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CASE REPORT

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Testicular diffuse large cell lymphoma with tubule preservation – molecular genetic evidence of transformation from previous follicular lymphoma

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Abstract Testicular lymphomas usually occur in older men and are mostly diffuse large B-cell lymphomas (DLBL). They may be primary manifestation of lymphoma or represent a relapse of a previous non-Hodgkin's lymphoma. This report details a testicular large cell lymphoma, which was proven to be large cell transformation of a low-grade follicular lymphoma biopsied 8 years earlier. Initially, a 38-year old man was diagnosed with cervical lymphadenopathy, and biopsy was interpreted as reactive follicular hyperplasia; no treatment was given, and the lymphadenopathy resolved spontaneously. Eight years later, the patient underwent surgery for a left testicular mass and gastroscopy for gastric symptoms. The patient died 7 months later with evidence for intra-abdominal and central nervous system lymphoma after a brief but temporary response to M-BACOD chemotherapy. Orchiectomy specimen and gastroscopic biopsy showed diffuse large B-cell lymphoma (CD20+), which infiltrated between well-preserved tubules in the testis. Histological comparison with 20 testicular lymphomas without previous lymphoma showed tubule infiltration in all cases, suggesting that the tubule-preserving infiltration pattern could be a histological marker for secondary lymphoma involvement in testis. On re-examination, the lymph node 8 years prior was verified as follicular, predominantly small, cleaved cell lymphoma with bcl2-positive follicles. The earlier follicular lymphoma and the subsequent diffuse large cell lymphoma were analyzed using polymerase chain reaction and showed identical sequences of the t(14;18) translocation and immunoglobulin heavy chain gene rearrangement. Analysis of the VH-gene sequences from the follicular lymphoma revealed sequence heterogeneity consistent with ongoing mutation. However, the transformed diffuse large cell lymphoma had no intraclonal variation, with the sequence matching with one of the subclones from the low-grade follicular lymphoma. These results confirm that the large cell transformation of follicular lymphoma occurs in a single follicular lymphoma cell. This case also indicates that the selection of the transformed clone can be part of the natural history of disease and can occur without exposure to chemotherapy.

Key words Lymphoma · IgH-gene rearrangement · Bcl2

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Introduction

Diffuse large B-cell lymphomas (DLBL) are the most common testicular tumors found in older men. These lymphomas can involve testis either primarily without previous disease elsewhere or secondarily following lymphoma involvement in other body sites [6, 9, 11, 24, 27, 30, 35, 37].

Follicular lymphoma (FCL) is among the most common nodal non-Hodgkin's lymphomas in North America and includes a spectrum of small and large cell variants. Although the course of disease is typically slow in the low-grade variants, diffuse large cell transformation tends to occur over time, imparting increased disease aggressiveness [1, 3, 13, 33, 36]. The t(14;18) translocation involving the bcl2 and IgH genes is a molecular marker for FCL that can be demonstrated in 60–80% of cases [13, 19, 34].

Sequences of the third complementarity-determining region (CDR3) of the immunoglobulin heavy chain (IgH) gene rearrangement offer excellent markers to identify and follow specific lymphoid clones and evaluate the possible relationship between two histologically different lymphomas diagnosed in one patient. Relatedness of the histologically different B-cell lesions has

been demonstrated in low-grade mucosa-associated lymphoid tissue (MALT)-lymphomas on the basis of identical IgH gene rearrangements [8].

Analysis of the VH-gene mutation pattern is helpful in establishing the cell of origin for B-cell lymphomas [18, 22]. Lymphomas derived from the follicular B cells show frequent somatic mutations in the VH gene [4, 5, 22] and accumulate mutations resulting in intraclonal heterogeneity [4, 5]. Comparison of the VH-gene mutation pattern in subsequent lesions helps us to understand the clonal selection during the progression of disease [28]. Intraclonal heterogeneity in FCL and DLBL can narrow toward homogeneity after intensive chemotherapy [39] but continue to exist throughout the course of disease if remission is not achieved by chemotherapy [23].

