

ORIGINAL ARTICLE

Lymphoma-Specific Genetic Aberrations in Microvascular Endothelial Cells in B-Cell Lymphomas

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ABSTRACT

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BACKGROUND

The growth of most tumors depends on the formation of new blood vessels. In contrast to genetically unstable tumor cells, the endothelial cells of tumor vessels are considered to be normal diploid cells that do not acquire mutations.

METHODS

Using a combined immunohistochemical and fluorescence in situ hybridization assay, we examined the endothelial cells in 27 B-cell lymphomas for cytogenetic alterations that are known to be present in the lymphoma cells.

RESULTS

We found that 15 to 85 percent (median, 37 percent) of the microvascular endothelial cells in the B-cell lymphomas harbored lymphoma-specific chromosomal translocations. In addition, numerical chromosomal aberrations were shared by the lymphoma cells and the endothelial cells.

CONCLUSIONS

Our findings suggest that microvascular endothelial cells in B-cell lymphomas are in part tumor-related and therefore reflect a novel aspect of tumor angiogenesis.

LIKE NORMAL TISSUES, TUMORS require an adequate supply of oxygen and metabolites and effective removal of waste products.¹ Although normal cells and tissues rely on physiologic angiogenesis, tumor cells induce their own blood supply from the preexisting vasculature.^{2,3} In a process that is very similar to physiologic angiogenesis, tumors can elicit the sprouting of new blood vessels from preexisting capillaries.² Moreover, some aggressive cancers can mimic the activities of endothelial cells by participating in the formation of fluid-conducting, vascular-like networks.⁴ This phenomenon occurs without the participation of endothelial cells and is independent of angiogenesis. The mechanism used by the early embryo to form blood vessels has also been observed in tumors.³ In this process, endothelial precursor cells are mobilized from the bone marrow and transported through the bloodstream to become incorporated into the walls of growing blood vessels.⁵ In mice with a mutation that causes defective angiogenesis and that results in resistance to transplanted tumors, Lyden et al. have shown that transplantation of wild-type bone marrow or vascular endothelial stem cells restores tumor angiogenesis and tumor growth.⁶

These observations prompted us to investigate the origin of the microvascular endothelial cells with the use of sex-chromosome analysis in a patient with a post-transplantation lymphoproliferative disorder that developed in the donor organ after sex-mismatched liver transplantation. Unexpectedly, we were unable to determine whether the cells from which the lymphoma developed had originated in the male recipient or in the female donor, because the tumor cells exhibited only one X chromosome. We were further surprised to find that the microvascular endothelium in the lymphoma also carried only one X chromosome.

This finding suggested a genetic relationship between the lymphoma cells and the microvascular endothelial cells, contradicting the assumption that endothelial cells are normally diploid cells that do not acquire mutations.³ To test this hypothesis further, we examined B-cell lymphomas that carried specific chromosomal translocations. In each case, a varying proportion of the microvascular endothelial cells of the lymphoma exhibited the lymphoma-specific genetic aberration, suggesting a close relationship between the two types of cells.

METHODS

CASES

We studied cases of lymphoma that met the histologic and immunohistologic criteria for lymphomas, according to the classification of the World Health Organization.⁷ The lymphomas included 1 case of post-transplantation lymphoproliferative disorder and 27 cases of non-Hodgkin's B-cell lymphoma. The presence of tumor cells was evaluated in each tissue block in sections stained with hematoxylin and eosin that were cut before and after the sections that were used for fluorescence in situ hybridization (FISH), which was performed on single-cell suspensions and on 5- μ m-thick tissue sections. Tissue specimens from 20 reactive lymph nodes and from an uninvolved lymph node in 1 of the 14 cases of follicular lymphoma served as negative controls for the FISH analyses. All patients gave written informed consent to an ethics committee-approved protocol.

CYTOGENETIC ANALYSIS

In 14 cases (Table 1), fresh tumor samples were available for cytogenetic analysis of short-term cultures. The methods of cell cultivation and of chromosome preparation and staining by the Giemsa-banding technique have been described previously.⁸

FISH ANALYSIS

For 13 B-cell lymphomas (Table 1) and for the case of the post-transplantation lymphoproliferative disorder, no fresh tumor samples were available. We used formalin-fixed, paraffin-embedded tissue for the FISH analysis. For a reliable interpretation of the hybridization signals, we preferred to use the analysis of single-cell suspensions to that of thin sections.⁸

The FISH analysis was performed on cells in interphase with the use of dual-color, dual-fusion rearrangement probes (Vysis) for *IGH/BCL2* in two follicular lymphomas, for *IGH/BCL1* in seven mantle-cell lymphomas, and for *IGH/MYC* in three Burkitt's lymphomas. In a marginal-zone B-cell lymphoma, the cells in interphase were investigated with probes spanning *MALT1* and *IGH*.⁸ In the case of the post-transplantation lymphoproliferative disorder, the hybridization was performed with centromere-specific probes for the sex chromosomes in both the hepatic tumor cells and the bone marrow cells. For

