Gain of Chromosome Arm 9p Is Characteristic of Primary Mediastinal B-Cell Lymphoma (MBL): Comprehensive Molecular Cytogenetic Analysis and Presentation of a Novel MBL Cell Line

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Primary mediastinal B-cell lymphoma (MBL) is an aggressive Non-Hodgkin's Lymphoma, which has been recognized as a distinct disease entity. We performed a comprehensive molecular cytogenetic study analyzing 43 MBLs. By comparative genomic hybridization (CGH), the most common aberrations were gains of chromosome arms 9p and Xq, which were present in 56% and 40% of cases, respectively. Based on the limited resolution of CGH, this technique may underestimate the real incidence of aberrations. Therefore, we also did an interphase cytogenetic study with eight DNA probes mapping to chromosome regions frequently altered in B-cell lymphomas. With this approach, both 9p and Xq gains were found in more than 70% of cases (75% and 87%, respectively). The findings were compared with results obtained in 308 other B-cell lymphomas. Gains in 9p were identified in only six of the 308 cases, and only one of these lymphomas with 9p gains was not primarily extranodal in origin ($P < 10^{-20}$ for CGH data and $P < 10^{-11}$ for fluorescence in situ hybridization data). We also present a novel MBL cell line, MedB-1, which carries the genetic aberrations characteristic of this entity.

INTRODUCTION

Primary mediastinal B-cell lymphoma (MBL) is a distinct clinicopathologic entity among diffuse large-cell lymphomas (Harris et al., 1994). It predominantly affects young adults, taking the form of a bulky tumor in the anterior mediastinum and often invading contiguous thoracic structures (Kirn et al., 1993; Lazzarino et al., 1997; Paulli et al., 1997; Zinzani et al., 1999). The tumor cells exhibit rearrangements of immunoglobulin genes and express B-cell antigens, such as CD19 and CD20. In addition, severe defects in the expression of immunoglobulin constituents and HLA class I and II molecules are present (Menestrina et al., 1986; Möller et al., 1987; Scarpa et al., 1987).

Little is known about genetic alterations in MBL. Abnormalities of the *MYC* and *REL* proto-oncogenes as well as of the *TP53* and *CDKN2A* tumor suppressor genes have been reported in a few cases (Scarpa et al., 1991, 1999; Joos et al., 1996; Tsang et al., 1996). Recently, it was shown that MAL, a specific transmembrane protein, is expressed differentially in MBL, but not in other diffuse large-cell lymphomas (Copie-Bergman et al., 1999). This protein is involved in both membrane trafficking and signaling. So far, the only information with regard to chromosomal aberrations in MBL has been provided by a study of our group using the molecular cytogenetic technique of comparative genomic hybridization (CGH) (Joos et al., 1996). The most common recurrent aberrations were gains of chromosomes 9, X, and 12, which were present in 30–50% of the cases. Owing to the limited spatial resolution of CGH (Bentz et al., 1998), however, the frequencies of specific aberra-

Supported by: Deutsche Krebshilfe; Grant numbers: M 47/95 Be I and 70-2310-Ba I; Deutsche Forschungsgemeinschaft; Grant number: Be 1454/5-2; Medical Faculty of the University of Ulm; Grant number: P389.

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Received 27 June 2000; Accepted 5 October 2000

tions may be underestimated by this technique. This hypothesis was supported by a recently published study comparing data obtained by CGH and by arbitrarily primed polymerase chain reaction fingerprinting in five cases (Scarpa et al., 1999).

To determine the incidence rates of specific chromosomal aberrations in MBL more precisely, we extended our series and, in addition to CGH analysis, also performed interphase cytogenetic studies of the chromosomal regions most frequently altered in B-cell lymphomas. To assess the specificity of chromosomal aberrations, we compared the molecular cytogenetic data of 43 MBLs with data obtained in 308 other cases of B-cell neoplasms. We also present a novel MBL cell line carrying the chromosomal aberrations most prominent in this lymphoma entity. This cell line will be instrumental for a detailed analysis of the functional consequences of specific genetic events in MBL.