In this report, we prove that a testicular DLBL represented a large cell transformation of a nodally presenting low-grade FCL 8 years prior. Both the earlier FCL and the subsequent DLBL showed the same t(14;18) translocation and IgH gene rearrangement. The VH-gene mutation pattern of one of the subclones obtained from the low-grade FCL matched with the mutation pattern obtained from the DLBL subclones. These data indicate that transformation occurred in a single cell of low-grade FCL and suggest that the selection of the transformed cell occurred naturally during the long duration of disease.

Case report

A 38-year-old man had an enlarged lymph node in the neck, which was partially biopsied to rule out lymphoma in April 1983. The biopsy showed a follicular lesion, which was diagnosed as reactive follicular hyperplasia, and no further therapy was given. Ultimately, the residual node clinically regressed.

Eight years later, in May 1991, the patient noticed a lump in the left testicle, and soon thereafter he had episodes of black stools. Ultrasound examination revealed multiple abnormal masses in the left testicle, and gastroscopy showed neoplastic infiltration. Orchiectomy and gastric biopsy were performed and revealed DLBL. Abdominal ultrasound and computed tomography (CT) scans showed a large gastric tumor with enlarged mediastinal and periaortic lymph nodes and tumor around the left kidney. A 20×15-cm tumor was palpable in the upper abdomen extending to the umbilical area. A 0.5-cm hard lymph node was palpated in the retroauricular area, but there were no enlarged cervical, axillary or inguinal nodes. Because of the apparently disseminated disease, there was no further surgery. Instead, systemic polychemotherapy was instituted according to the M-BACOD protocol. Although the tumor mass appeared to regress within 2–3 months, it showed progression 4 months after initiation of the chemotherapy. The patient developed central-nervous-system symptoms, and dissemination of lymphoma in the cerebrospinal fluid was verified by means of cytology. At the same time, elevated serum lactate dehydrogenase (LDH) also indicated activation of disease. Further chemotherapy was unsuccessful, and the patient expired 7 months after histological diagnosis of DLBL, 9 months after having noticed the lump in the left testicle, and 8.5 years after cervical lymph-node biopsy. Permission for autopsy was not granted.

Materials and methods

Histological observations

Hematoxylin and eosin stained sections were evaluated from an earlier follicular lymph-node lesion and the subsequent testicular and gastric lymphoma. For comparative purposes, 20 previously reported primary testicular lymphomas (14) from men without evidence of a previous lymphoma were studied.

DNA extraction and evaluation of template quality

DNA for polymerase chain reaction (PCR) amplification was obtained from ten 5-µm histological sections of formalin-fixed and paraffin-embedded tissue by phenol–chloroform extraction, as previously described [17]. The adequacy of DNA templates was verified by amplification of 336-bp and 268-bp fragments of genomic DNA, as previously described [12, 20].

Evaluation of t(14;18) translocation

The junctional sequences of the t(14;18) translocation involving the major breakpoint region (MBR) and minor cluster region (MCR) were amplified by multiplex PCR using previously described primers for MBR, MCR (MC-9F) and JH [7, 20] in a final concentration 0.35 µM of each primer. The reaction conditions were as previously described [7] with a 60°C annealing temperature. PCR products were size fractionated on 8% polyacrylamide gels, stained with ethidium bromide and ultraviolet light (UV) illuminated. To confirm the specificity of the amplification, PCR products were cloned into plasmid vector and sequenced.

Evaluation of the IgH gene rearrangements in FCL and testicular DLBL

The CDR3s were evaluated using two previously described PCR procedures [25, 32] with a minor modification [17, 20]. PCR products were analyzed on 8% polyacrylamide gels when using the third framework (FR3)-based primer system [32] and on 5% polyacrylamide gels when using the second framework (FR2)-based primer system [25]. The gels were stained with ethidium bromide and UV illuminated.