Table 1. Cytogenetic Findings in 27 B-Cell Non-Hodgkin's Lymphomas and the Corresponding Tumor Endothelial Cells.*

Case No.	Diagnosis	Site	Patient's Age and Sex	Cytogenetic Aberrations		Endothelial-Cell Markers	Endothelial Cells with Genetic Aberrations
				In Lymphoma Cells (Stem-Cell Line)	In Endothelial Cells		
1	FL 1†	Lymph node	55 yr, M	49,XY,+X,+11,t(14;18)(q32;q21),+21	t(14;18)(q32;q21),+X,+11,+21	CD31, WF	21
2	FL 3†	Lymph node	43 yr, M	53,XY,+2,+3,+7,+7,+8,+11,+12,t(14;18)(q32;q21)	t(14;18)(q32;q21),+2,+3,+7,+7,+8,+11,+12	CD31, UEL	32
3	FL 2†	Lymph node	61 yr, F	49,XX,+X,+5,der(5)t(1;5)(q11;q31),+i(6)(p10),t(14;18)(q32;q21)	t(14;18)(q32;q21),+X,+5	CD31, WF	28
4	FL 2†	Lymph node	83 yr, F	47,XX,+7,t(14;18)(q32;q21)	t(14;18)(q32;q21),+7	CD31, CD34	29
5	FL 1†‡	Lymph node	32 yr, M	46,XY,t(14;18)(q32;q21)	t(14;18)(q32;q21)	CD31, WF, UEL, CD34	80
6	FL 3	Lymph node	60 yr, F	t(14;18)(q32;q21)(IGH con BCL2×2)	t(14;18)(q32;q21)	CD31, WF, UEL, CD34	53
7	FL 1†	Lymph node	48 yr, M	46,XY,t(14;18)(q32;q21)	t(14;18)(q32;q21)	CD31, UEL	48
8	FL 1†	Lymph node	54 yr, F	49,XX,t(1;X)(q43;q24),+2,der(4)t(4;12)(p15;q13),del(6)(q21),+7,dup(9)(q21q32),+13,t(14;18)(q32;q21)	t(14;18)(q32;q21),+2,+7,+13	CD31, WF	50
9	FL 1†	Lymph node	39 yr, F	46,XX,t(14;18)(q32;q21)	t(14;18)(q32;q21)	CD31, WF	63
10	FL 1†	Lymph node	40 yr, M	46,XY,t(14;18)(q32;q21)	t(14;18)(q32;q21)	CD31, CD34	27
11	FL 1†	Lymph node	46 yr, M	46,XY,t(14;18)(q32;q21),del(13)(q12q31)	t(14;18)(q32;q21),del(13)(q14)(RB1×1)	CD31, WF, UEL, CD34	18
12	FL 1†	Lymph node	60 yr, F	48,XX,+5,+5,t(14;18)(q32;q21)	t(14;18)(q32;q21),+5,+5	CD31, WF, UEL, CD34	21
13	FL 1†‡	Lymph node	68 yr, M	47,XY,+7,t(14;18)(q32;q21)	t(14;18)(q32;q21),+7	CD31, WF, UEL, CD34	29
14	FL 1	Rectum	67 yr, M	t(14;18)(q32;q21)(IGH con BCL2×2)	t(14;18)(q32;q21)	CD31, CD34	64
15	MCL	Lymph node	70 yr, M	t(11;14)(q13;q32)(BCL1 con IGH×2-×3)	t(11;14)(q13;q32)×2-×3	CD31, WF, UEL, CD34	77
16	MCL	Lymph node	62 yr, M	t(11;14)(q13;q32)(BCL1 con IGH×2)	t(11;14)(q13;q32)	CD31, CD34	41
17	MCL	Lymph node	75 yr, M	t(11;14)(q13;q32)(BCL1 con IGH×2)	t(11;14)(q13;q32)	CD31, WF, UEL, CD34	77
18	MCL	Lymph node	76 yr, M	t(11;14)(q13;q32)(BCL1 con IGH×2)	t(11;14)(q13;q32)	CD31, WF, UEL, CD34	85
19	MCL	Lymph node	70 yr, M	t(11;14)(q13;q32)(BCL1 con IGH×2)	t(11;14)(q13;q32)	CD31, WF	42
20	MCL†	Lymph node	59 yr, M	82,XXYY,der(1)t(1;6;9)(p11;q?;?)×2,t(4;7)(q12;q11)×2,t(11;14)(q13;q32)×2,t(12;18)(q13;q21),inc	t(11;14)(q13;q32)×2	CD31, CD34	48

each hybridization, 500 cells in interphase were analyzed. The cutoff value for the diagnosis of each set of probes was the mean percentage of cells with a false positive signal constellation plus 3 SD as assessed with the use of tissue specimens obtained from 20 reactive lymph nodes.