MATERIALS AND METHODS

Patients

MBLs

Freshly frozen tumor samples from 43 patients (26 men, 17 women; age range, 17–73 years; median age, 38 years) were analyzed. All cases were analyzed by CGH. In a subset of 31 cases, interphase cytogenetic analysis was performed with eight DNA probes (see later discussion). In the other 12 cases, sufficient material for extensive fluoresence in situ hybridization (FISH) studies was not available. In nine of these cases, however, cells for one FISH experiment analyzing the 9p region were obtained. The diagnosis of MBL was based on histomorphologic features and on the expression of surface antigens.

Other B-cell neoplasms

For specific genetic aberrations, data from 78 follicular lymphomas, 72 nodal diffuse large-cell lymphomas, 60 B-cell chronic lymphocytic leukemias, 40 mantle cell lymphomas, 31 primary large B-cell gastrointestinal lymphomas, and 27 typical and atypical Burkitt lymphomas were included in the analysis.

Comparative Genomic Hybridization

Genomic DNA was prepared from fresh tumor tissue as previously described (Sambrook et al., 1989), using proteinase K digestion and phenol-chloroform extraction. CGH was performed as previously reported (Lichter et al., 1995). Briefly, tumor DNA was labeled with biotin-16-dUTP, and normal human control DNA was labeled with digoxigenin-11-dUTP (Boehringer Mannheim, Mannheim, Germany) by a standard nick translation reaction. One microgram of biotin-labeled tumor DNA, 1 μg of digoxigenin-labeled control DNA, and 70 μg of human Cot-1 DNA (BRL Life Sciences, Gaithersburg, MD) were co-hybridized to slides with metaphase cells from blood of a healthy donor. After hybridization for 1–2 days and post-hybridization washes, control and test DNA samples were detected via rhodamine and fluorescein isothiocyanate (FITC), respectively. For identification, chromosomes were counterstained with DAPI (4,6-diamidino-2-phenylindole).

Digital Image Analysis

For 26 of the MBL cases (nos. 1–26), methodology and CGH data were published previously (Joos et al., 1996). In the other MBL cases and the cell line, image analysis was done using an epifluorescence microscope (Axioplan, Zeiss, Jena, Germany) and the commercially available image analysis system ISIS (MetaSystems, Altlussheim, Germany). Ratio values of 1.25 and 0.8 were used as upper and lower thresholds for the identification of chromosomal imbalances. Overrepresentations were considered high-level amplifications when the fluorescence ratio values exceeded 2.0 or when the FITC fluorescence showed strong focal signals and the corresponding ratio profile was diagnostic of overrepresentation. The extension of imbalanced regions was assessed by comparison of the fluorescence ratio profiles with the corresponding regions in chromosome ideograms. Assignment of highly amplified sequences to chromosomal bands was done by comparison of signal intensities and DAPI banding on individual chromosomes. Certain chromosomal regions are known to be critical in CGH analysis, and therefore they were not considered for quantitative image analysis. These regions were the whole chromosome 19 (Du Manoir et al., 1995) as well as regions with a high content of repetitive sequences (heterochromatin blocks of centromeric regions or the long arm of the Y chromosome), which exhibit very low signal intensities resulting from suppression with Cot-1 DNA. This may lead to gross variations of the ratio profiles from only small absolute variations of the hybridization intensities. Part of the CGH data of the other B-cell neoplasms were published previously (Bentz et al., 1995, 1996, 2000; Werner et al., 1997; Barth et al., 1998).

FISH Using a Set of Specific DNA Probes

For interphase cytogenetic analysis, a set of eight DNA probes was used in each case. These probes mapped to chromosomal regions frequently altered in B-cell Non-Hodgkin's Lymphoma and were as follows: YAC clones 866e7 mapping to 3q26, 963d6 mapping to 6q21 (Stilgenbauer et al., 1999), and 754a1 mapping to 12q14 (all obtained from the Centre d'Etude Polymorphisme Humain (CEPH)) (Cohen et al., 1993); YAC clones 755b11 mapping to 11q22.3–23.1 (Stilgenbauer et al., 1996; Döhner et al., 1997) and A24CH4 mapping to Xq28 (Fischer et al., 1996); cos p16, consisting of a pool of eight overlapping cosmid clones covering approximately 250 kb of the *CDKN2A* gene on 9p21 (Dreyling et al., 1997); cos p53, containing four overlapping cosmid clones mapping to 17p13 and spanning the *TP53* tumor suppressor gene (Döhner et al., 1995); and probe c13S25, consisting of two cosmid clones (ICRFc108I155 and ICRFc108L2145) mapping to the D13S25 locus on 13q14 (Stilgenbauer et al., 1998). Eight cases, which exhibited two hybridization signals for cos p16, also were hybridized with the YAC clone 776a11 mapping to chromosomal bands 9p23–24 (obtained from the CEPH YAC library).