Amplification of the VH gene

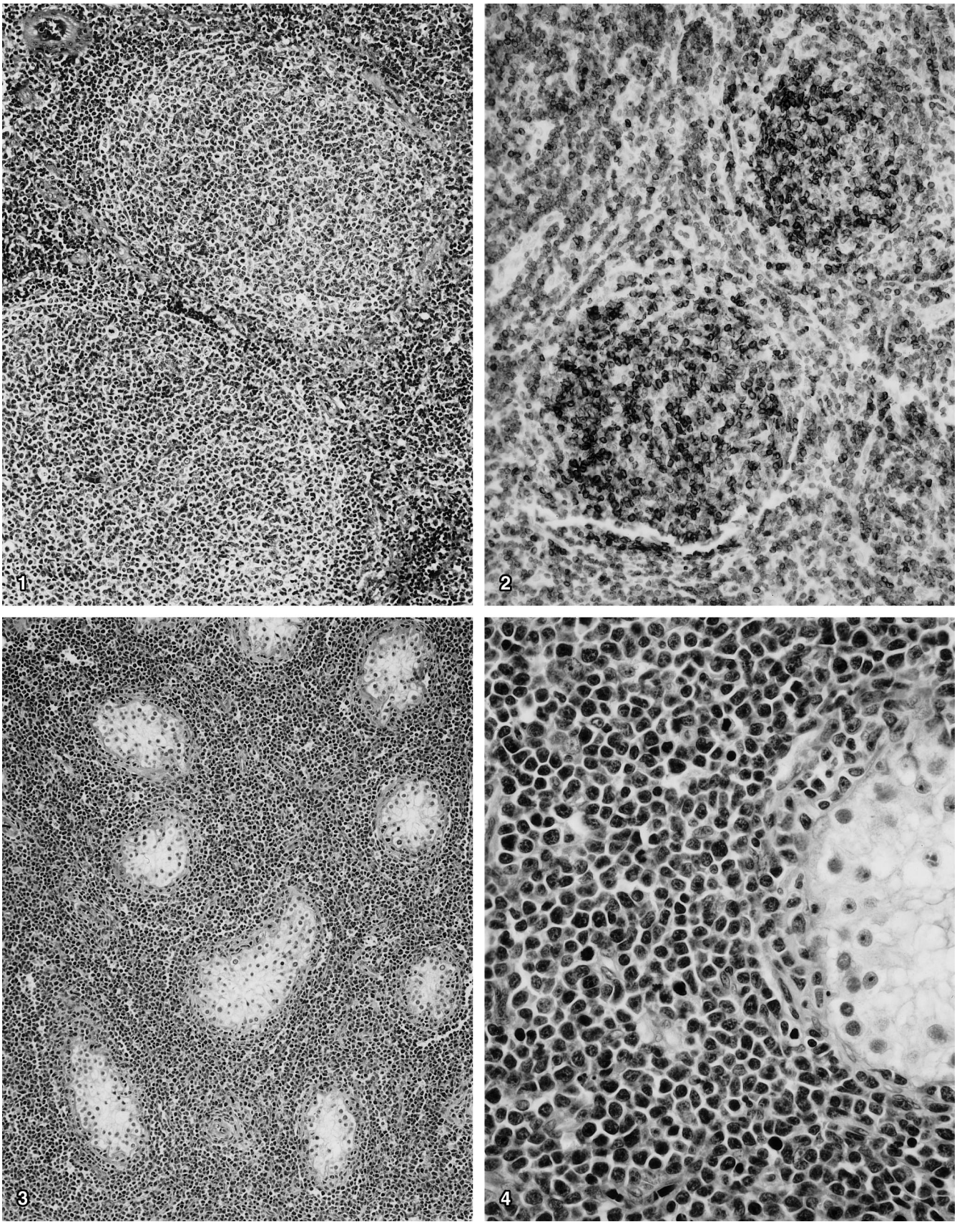
Analysis of the VH gene sequences obtained using the FR2-based PCR system revealed the highest homology to the VH3 gene family. Thus, a VH3-family-specific primer [10] and JH-consensus (LJH) primer [31] were used for PCR amplification to obtain more complete sequences of the VH gene. Samples of 0.5 µg DNA were amplified for 40 cycles in a 50-µl reaction, using standard PCR conditions (Perkin-Elmer) and a 60°C annealing temperature. PCR products were analyzed on 5% polyacrylamide gels, stained with ethidium bromide and UV illuminated.