IMMUNOHISTOCHEMICAL AND FISH ANALYSIS

Fluorescence immunophenotyping and interphase cytogenetics (FISH), a technique combining im-

munohistochemistry and FISH, was carried out on 5-µm-thick paraffin sections of all the lymphomas.⁹ For the identification of endothelial cells, adjacent sections were stained with two to four of the following endothelial-cell markers: anti-CD31 antibody, anti-CD34 antibody, anti-von Willebrand factor antibody, and *Ulex europaeus* lectin (Dako) (Table 1). For a negative control, T cells in the tissue specimens were stained for the α/β T-cell antigen receptor with the antibody βF1 (Endogen). In addition, in the case

Table 1. (Continued.)

Case No.	Diagnosis	Site	Patient's Age and Sex	Cytogenetic Aberrations		Endothelial-Cell Markers	Endothelial Cells with Genetic Aberrations
				In Lymphoma Cells (Stem-Cell Line)	In Endothelial Cells		
21	MCL	Colon	62 yr, M	t(11;14)(q13;q32)(BCL1 con IGHx2)	t(11;14)(q13;q32)	CD31, UEL	28
22	MCL	Colon	54 yr, M	t(11;14)(q13;q32)(BCL1 con IGHx2)	t(11;14)(q13;q32)	CD31, WF	41
23	BL	Lymph node	67 yr, M	t(8;14)(q24;q32)(MYC con IGHx2)	t(8;14)(q24;q32)	CD31, WF, UEL, CD34	28
24	BL	Lymph node	9 yr, M	t(8;14)(q24;q32)(MYC con IGHx2)	t(8;14)(q24;q32)	CD31, WF	37
25	BL	Lymph node	16 yr, M	t(8;14)(q24;q32)(MYC con IGHx2)	t(8;14)(q24;q32)	CD31, UEL	26
26	MZL	Lymph node	82 yr, F	t(14;18)(q32;q21)(IGH con MALT1x2)	t(14;18)(q32;q21)	CD31, WF, UEL, CD34	15
27	DLBCL†‡	Lymph node	45 yr, F	48,X,der(X)t(X;9)(Xpter→Xp11::9q31→9p13::Xp11→Xqter), t(1;10)(q11;q11),+der(1)t(1;5)(q11;p11)x2,+der(5)t(1;5)(q11;q11),t(2;22)(p21;q13),-9	5p15x4,5q33x3	CD31, WF, UEL, CD34	22

* FL denotes follicular lymphoma with a grade of 1, 2, or 3; MCL mantle-cell lymphoma; BL Burkitt's lymphoma; MZL marginal-zone B-cell lymphoma; DLBCL diffuse large-B-cell lymphoma; WF von Willebrand factor; and UEL *Ulex europaeus* lectin.

† The karyotype was assessed from cultured lymphoma cells in metaphase.

‡ Magnetic-bead sorting and endothelial-cell culture were performed.

of the post-transplantation lymphoproliferative disorder, the bone marrow cells were stained with an antibody against CD5 (Novocastra). After staining, FISH was performed. Only the microvascular endothelial cells were investigated, because the quality of the FISH signals in large tumor vessels was unreliable.

Chromosomal translocations were evaluated with the use of dual-color, dual-fusion translocation probes and *IGH* dual-color break-apart rearrangement probes (Vysis). Moreover, in eight follicular lymphomas and in the diffuse large-B-cell lymphoma, probes for *RB1* (chromosomal band 13q14), *CSF1R* (5q33–34), *D5S23*, *D5S721* (5p15), *ALK* (2p23), and *AML1* (21q22) and for the centromeres of chromosomes 3, 7, 8, 11, 12, and X were applied (Vysis). In the case of the post-transplantation lymphoproliferative disorder, centromere-specific probes for the sex chromosomes were used (Vysis). At least 250 endothelial cells were analyzed per slide. Cutoff levels for all probes were calculated as in the FISH analysis.

SHORT-TANDEM-REPEAT ANALYSIS

Genomic DNA was isolated according to standard procedures in specimens of the explanted liver and of the lymphoma from the patient with post-transplantation lymphoproliferative disorder. Nine short-

tandem-repeat loci and the amelogenin locus were amplified with the use of the AmpFLSTR Profiler PCR Amplification Kit and analyzed with the use of the 310 Capillary DNA Sequencer/Genotyper (Applied Biosystems).

MAGNETIC-BEAD SORTING AND ENDOTHELIAL-CELL CULTURE

Lymph-node tissue from specimens of three lymphomas was incubated in 20 mM phosphate-buffered saline containing 50 mg of collagenase H per milliliter for 30 minutes at 37°C in a shaking incubator. After centrifugation, the pellet was resuspended in EGM-2MV microvascular endothelial-cell medium (Clonetics) and plated on petri dishes. After five hours, the nonadherent cells were removed, and fresh medium was added to the adherent cells. The medium was changed every three days. After 5 to 10 days, the adherent cells were detached from the petri dish by incubation with 0.25 percent trypsin and 1 mM EDTA (GIBCO) and incubated with magnetic beads that were coated with anti-CD31 antibody (Dynal), according to the manufacturer's instructions. Microbeads with bound cells were replated on petri dishes. The beading procedure was repeated after another 5 to 10 days of culture. After becoming confluent, cells were passaged again and plated on chamber slides (Becton Dickinson).