Hybridization was done as described previously (Lichter et al., 1995). Preparations were evaluated only for a specific DNA probe, if the co-hybridized and differentially detected control probe exhibited two hybridization signals in more than 90% of interphase cells. Thus, a high level of hybridization efficiency was assured. Experiments were evaluated using an epifluorescence microscope connected to a charged-coupled device camera. In each case, at least 200 cells were enumerated.

Generation of the Cell Line MedB-1

Patient and tumor

In March 1990, an MBL was diagnosed in a biopsy specimen obtained from a mediastinal tumor in a 27-year-old man (patient no. 14). In July 1990, after an initial response to combined chemo- and radiotherapy, the tumor progressed, invading parts of the left lung. In a second thoracotomy, debulking of the mediastinal mass was undertaken, and the left lung was resected. The patient died in January 1991 from extensive local tumor progression.

Establishment of the cell line

From the surgical specimen of July 1990, fresh and sterile tumor tissue was obtained immediately after removal. For the establishment of the cell line, a 3-cm^3 specimen of the resected tissue was washed several times (minimal essential medium containing 10 μg/mL amphothericin and 100 U/100 μg/mL penicillin/streptomycin), minced into pieces about 1 mm in diameter, and centrifuged. After ammonium chloride lysis of the erythrocytes, the cell suspension was placed into Iscove medium (Serochrom/Biochrom, Berlin, Germany), supplemented with 10% fetal calf serum and antibiotics, as described earlier. The cultures were maintained at 37°C in a humidified atmosphere of 5% carbon dioxide, and the medium was replaced once a week. Outgrowing fibroblasts gradually decreased and finally disappeared. Since 1991, the tumor cells grow as a single cell suspension with a doubling time of approximately 3 days.

Genetic Chararacterization of the Cell Line

Banding analysis of cell lines has been described in detail before (Brüderlein et al., 1990). Briefly, the cell suspension was treated with Colcemid (0.5 μg/mL), suspended in hypotonic KCl (0.075 M), fixed with methanol/acetic acid (3:1), and spread on glass slides. Chromosomes were stained by standard trypsin-Giemsa banding techniques. Metaphases were photographed immediately after staining. Isolation of DNA and CGH analysis were performed as described earlier for the primary tumor tissues.

RESULTS

Clinical Characteristics of Patients

Clinical data were available for 32 of the 43 patients. At diagnosis, one patient had stage I, 25 patients had stage II, two patients had stage III, and four patients had stage IV disease. The lactate dehydrogenase values ranged from 156 U/L to 1,950 U/L (median, 313 U/L). Performance status was measured according to the system of the World Health Organization (WHO). It was WHO 0 in 18 patients, WHO 1 in six patients, WHO 2 in two patients, and WHO 3 in two patients. The patients' characteristics are listed in Table 1.

Comparative Genomic Hybridization

In 38 of 43 cases, chromosomal imbalances were detected by CGH. The median number of aberrations per case was four (range, 0–12 aberrations). Gains of chromosomal material were much more common than losses (155 gains and 27 losses). The most frequent gains mapped to chromosome arms 9p (24 cases; 56%) and Xq (17 cases; 40%) and