Taq-polymerase error and negative controls

All PCR reactions were performed using reagents commercially available from Promega (Madison, Wis.). *Taq*-polymerase used in this study was previously evaluated by us for amplification error [14]. This *Taq*-polymerase yields one error per approximately 3400 bp (0.03%). Besides standard precautions to prevent cross-contamination, multiple negative controls were used in each experiment.

Cloning and sequence analysis of PCR products

All PCR products (a single sharp band in each case) were purified from the gels and cloned into a plasmid vector as previously described [17]. Double-stranded DNA templates were prepared from randomly selected recombinants using Qiagen plasmid kit (Qiagen Inc., Chatsworth, Calif.) and sequenced on a 373 DNA sequencer (Applied Biosystems, Foster City, Calif.). All clones were se-


1
2
3
4

◀ **Fig. 1** This axillary follicular lymphoma shows follicular structures composed of predominantly small cells

Fig. 2 Immunohistochemistry of the axillary follicular lymphoma shows strong bcl2-reactivity in the follicles

Fig. 3 Testicular transformed large cell lymphoma showing infiltration between well-preserved seminiferous tubules

Fig. 4 High magnification of the testicular transformed large cell lymphoma reveals uniform large non-cleaved lymphoid cells around the well-preserved seminiferous tubule

quenced from both directions using the forward and reverse primers. Computer analysis of the resulting DNA sequences was performed using the Lasergene software (DNASTAR, Madison, Wis.) in connection with the data of the GeneBank 110/EMBL 55 database (January 1999 edition) and the published germline sequences [15, 16, 26, 29].

Results

Histological features

The cervical lymph-node biopsy taken from 1983 measured 1.2×0.7×0.7 cm and was histologically composed of relatively small follicular structures of uniform size and intervening small lymphocytes. The follicles showed small cells with frequent nuclear cleavages. Rare tingible bodies were observed, and there was mild mitotic activity (Fig. 1). Immunohistochemically, the follicles were strongly reactive with bcl2 (Fig. 2) and were also positive for CD20. Numerous CD3-positive lymphocytes were seen between the follicles, and some inside them.

The testicular tumor operated on in 1991 was a diffuse large cell lymphoma, which showed an interstitial infiltration pattern between well-preserved seminiferous tubules with no or minimal tubular wall infiltration (Fig. 3). The lymphoma cells had large, non-cleaved nuclei and showed no tendency to follicle formation (Fig. 4). The lymphoma cells were positive for CD20 and negative for CD3 and CD30. Scattered CD3-positive small lymphocytes were seen admixed with the lymphoma cells. The gastric biopsy from 1991 showed similar features of diffuse large cell lymphoma.

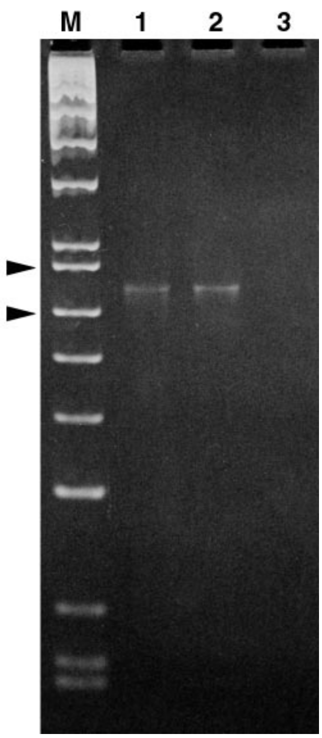


Fig. 5 Gel electrophoretic patterns of the polymerase-chain-reaction amplification of the t(14;18) translocation of the follicular lymphoma and the subsequent large cell transformation. *Lane 1*: follicular lymphoma; *lane 2*: transformed large cell lymphoma; *lane 3*: negative controls. *Arrows* at the DNA size marker (*M*) shown on the side indicate 118 bp and 140 bp. 8% polyacrylamide gel stained with ethidium bromide. Note identical amplification in the follicular lymphoma and the transformed large cell lymphoma

Histological comparison with primary testicular lymphomas

All 20 primary testicular diffuse large B-cell lymphomas (CD20-positive) showed tubular invasion of lymphoma. Sixteen cases showed prominent and four cases focal tubular invasion with common intratubular growth of lymphoma. All cases were negative for the t(14;18) translocation by PCR as previously reported [14].