The FICTION analysis was performed after the cells had grown to approximately 70 percent confluency in all three of the lymphomas cultured. In one case (Case 13), the endothelial cells were passaged twice more and investigated with the FICTION procedure after reaching 70 percent confluency. At least 200 endothelial cells were analyzed per cell culture. Cut-off levels for all probes were calculated as in the FISH analysis.

RESULTS

POST-TRANSPLANTATION LYMPHOPROLIFERATIVE DISORDER

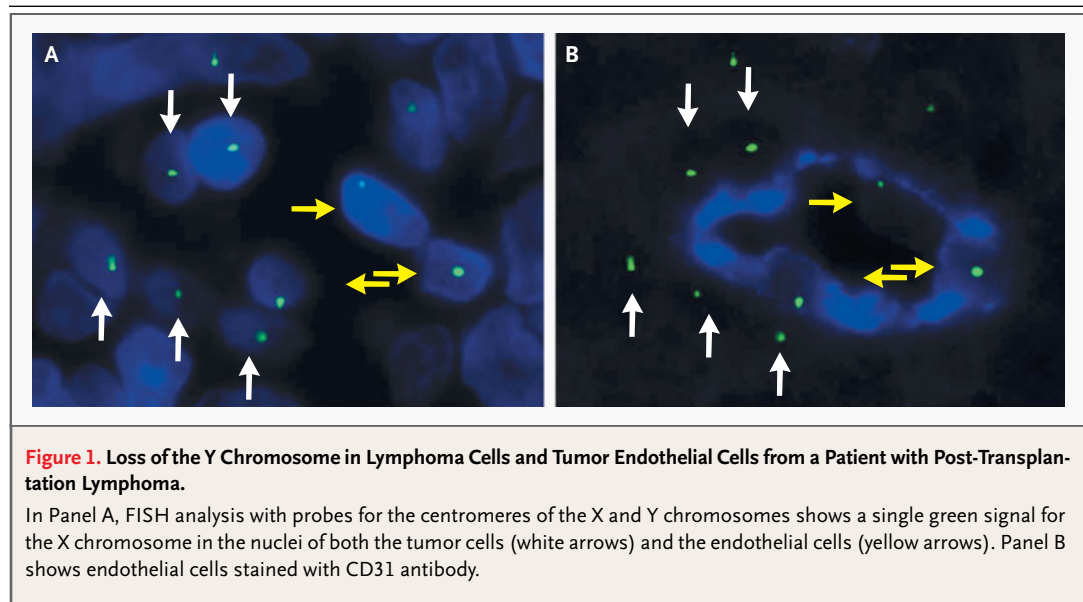
A 69-year-old man with primary biliary cirrhosis had received a liver transplant from a female donor. Four years later, a post-transplantation lymphoproliferative disorder, classified as diffuse large-B-cell lymphoma, was diagnosed in the transplanted liver. A FISH analysis of a single-cell suspension of a specimen of the lymphoma, performed with the use of centromere-specific probes for both sex chromosomes, showed only a single signal for the X chromosome.

Next we performed the FICTION procedure. To identify microvascular endothelial cells, we successfully used four markers, anti-CD31, anti-CD34, and anti-von Willebrand factor antibodies and *U. europaeus* lectin, on subsequent paraffin sections of the lymphoma tissue. These slides were then hybridized with centromere-specific X and Y probes, re-

spectively. All the tumor cells and 90 percent of the endothelial cells showed an X karyotype (Fig. 1). Short-tandem-repeat analysis showed that the lymphoma was of recipient (male) origin. These findings indicated that the X karyotype must have resulted from loss of the Y chromosome. To rule out constitutive loss of the Y chromosome, we examined a specimen of the patient's bone marrow, which contained about 15 percent lymphoma cells. Because the lymphoma cells aberrantly expressed CD5, an antibody against CD5 was used to identify them in combination with centromere-specific probes for both sex chromosomes. Again, the Y chromosome was not detected in the malignant cells, whereas the nonmalignant bone marrow cells had a normal XY karyotype. Thus, the X genotype was regarded as tumor-specific.¹⁰ These findings indicated that the microvascular endothelial cells in the post-transplantation lymphoproliferative disorder were genetically related to the lymphoma cells.

B-CELL LYMPHOMAS

To determine whether the endothelial cells in other types of B-cell lymphoma share genetic aberrations with the lymphoma cells, we selected 27 B-cell lymphomas with known cytogenetic alterations (Table 1). These 27 lymphomas comprised 14 follicular lymphomas with t(14;18)(q32;q21) involving *IGH* and *BCL2*, 8 mantle-cell lymphomas with t(11;14)(q13;q32) involving *BCL1* and *IGH*, 3 Burkitt's lymphomas with t(8;14)(q24;q32) involving



c-MYC and *IGH*, 1 marginal-zone B-cell lymphoma with $t(14;18)(q32;q21)$ involving *IGH* and *MALT1*, and 1 diffuse large-B-cell lymphoma with complex rearrangements, including a gain of chromosome 5.

