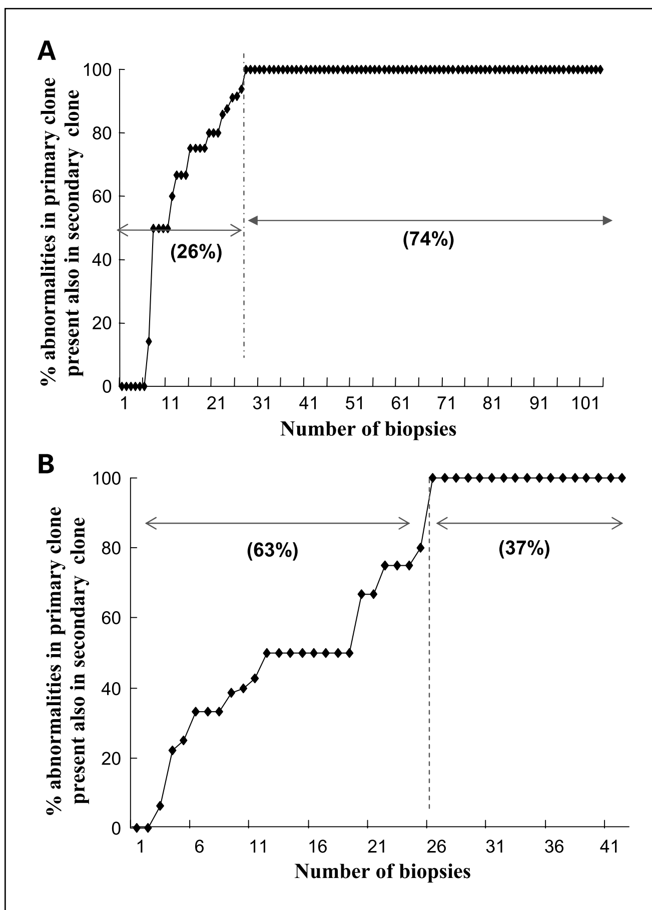

Fig. 2. Comparison of clonal abnormalities in (*A*) the same biopsy and (*B*) sequential biopsies. In the same biopsy, 74% of the secondary clones contain all the abnormalities identified in the corresponding primary clones. In sequential biopsies, only 37% of the secondary clones contain the entire set of abnormalities identified in the corresponding primary clones.

number of the gains/losses in chromosomes 1, 6, and 17 were due to isochromosomes and their occurrence is indicated in Table 1. In fact, the majority of the 6p and 17q gains with an equal number of consequent 6q and 17p losses were accounted for by isochromosomes.

Distribution and temporal order of genetic imbalances. The NIPT may reflect the biological age of tumor. The majority of cases had three or less imbalances with a peak value at 1. About 10% of the tumors had >10 NIPT (Supplementary Fig. S1).

To determine the most frequent early events in follicular lymphoma, which are most likely represented by clones with few karyotypic changes, we examined karyotypes with one or two genomic abnormalities in addition to the t(14;18). An additional copy of der(18)t(14;18) [a +der(18)t(14;18)] was the most frequent single abnormality followed by +7 and del(6q) (Table 2). It is interesting to note that, although infrequent, balanced translocations, most often involving chromosomes 1 and 3, occurred as single abnormalities. A total of 122 t(14;18)-positive cases contained up to two imbalances, including +7, del(6q), +der(18)t(14;18), +18, +X, del(10q), and del(1p) in descending order (Supplementary Table S1). The distribution of frequencies of these early events plotted against NIPT is shown in Supplementary Fig. S2. It is noteworthy that +der(18)t(14;18) was seen most frequently as a single event (mode of NIPT = 1), with +X and del(10q) peaking slightly later, whereas the peaks for del(6q) and +7 occurred at higher NIPT values (mode = 3).