Evaluation of the t(14;18) translocation

The FCL and the subsequent testicular lymphoma were analyzed. DNA from both lesions yielded a visible band upon PCR amplification of the t(14;18) translocation (Fig. 5). Sequencing confirmed the presence of identical junctional sequences of the bcl2 gene and JH5 segment of the Ig gene in both lesions (Fig. 6).

Evaluation of IgH gene rearrangements in FCL and testicular DLBL

The CDR3s were PCR amplified from both lesions. When the FR3-based primer system was used for amplification, the axillary FCL revealed a sharp band, whereas the testicular DLBL showed a smear of bands (Fig. 7A). PCR amplification with the FR2-based primer system showed similar-sized bands from both lesions (Fig. 7B). Sequencing of all three PCR products showed the same clone-specific IgH VDJ rearrangement in both lesions (Fig. 8). However, multiple nucleotide mismatches were seen in different subclones indicating intraclonal variation. Figure 8 shows alignment of the IgH-gene rear-

Fig. 6 Junctional sequences of the t(14;18) translocation from follicular lymphoma and transformed large cell lymphoma obtained using polymerase chain reaction amplification. Note 100% homology of the sequences. NDN sequence is shown in *small characters*. Primer sequences are not included

	bcl2	N D N	JH 5
FCL	C A A C A C A G A C C C A C C C A G A G C C C T C C T G C C C T C C T T C	c c c c t c c c c c c c g a	A C T G G T T C G A C C C C T G G G
DLBL	C A A C A C A G A C C C A C C C A G A G C C C T C C T G C C C T C C T T C	c c c c t c c c c c c c g a	A C T G G T T C G A C C C C T G G G

rangement sequences from two FCL and seven DLBL subclones with closest similarities. The lack of specific amplification by the FR3-based primer system in the testicular DLBL could be explained by the presence of an additional third mutation in the 3'-end of the FR3-priming area. Two mutations in the target area for FR3 primer were already seen in the FCL.

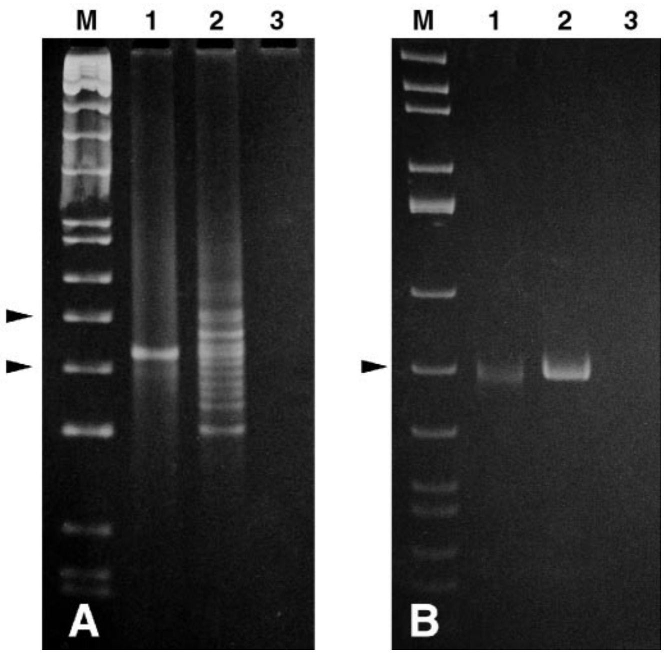


Fig. 7 Gel electrophoretic pattern of the FR3- (**A**) and FR2- (**B**) -based polymerase-chain-reaction amplification of immunoglobulin heavy chain (IgH) gene rearrangement of follicular lymphoma and the transformed large cell lymphoma. *Lane 1*: follicular lymphoma; *lane 2*: transformed large cell lymphoma; *lane 3*: negative control. *Arrows* at the DNA size marker (*M*) shown on the side indicate 82 bp and 100 bp (**A**) and 249 bp (**B**). Polyacrylamide gel stained with ethidium bromide (A 8%; B 5%)