With the FICTION technique, the endothelial cells were clearly distinguishable from the lymphoma cells. In each tissue specimen, staining of the endothelial cells was positive for all the markers used (Table 1). In all 27 lymphomas, varying proportions of the endothelial cells (15 to 85 percent; median, 37 percent) in the tumors harbored the chromosomal rearrangement that was present in the lymphoma cells (Table 1 and Fig. 2). To confirm the translocations, we used two different sets of probes in the analysis of each lymphoma. When microvessels were cut in the longitudinal axis, genetically normal and aberrant endothelial cells could be observed side by side in some cases. However, a calculation of mosaic vessels was not possible, because of technical limitations. Large parts of the nuclei are lost when 5- μm slices are cut for the FICTION procedure. Consequently, a high proportion of cells do not show all the hybridization signals that are nec-

essary to establish the karyotype. In all 27 cases, the lymphoma-specific rearrangements were not observed in the T cells with the α/β receptors that were seen among the lymphoma cells.

SECONDARY CHROMOSOMAL ALTERATIONS IN ENDOTHELIAL CELLS IN FOLLICULAR LYMPHOMAS

The $t(14;18)(q32;q21)$ translocation is the primary chromosomal aberration in follicular lymphoma, but secondary, predominantly numerical aberrations are usually found at presentation or emerge during the course of the disease.¹¹ We studied eight lymphomas (Cases 1, 2, 3, 4, 8, 11, 12, and 13) to determine whether the endothelial cells in follicular lymphomas carry secondary chromosomal aberrations. These lymphomas were shown by Giemsa-banding analysis to harbor both $t(14;18)(q32;q21)$ and a gain of chromosome 2, 3, 5, 7, 8, 11, 12, 13, 21, or X in seven cases and loss of *RB1* in one case (Table 1). In all eight cases, the endothelial cells carried the same secondary aberrations, as shown in Figure 3 for a follicular lymphoma with trisomy 7.

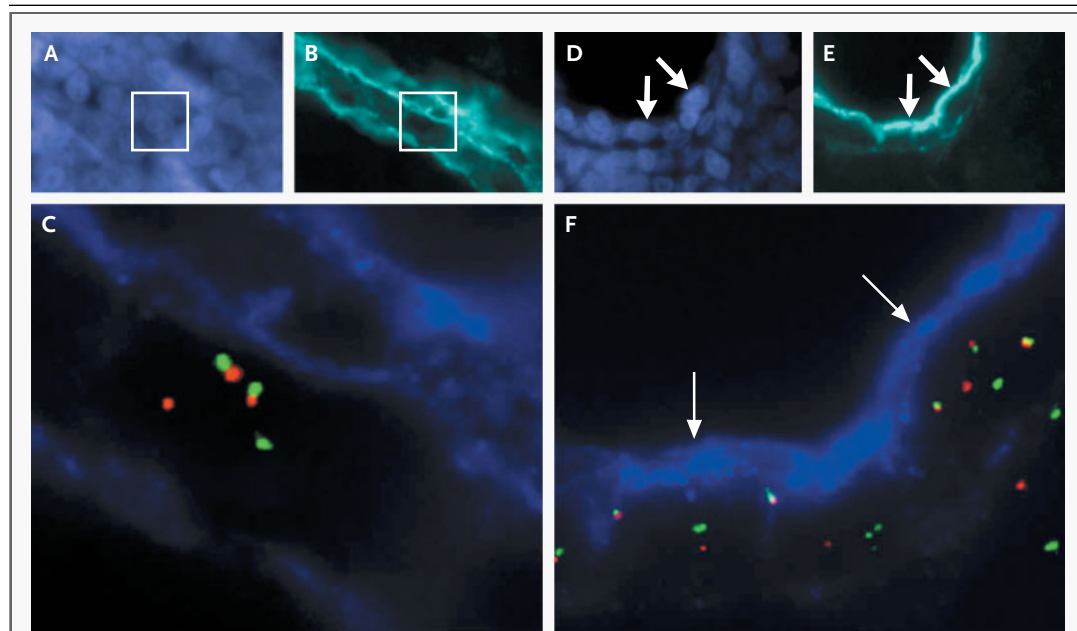


Figure 2. *IGH* Translocations in Endothelial Cells in Follicular Lymphoma and Mantle-Cell Lymphoma.