Association among recurrent genetic imbalances. We examined associations between the common cytogenetic imbalances. Strong associations between del(1p)/dup(1q), dup(6p)/del(6q), and del(17p)/dup(17q) were observed due to the frequent presence of isochromosomes 1, 6, and 17. Taking isochromosomes as a single event, we reanalyzed the results (Supplementary Table S2). The common gains and losses [+X, del(1p), +12, del(10q), +21, -13, i(6p), +8, del(1p), +5, and i(17q)] were often associated with one another and with del(6q) or +7 abnormalities (Supplementary Table S2A). The most striking exception is the paucity of association between +18 and +der(18)t(14;18) with these common abnormalities. Statistical analysis suggested that the occurrence of some of these associations was not a chance event ($P < 0.05$; Supplementary Table 2B). Further, we analyzed the data for hierarchical clustering taking isochromosomes as a single event in the analysis. Two-dimensional hierarchical clustering, using common cytogenetic events ($n = 44$; >2.5% frequency), is shown in Fig. 1B. Many of the common events consisted of whole chromosome gains (total, $n = 18$), many of which formed a tight cluster (from +11 to +8). An adjacent cluster representing moderately common events and consisting of whole chromosome losses (-6 to -2) was also seen. Many of the events within these two clusters had also been observed to form pairs of events with significant association as noted earlier. +der(18)t(14;18) and +18 again did not cluster with these two groups of abnormalities, indicating that they may be biologically distinct events.

Balanced translocation and breakpoint analysis. Balanced translocations other than t(14;18)(q34;q21) were uncommon (Supplementary Fig. S3A) and involved a variety of chromosomal regions (Supplementary Fig. S3B), most frequently involved chromosomes 1, 2, 3, 6, 10, and 11, but the breakpoints are quite variable. There were a cluster of translocations involving 3q with 3q29 as the most common breakpoint. 3q27 was observed in only four karyotypes. The 8q24.1 translocation might involve *c-myc*, but none of them involved 14q32 and the IgG light-chain loci were implicated in one case each (the λ locus in 2p12 and the κ locus at 22q11.2). The 14q32 and 18q21 were frequently involved in structural abnormalities other than the t(14;18). Part of these was represented by +der(18)t(14;18). Other frequent breakpoints, as shown in Supplementary Fig. S3B, are 1p36.3, 1q21, 10q22, 10q24, 12q13, and a cluster of breakpoints on 6q (6q21, q23, q15, and q25). The p10 and q10 breakpoints represented the formation of isochromosomes.

Association between primary (stemline) and secondary (sideline) clones. There were 106 cases with karyotypes showing two or more clonal populations and multiple sequential biopsies were studied in 43 cases. This allowed us to study the relationship between the clones from the same tumor concurrently and sequentially (Fig. 2A and B). We operationally defined the clone with the lowest cytogenetic complexity as the "primary" or "stemline" clone and compared the other abnormal clones with it. If the other clones contained all the abnormalities of the corresponding primary clone, we considered them clones that had evolved from the primary clone, also called secondary or sideline clones. If not, we quantitate the