Fig. 8 Alignment of the immunoglobulin heavy chain (IgH) gene rearrangement sequences from two follicular lymphoma (FCL) and seven diffuse large B-cell lymphoma (DLBL) subclones showing the closest sequence similarity. The complementarity-determining regions (CDRs) are according to Kabat et al. [16]. *Numbers above* the sequences indicate codons. DH- and JH-gene germlines involved in the IgH gene rearrangement are shown in CDR3. N-sequences are shown in *small characters*. The FCL sequence is shorter, because it is based on FR2 primer amplification. The DLBL sequence is based on FR1 primer amplification. Primer sequences are not included. *Black squares* indicate two differences between the sequences

VH-gene utilization

DNA from the testicular lymphoma was better preserved and the use of a VH-family-specific primer was possible for the amplification of almost the entire VH segment. Analysis of the sequences revealed highest homology (similarity index 87) to the DP50 VH-gene germline.

Intraclonal variation

Intraclonal variation was seen in all subclones of the FCL. The number of differences varied between 1 and 7 in each of seven related sequences when FR2-based PCR products were compared with the FCL consensus sequence (Fig. 9A). Comparison of the subclone sequences from the testicular DLBL showed only a single nucleotide difference in three of the ten analyzed subclones when compared with DLBL consensus sequences (Fig. 9B).

Discussion

Testicular DLBL can be either primary lymphoma manifesting in the testis or involve testis secondarily after previous lymphoma at other body sites. According to our observations and those of previous investigators [6, 9, 11, 14], primary testicular DLBL occurs chiefly in older men (usually from the sixth decade and older) and almost invariably shows significant infiltration in the walls of seminiferous tubules. In contrast, the testicular relapses of lymphoblastic leukemia typically occur in young patients and show an interstitial infiltration pattern with preservation of the tubules in between the leukemia infiltration [35]. The secondary testicular lymphoma (transformed FCL) in this case occurred in a 45-year-old man and showed an interstitial infiltration pattern with well-preserved tubules in between the lymphoma infiltration. Therefore, the interstitial tubule-sparing pattern could represent signs for secondary nature of the testicular lymphoma. Also, the patient was of younger age than patients with testicular lymphomas (14).

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      25                     CDR1      35                          45
FCL   xxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxAAGGGACTGGAGTGG
DLBL  TCTGGATTCACCTTCAAGAACTACGGCATGAACTGGGTCCGCCAGGCCCCTGGCAAGGGACTGGAGTGG

                       55 CDR2                          65
FCL   GTGGCGGTTATTCGATATGGTGGAAGTTACAAAGATTATGCAGGCTCTGTGAAGGGCCGGGTCACCATC
DLBL  GTGGCGGTTATTCGATATGGTGGAAGTTACAAAAATTATGCAGGCTCTGTGAAGGGCCGGGTCACCATC

                 75                             85
FCL   TCCGGCGACATTTCCAAGAACACAGTGTCTCTGCAAATGAGTACCCTGAGAGTACACGGCTGTCTATTA
DLBL  TCCGGCGACATTTCCAAGAACACAGTGTCTCTGCAAATGAGTACCCTGAGAGTACACGGCTCTGTATTA