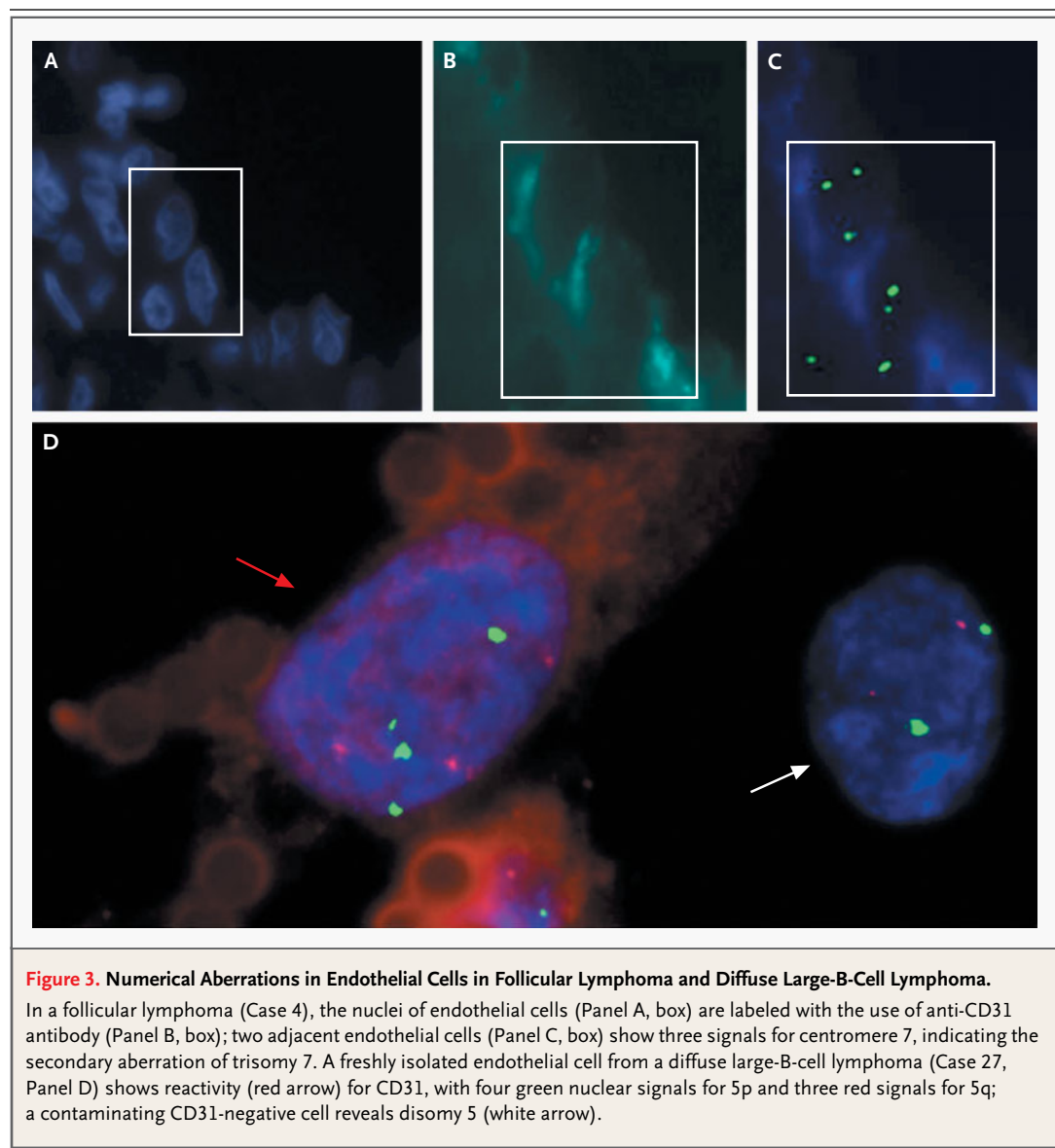
In a follicular lymphoma (Case 11), the nucleus of an endothelial cell (Panel A, box) that is labeled with the use of anti-von Willebrand factor antibody (Panel B, box) reveals two fusion signals for the green *IGH* probe and the red *BCL2* probe (Panel C), indicating $t(14;18)(q32;q21)$. In a mantle-cell lymphoma (Case 20), arrows indicate nuclei that belong to the endothelial cells of a cross-sectioned vessel (Panel D) with staining for CD34 (Panel E). Two CD34+ endothelial cells (Panel F, arrows) show two and three fusion signals for $t(11;14)(q13;q32)$, respectively.

The percentage of endothelial cells exhibiting secondary alterations corresponded to the rate of detection of $t(14;18)(q32;q21)$. These findings indicate that the endothelial cells that line the microvessels in follicular lymphoma share secondary chromosomal aberrations with the respective lymphoma cells.