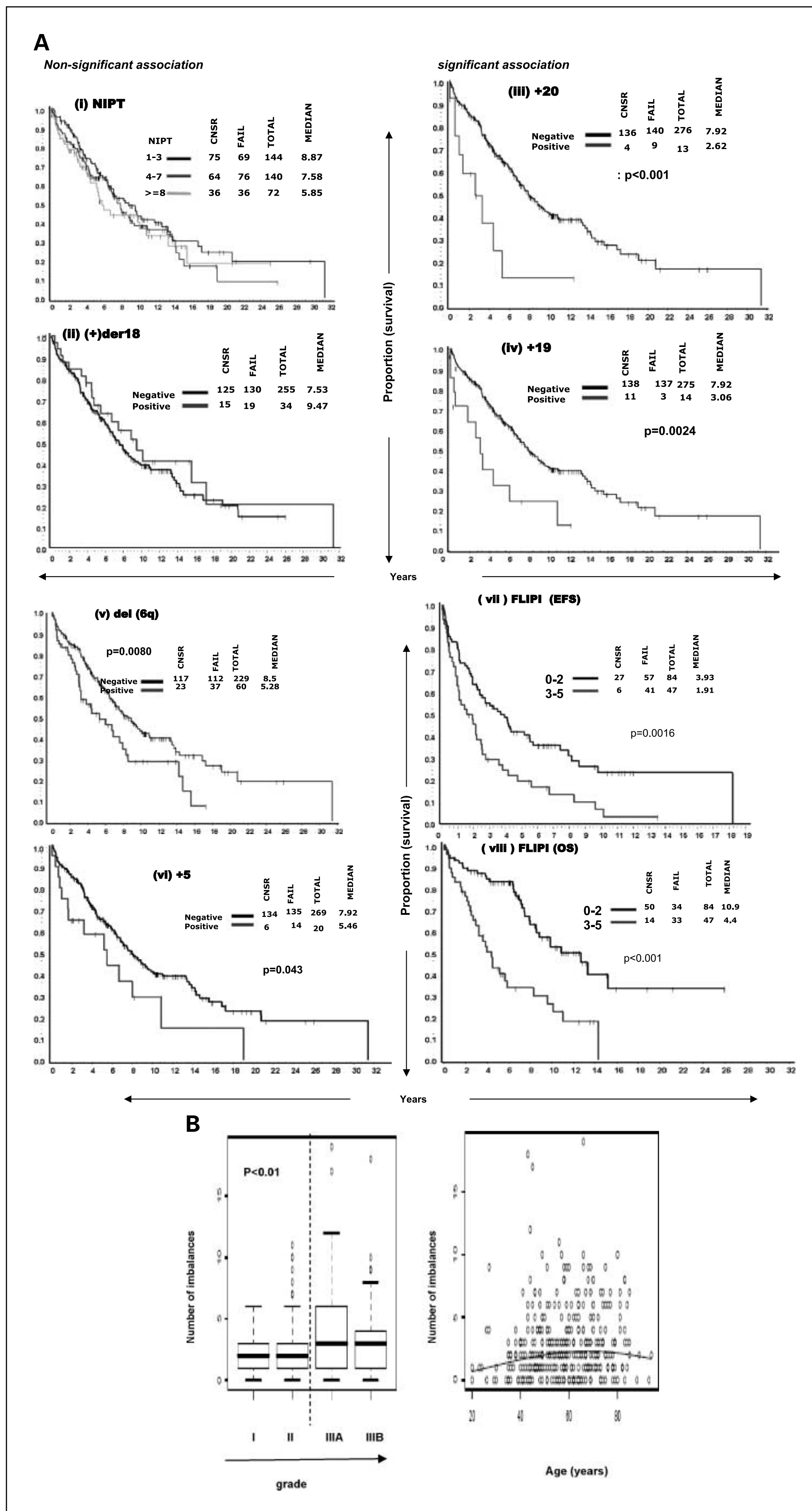

Fig. 3. Correlation of cytogenetic abnormalities with OS, tumor grade, and age in follicular lymphoma. *A,* significant and selected nonsignificant associations with clinical outcome. *B,* correlation with tumor grade and age.