        95            D4-17  CDR3          JH3
FCL   CTGTGCGAGAgaccTGACTCACGGTGAttattggggcGCTTTTGATAT
DLBL  GTGTGCGAGAgaccTGACTCACGGTGAttattggggcGCTTTTGATAT
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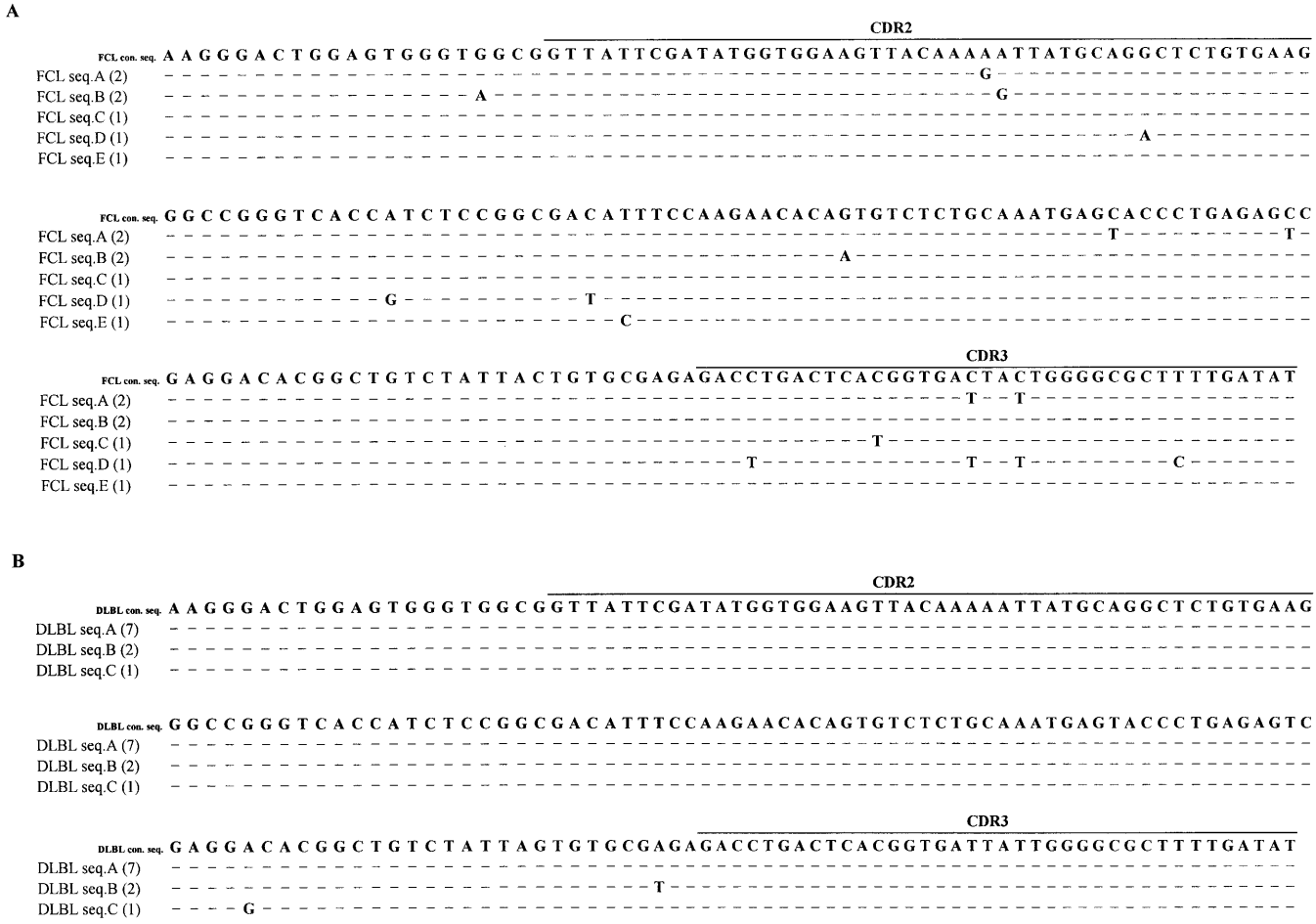

Fig. 9 FR2-based VH gene sequences showing intraclonal variation in follicular lymphoma (A) and transformed large cell lymphoma (B). The sequences are grouped A–E. The number of subclones in each group is shown in *parentheses*. Note nucleotide differences between the subclone sequences. Primer sequences are not included

FCL is one of the most common non-Hodgkin's lymphomas in North America. Although low-grade FCL typically has an indolent course, transformation to diffuse large cell lymphoma ultimately occurs in a significant number of cases and is a sign for accelerated disease with adverse prognosis [1, 3, 13]. In the present case, a low-grade FCL diagnosed in a cervical lymph node remained asymptomatic for 8 years without treatment, but was then followed by a disseminated recurrence with a mass-forming testicular involvement simulating a primary testicular tumor. This disseminated recurrence with evidence for central-nervous-system involvement proved fatal within 7 months, despite intensive chemotherapy.