CULTURED ENDOTHELIAL CELLS FROM LYMPHOMAS

To confirm the in situ observations outlined above, we investigated freshly isolated cultured endothelial cells obtained from one diffuse large-B-cell lympho-

ma (Case 27) and two follicular lymphomas (Cases 5 and 13). Cytogenetic analysis of short-term cultures of the lymphoma cells was performed in parallel. As expected, both the lymphoma cells and the endothelial cells were characterized by a gain of chromosome 5 in Case 27 (Fig. 3), $t(14;18)(q32;q21)$ in Cases 5 and 13, and an additional trisomy 7 in Case 13 (Fig. 4). When the proportion of genetically aberrant endothelial cells in the third passage of cell culture was compared with that in tissue sections, the percentage of aberrant cells was much higher in the tissue sections in all cases (Case 27, 22 percent vs. 9 percent; Case 5, 80 percent vs. 27 percent; and



Case 13, 29 percent vs. 12 percent). The value of this comparison, however, is limited by the fact that the percentage of tumor cells in the specimens obtained for culture was unknown. In Case 13, 10 percent of the endothelial cells carried the genetic aberrations after two further passages.

DISCUSSION

The observation that tumors are involved in the assembly of their blood vessels is not entirely new. As early as 1948, Willis proposed that tumor cells can acquire a new phenotype and participate in the formation of blood channels.¹² Several reports added ultrastructural evidence of the contribution of can-

cer cells to the walls of tumor vessels.¹³⁻¹⁶ In multiple myeloma, endothelial cells in the bone marrow were reported to differ markedly from umbilical-vein endothelial cells, their quiescent counterpart, with regard to the secretion of growth factors, growth properties, the genetic profile, and ultrastructural features.¹⁷ These findings raised the possibility of myeloma-induced endothelial-cell growth in the bone marrow.

We found that microvascular endothelial cells in B-cell lymphomas harbored lymphoma-specific genetic aberrations. These rearrangements are not only B-cell-specific translocations of *IGH* but also secondary genetic alterations in follicular lymphomas. Both primary and secondary chromosomal al-

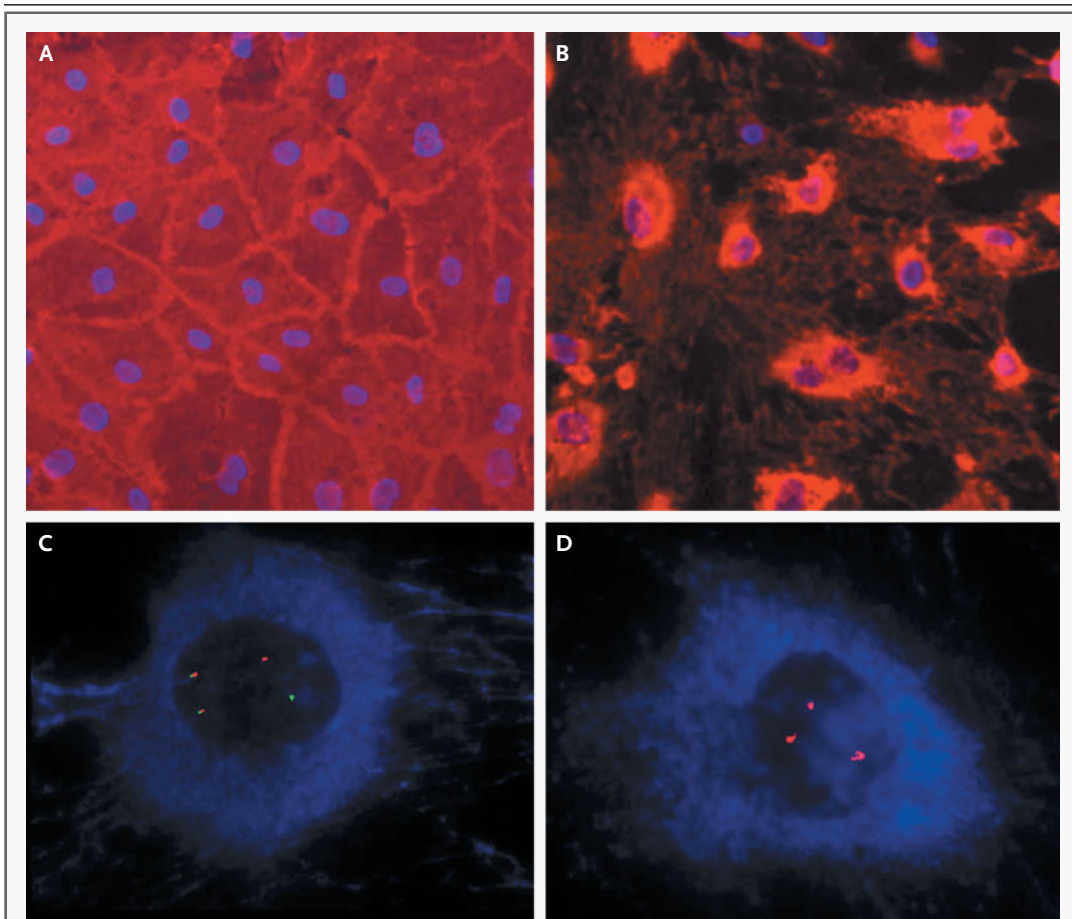


Figure 4. Cultured Endothelial Cells from a Follicular Lymphoma Showing *t*(14;18)(q32;q21) and Trisomy 7. Confluent endothelial cells from the fourth passage are positive for CD31, exhibiting the typical cobblestone structure (Panel A), and for von Willebrand factor (Panel B). The endothelial cell that is positive for von Willebrand factor reveals two fusion signals for the green *IGH* probe and the red *BCL2* probe, indicating *t*(14;18)(q32;q21) (Panel C), and three signals for centromere 7, indicating trisomy 7 (Panel D).

terations remained detectable in cultured endothelial cells after three to five passages.