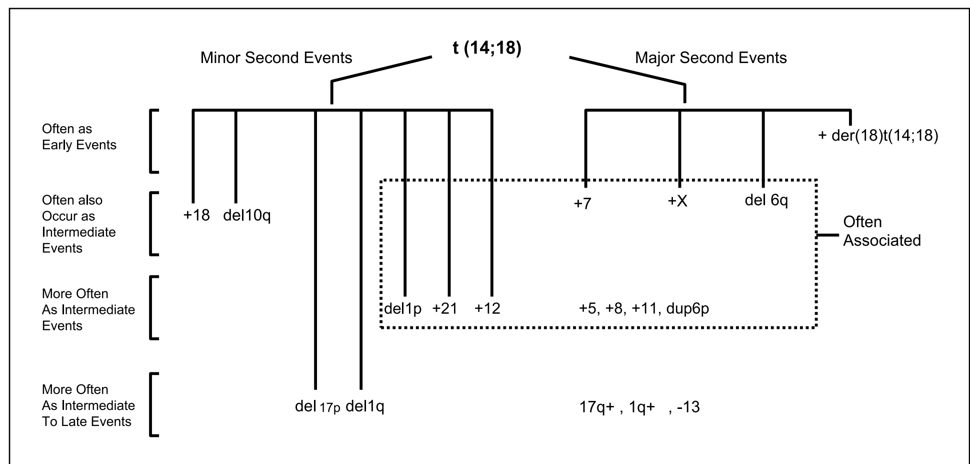

Fig. 4. Diagram illustrating how the common genetic imbalances interrelate with each other in the genetic evolution of follicular lymphoma.

number of genetic events that were not represented in these second clones and the two clones were considered to have arisen from a common precursor.

In many instances, we observed that the concurrent second clones (26%) or abnormal clones in sequential biopsies (63%) did not share all the abnormalities of the primary clones, indicating that they have not "evolved" from these primary clones (Fig. 2A and B). On the other hand, the multiple related clones allowed us to directly observe the genetic evolution of the primary clones. The vast majority of the imbalances that were added to the primary clones were common abnormalities observed in the entire cohort.

Association of cytogenetic abnormalities with clinical outcome. Among the 356 patients with clinical data, 49% (n = 175) were alive at last follow-up and 51% (n = 181) had died. The median follow-up time for the 175 living patients was 5.3 years (range, 0-29.7 years). There was no association of NIPT and OS (P = 0.34; Fig. 3A, *i*). Survival analysis of the recurrent imbalances (n = 42) revealed significantly negative association of del(6q) (P = 0.008), +5 (P = 0.04), +19 (P = 0.002), and +20 (P < 0.001) with OS (Fig. 3A, *iii-vi*). Neither +der(18)t(14;18) nor +18 (data not shown) was associated with OS (Fig. 3A, *ii*). When association with EFS was examined (n = 145), +18 (P = 0.07) and del(4q) (P = 0.045) were marginally associated with better EFS, whereas del(17p) (P = 0.05), i(17q) (P = 0.026), and del(3q) (P = 0.07) were the opposite. The imbalances that showed significant association with OS also showed similar trend with EFS, but were not significant, probably related to the lower number of cases. Imbalances that showed significant association with OS from the univariate model were also significant in the multivariate model (Supplementary Table S3). FLIPI data were available in 131 cases and showed significant association with both OS and EFS (Fig. 3A, *vii* and *viii*), and of these, only 106 cases had one of the recurrent chromosomal abnormalities. Among these, only del(6q) and add (1p) showed marginal association with higher FLIPI scores (refs. 3–5; P = 0.06 and 0.05, respectively).

In addition, none of the aberrations was significantly associated with tumor grade. However, NIPT showed significant association with grade, grades 1 and 2 showing lower numbers compared with 3A and 3B (P < 0.01; Fig. 3B). There was no association of NIPT with age at presentation.

Discussion

We report here a large cytogenetic study on follicular lymphoma with data generated from a single laboratory and employed computational analysis to dissect the pattern of abnormalities observed. This study is confined to cases with t(14;18)(q32;q21), which is typically present in about 85% to 90% of follicular lymphoma cases.

Several genetic imbalances occurred at high frequencies in tumors with low cytogenetic complexities and therefore might represent early events in the evolution of follicular lymphoma. The temporal sequence of events in our study (Fig. 1A) showed significant differences from that reported in an earlier report (12), most likely resulting from the much larger number of cases that were all analyzed in a single institution in our study. Of the early events, the +der(18)t(14;18) was a highly unique abnormality that was frequently observed as a sole additional abnormality among the t(14;18)-positive follicular lymphoma cases. We also noted that it was less frequently associated with other common cytogenetic changes that occurred in abnormal clones with low or intermediate complexity. The +der(18)t(14;18) therefore appeared to represent a distinct gateway into a separate pathway of genetic evolution of follicular lymphoma. This is in agreement with earlier observations (11, 12).