The demonstration of the bcl2 immunoreactivity throughout the follicles in this case supported the histological diagnosis of low grade FCL; most of these lymphomas show prominent bcl-2 reactivity in the follicles, in contrast to lack of bcl2 reactivity in reactive follicular hyperplasia, which has been proven a practical diagnostic aid [30].

Primary FCLs have been occasionally reported in the testis, but are rare. Ferry at al. [11] found a single testicular FCL among 64 male genital lymphomas (1.6%). Moertel et al. reported a case of testicular FCL in an 11-year-old child, although immunostaining failed to detect bcl2 protein expression, and Southern-blot analysis showed no evidence of bcl2-gene rearrangement [21]. In our previous study, none of the 20 testicular diffuse large B-cell lymphomas analyzed for t(14;18) using PCR were positive, indicating that derivation of these DLBLs from FCLs must be rare [14]. However, it is possible that disseminated FCLs involve testis more frequently, but are not biopsied and seen in surgical-pathology-based material because of the implied clear diagnosis.

The t(14;18) chromosomal translocation occurs in up to 80% of FCLs and 20% of DLBLs, suggesting that some of the DLBLs represent transformation from FCL [2, 34]. This translocation juxtaposes the bcl2 locus on the long arm of chromosome 18 with the joining region of the IgH gene on the long arm of chromosome 14, resulting in upregulated expression of the bcl2 gene [19, 33]. Although the breakpoints vary, the majority of them are distributed in two narrow areas, called MBR and MCR and, therefore, can be conveniently analyzed using PCR amplification of genomic DNA [7, 20]. Therefore, PCR amplification of the lymphoma-specific bcl2–JH junctional sequences can be used as a unique molecular

marker to follow the natural history of the disease and trace minimal residual disease [7]. In the present study, we showed identical sequences of the bcl2-JH-translocation in a low grade, untreated FCL and subsequent DLBL 8 years later, supporting their relationship.

Analysis of the IgH gene rearrangements offers an additional excellent tool to compare the relatedness of two histologically different B-cell lymphomas, as the IgH-gene rearrangement remains the same in B lymphocytes and their clonal descendants [8, 39]. In this study, the testicular DLBL following an FCL in axillary lymph node 8 years prior showed identical IgH-gene rearrangement, verifying the same clonal origin of the two histologically divergent processes.

The VH genes in FCLs are typically highly mutated and show intraclonal variation [5]. Comparison of the VH-gene mutation pattern in subsequent lymphoma lesions is helpful for understanding of the natural history of this disease [28]. Previous studies have indicated that VH-gene mutation pattern can change during the disease progression [23, 38]. In one case of FCL, which relapsed 5 years after complete remission achieved by chemotherapy, lack of intraclonal variation was reported [39]. In another case of FCL, in which clinical remission was not achieved, intraclonal variation persisted during the course of disease [23]. These observations could suggest that chemotherapy plays a role in the clone selection during disease progression. In this study, we reported a case of clinically silent FCL, which was not treated with chemotherapy and transformed to DLBL after 8 years. In the present case, intraclonal variation was seen in FCL but not in the transformed DLBL, indicating that one of the subclones was responsible for disease progression. Our case proves that clonal selection in lymphoma can occur independently of chemotherapy and can be part of the natural course of the disease.

In summary, we have demonstrated the transformation of FCL from clinically silent low-grade lymphoma to a disseminated DLBL mimicking primary testicular lymphoma 8 years later. We confirmed previously published observations [38, 39] indicating that the large transformation in FCLs occurs in a single cell. However, this study shows that the selection of the transformed clone can occur naturally during the disease progression and is independent of possible selection by chemotherapy. We also suggest that the interstitial tubule-sparing pattern could be considered a histological marker for secondary testicular lymphoma. Comparison of the clinicopathologic features of larger series of primary and secondary testicular lymphomas would be of interest.

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