In breast carcinoma, stromal cells carry genetic alterations that have been associated with carcinogenesis in solid tumors.^{18,19} These aberrations were found in the neoplastic epithelium alone, the surrounding stroma alone, or both compartments, suggesting that genetic alterations can occur as independent events in tumor cells and in stroma. In contrast to those findings, identical primary and secondary genetic aberrations were detected simultaneously in endothelial cells and tumor cells in all of our cases of lymphoma, suggesting a constant and therefore very close relationship between the genetic events in these two types of cell.

Four mechanisms should be considered as possible explanations for our findings. First, lymphoma cells and endothelial cells may derive from a multipotent hemangioblastic precursor cell. Gunsilius et al. have shown that the *BCR-ABL* translocation can be detected in a minor subgroup of endothelial cells generated in vitro from bone marrow and peripheral-blood specimens obtained from patients with chronic myeloid leukemia,²⁰ suggesting the existence of a common malignant precursor cell. This interpretation is plausible in chronic myeloid leukemia, which arises in a multipotent hematopoietic stem cell; moreover, the *BCR-ABL* translocation has been detected in nonmyeloid hematopoietic lineages.²¹ Although the chromosomal translocations we found are regarded as lymphoid-specific, we cannot exclude the possibility that the genetic transformation of a common precursor cell accounts for the identical genetic anomalies found in lymphoma cells and in associated endothelial cells.

Second, the endothelial cells that carry the genetic alterations of the lymphoma may have arisen from a cell that was already committed to the lymphoid lineage. There is strong evidence that reciprocal translocations involving the immunoglobulin loci require enzymes that are normally expressed only in lymphoid cells. Although the *t(14;18)(q32;q21)* found in follicular lymphoma and the *t(11;14)(q13;q32)* found in mantle-cell lymphoma are at least partly mediated by V(D)J recombination (catalyzed by the enzymes RAG1 and RAG2), the *t(8;14)(q24;q32)* in Burkitt's lymphoma occurs predominantly as a consequence of aberrant switch recombination, a late event in B-cell maturation.²²⁻²⁶ Furthermore, the kinds of secondary genetic changes we found are typical of the later stages

of lymphoma.²⁷ Immature B cells have been shown to possess extraordinary developmental plasticity when the *Pax-5* gene is deleted. In *Pax-5*-deficient mice, B-cell development is blocked at an early stage.^{28,29} Such B cells can differentiate, in vitro and in vivo, into all known hematopoietic lineages (except mature B cells) and even into dendritic cells and osteoclasts.^{30,31} Moreover, human pro-B cells, which have very low levels of *Pax-5* messenger RNA, can give rise to macrophages, natural killer cells, and T cells.³²

Nevertheless, stimuli that could induce lymphoma cells to switch to an endothelial-cell phenotype are unknown. In solid tumors, malignant cells can dedifferentiate and alter their gene-expression program by activating angiogenesis-related genes in response to hypoxia.³³⁻³⁵ A similar mechanism may play a role in some B-cell lymphomas.

Third, our findings may be explained by cell fusion.³⁶⁻³⁸ The fusion of lymphoma cells and endothelial cells, however, should result in a tetraploid karyotype. Tetraploidy is easily detectable with the use of the FICTION procedure but was observed in only 1 of the 27 cases, a mantle-cell lymphoma with a near-tetraploid karyotype that is characteristic of this disease entity.³⁹ Nevertheless, the recent observation that some tetraploid hybrids undergo reduction division, resulting in diploid daughter cells, suggests that cell fusion cannot be ruled out.³⁷

Finally, gene transfer by means of the uptake of apoptotic bodies from tumor cells by neighboring cells deserves consideration.^{40,41} Bovine endothelial cells have been shown to contain human DNA after coculture with apoptotic bodies from B-cell lymphomas.⁴⁰ However, gene uptake should not result in the loss of chromosomal material, as was observed in two of our cases (loss of the Y chromosome in the index case and loss of *RB1* in Case 11). Furthermore, most of the lymphomas we studied were follicular or mantle-cell lymphomas, which have very low rates of apoptosis, partly owing to the high expression of the anti-apoptotic protein Bcl-2.

Our findings suggesting that microvascular endothelial cells in B-cell lymphomas are in part tumor-related point to a novel aspect of tumor angiogenesis. The mechanisms by which the endothelial cells in a lymphoma acquire the specific genetic alterations of the lymphoma remain to be elucidated.

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