Many of the other "early" abnormalities were often observed as a part of multiple common imbalances. The most frequent of these early events were del(6q), +7, and +X but +12, +21, del(1p), +18, and del(10q) were also observed. Hierarchical clustering of imbalances with >2.5% frequency (Fig. 1B) showed a large cluster containing the early events +7, +X, and i(6p) and the early/intermediate events +8, +12, +21, +5, +9, +11, +19, and +20. By analyzing paired imbalances, many of the above form pairs with significant P values, again confirming their association and possible functional interaction. Other abnormalities may pair with one another or with members of the above clusters, but the association is less likely to be statistically significant.

Our results are not in accordance with the suggestion of Hoglund et al. (12) that del(6q) and +7 lead to distinct genetic subgroups; our results indicate that these two abnormalities did not lead to distinct genetic subgroups. There is actually a group of abnormalities that seem to provide multiple entry points

in the further evolution of the t(14;18)-positive clone, including del(6q)/i(6p) and +7 but also +X, +12, del(10q), del(1p), and +18. Unlike +der(18)t(14;18), del(6q)/i(6p), +7, +X, +12, and del(1p) commonly paired with each other and with the other events in the above-described cluster, suggesting that members of this cluster may cooperate to establish the follicular lymphoma (Fig. 4). It should be noted that, although infrequent, other events could also occur as early events or even the sole abnormalities aside from t(14;18)(q32;q21). These observations suggest that functional alterations important in the establishment of follicular lymphoma may be accomplished by multiple genetic alterations (20); some of these alterations are preferred and represented as the frequent early abnormalities.

An adjacent cluster containing whole chromosome losses was observed in hierarchical clustering involving chromosomes 2, 4, 6, 13, 15, and 17. Again, we observed significant paired associations between many of these losses. These are generally intermediate to late events and probably more important in the progression of follicular lymphoma.

Balanced translocations were uncommon events, but it is interesting to note that a few of these were sole additional events (Table 2). Balanced translocation involving 3q27 (possible *BCL6* breakpoint) was uncommon. However, this may be an underestimation of the frequency of *BCL6* translocation because there are breaks in this region not associated with balanced translocation and marker chromosomes were not characterized. It is interesting that, in follicular lymphoma, the frequency of different molecular breakpoint on *BCL6* may be different from that classically observed in diffuse large B-cell lymphoma (21, 22). Five cases with translocations at 8q24.1 were also observed, but none of these involved the 14q32 locus. Although none of the balanced translocations involved 14q32, the breakpoint 14q32 was frequently noted to be involved in other structural abnormalities. Other recurrent breakpoints included 18q21, 1p36, 1q21, 10q22, 10q24, 12q13, a cluster of breaks on 6q, and the breaks involved in the formation of isochromosomes 1q, 6p, and 17q. It is interesting that only 1q21, 10q22, and 10q24 also occur relatively frequently in balanced translocations. It would be highly interesting to determine the frequently translocated genes located on the common breakpoints. Long-distance inverse PCR may be used to discover genes translocated to the vicinity of the IgH gene locus (23). Novel technologies using high-density DNA microarray may be employed to characterize other breakpoints (24, 25).

Karyotypes containing ''adds'' and ''markers'' represent chromosome or chromosomal segments that cannot be characterized by conventional cytogenetic techniques and may harbor some important information. Techniques such as multicolor fluorescence *in situ* hybridization (M-FISH; ref. 26) have been useful in defining the origin of these ''undefined'' chromosomes or chromosomal segments. An example of this is the chromosomal band 1p36, commonly reported as add(1)(p36) in follicular lymphoma. Spectrokaryotyping (SKY)-based and M-FISH analysis revealed that the extrachromosomal segment at 1p36 could be derived from multiple chromosomal regions, including materials containing *BCL2/IgH* fusion (13, 27). Furthermore, there may be loss of genetic materials at 1p36, possibly including tumor suppressor genes contributing to tumor progression (14). However, SKY and M-FISH are expensive assays and require the availability of stored metaphase spreads or cell pellets to apply to archival cases. These represent significant barriers in using these technologies to study large retrospective series of cases.

Another important observation in this study is the clonal complexity of follicular lymphoma. The large number of primary and sequential biopsies in this study allowed us to assess the frequency of clonal divergence in follicular lymphoma. It was common to observe two or more abnormal clones in a karyotype. About a quarter of these clones were divergent in their cytogenetic profiles, indicating that they are progenies from a common precursor clone rather than one deriving from the other. The frequency was even higher in clones from sequential biopsies (63%). This has important implications on the concept of tumor evolution and transformation in follicular lymphoma. It seems that parallel clonal evolution is a common phenomenon in follicular lymphoma with multiple subclones emerging from a parental clone and even a single tumor site may be populated by one or more of these clones. Notably, clones from sequential biopsies were frequently different from the original biopsy. Clonal evolution of one of these subclones may give rise to a large cell lymphoma, but this subclone may not have the same profile of genetic changes as the subclone detected from a previous biopsy. Therefore, the common approach of investigating large cell transformation by comparing the transformed lymphoma with a previously obtained follicular lymphoma may not be entirely valid. Further, sequential biopsies may not be a good model for pairwise comparative study of follicular lymphoma progression. Our observation may also explain the inability of a recent gene expression profiling study to find outcome predictors related to the tumor cell population in the initial tumor biopsy. The host response to the tumor may be more consistent; hence, an association with prognosis is detected (28). Similarly, correlation of survival with cytogenetics findings of the initial biopsy may be subjected to the same limitations. The cytogenetics data in this study were collected over a long period (May 1982 to May 2005) and the treatment regimens were not standardized. Rituximab was administered to selected patients after 1999 but again not according to a single protocol. The survival data have to be interpreted in this context. In our series, we observed a significant association of only a few chromosomal abnormalities with OS. These abnormalities may have a strong effect on OS that is significant even with the limitations noted above.

+der(18)t(14;18) represents a unique pathway in the pathogenesis of follicular lymphoma, but follicular lymphoma with this abnormality has no significant difference in outcome compared with other cases, suggesting that this pathway does not have influence on the prognosis. Previous studies have shown del(1)(p36), +18q (29), del(6)(q23-q26), del(17p) (30), del(17p), +12 (12), +21, and NIPT (31) to be associated with poorer clinical outcome. In our series, del(6q) and del(17p) were also found to be associated with poorer outcome consistent with previous reports, but +19, +5, and +20 have not been reported before. The association of FLIPI with clinical outcome was expected. There are significant discrepancies in the literature regarding the association of specific genetic imbalance with clinical outcome. This may be due to the

limited number of cases in many previous studies, the different populations of patients who were not treated uniformly, or the clonal populations divergent from the one examined at the time of cytogenetic review as noted above. Despite all these limitations, del(6q) and del(17p) appear to be most consistently associated with poorer outcome and may be the most promising candidates for further investigation.

Large cytogenetic data set can provide very useful information, but the resolution is not sufficiently high to identify the precise boundaries of the gains and losses. Small deletions/duplications may have been missed and chromosomes and/or chromosomal segments may have remained unidentified due to suboptimal resolution. The data can be enriched by high-resolution array comparative genomic hybridization (32 – 34) that can help to resolve many of the ambiguities of cytogenetic studies and provide fine details on the abnormal loci that may lead to identification of candidate genes in the abnormalities in the future.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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