TABLE 1. Clinical Data of Patients with Primary Mediastinal B-Cell Lymphoma*

Case	Sex	Age at diagnosis (yr)	Stage[a]	LDH[b]	WHO[c]
1	f	34	—	—	—
2	f	42	—	—	—
3	f	17	2	220	0
4	m	28	2	491	3
5	m	39	—	—	—
6	m	41	2	318	1
7	f	59	4	236	0
8	m	34	2	471	3
9	f	56	2	296	0
10	f	43	2	1,950	0
11	m	43	2	156	0
12	m	21	4	388	0
13	m	29	—	—	—
14	m	27	2	—	—
15	m	42	2	—	—
16	m	62	2	159	2
17	f	73	2	329	1
18	m	48	—	—	—
19	m	52	3	370	0
20	f	17	2	517	1
21	m	17	4	247	0
22	f	46	2	205	0
23	m	24	2	219	0
24	m	54	2	252	2
25	m	26	2	215	0
26	m	26	2	313	0
27	f	36	—	—	—
28	f	30	—	—	—
29	m	38	2	281	0
30	f	29	2	664	1
31	m	58	2	565	0
32	f	23	2	517	1
33	m	26	2	335	0
34	m	41	2	289	1
35	m	40	2	—	0
36	m	17	4	448	—
37	f	63	—	237	—
38	f	70	—	—	—
39	m	68	—	—	—
40	m	20	3	380	0
41	m	38	2	272	0
42	f	38	1	895	0
43	f	43	—	—	—

*LDH, lactate dehydrogenase; WHO, World Health Organization; f, female; m, male.
[a]The dashes indicate that data were not available.
[b]Lactate dehydrogenase level in the serum (U/L).
[c]Performance status according to the classification system of the World Health Organization.

chromosomes 12 (14 cases; 33%), 2 (14 cases; 33%), and 16 (10 cases; 23%). The most frequent chromosomal deletion mapped to chromosome 13 (six cases; 14%). For the most common aberrations, the consensus regions are as follows: On chromosome 9, two commonly overrepresented regions were identified; 24 of the 26 cases with gains on this chromosome showed an overrepresentation of bands 9p23–24, and 17 cases had a gain mapping to bands 9q32–34. Similarly, on the X chromosome, two commonly overrepresented regions were found mapping to bands Xp11.4–21 (14 cases) and Xq24–26 (14 cases). Chromosome bands 12q23–24 were overrepresented in all 14 cases with gains of chromosome 12. For chromosome 2, two consensus regions were found mapping to bands 2p24–25 (eight cases) and 2p14–16 (eight cases). Eight high-level DNA amplifications were identified in a total of six cases. The amplified regions mapped to the following chromosomal bands: Xp11–21, Xq22–28, 2p24–25, 2p13–15 (two cases), 8q24, and 9p23–24 (two cases). The CGH data are illustrated in Figure 1.

FISH Using a Set of Specific DNA Probes

By interphase cytogenetic analysis, aberrations were detected in all but one (no. 8) of the 31 analyzed cases. The most frequent changes were gains of Xq28 (27 cases; gains in 15–77% of cells; median, 49%), gains of 9p21 (30 of 40 cases; gains in 8.5–77% of cells; median, 39.5%), gains of 12q14 (11 cases; gains in 11.8–79% of cells; median, 37.5%), and gains of 11q23 (seven cases; gains in 10.5–75% of cells; median, 29.5%). All these aberrations were present in more than 20% of the cases. The interphase cytogenetic data are listed in Table 2.

9p Gains Are Highly Characteristic of MBLs

To test whether any of the genetic aberrations were specific to MBL, the molecular cytogenetic data were compared with data obtained in 308 other B-cell lymphomas. Whereas gains of chromosomes X, 12, and 2 have been reported in other lymphomas (e.g., Xq gains in 10% of other lymphomas and 12q gains in approximately 15% of other lymphomas), a gain of chromosome arm 9p, which was present in 56% of the cases, was specific to MBL ($P < 10^{-20}$, Fisher exact test). By CGH, this aberration was far less common in other types of B-cell neoplasms: none of 138 indolent B-cell neoplasms (78 follicular lymphomas and 60 B-cell chronic lymphocytic leukemias) exhibited this aberration. Similarly, 9p gains were not identified in 27 mantle cell lymphomas and 27 Burkitt lymphomas. In a series of 72 diffuse large B-cell lymphomas and 31 primary gastrointestinal large B-cell lymphomas, 9p gains were found in four cases. Only one of these cases was a nodal lymphoma.

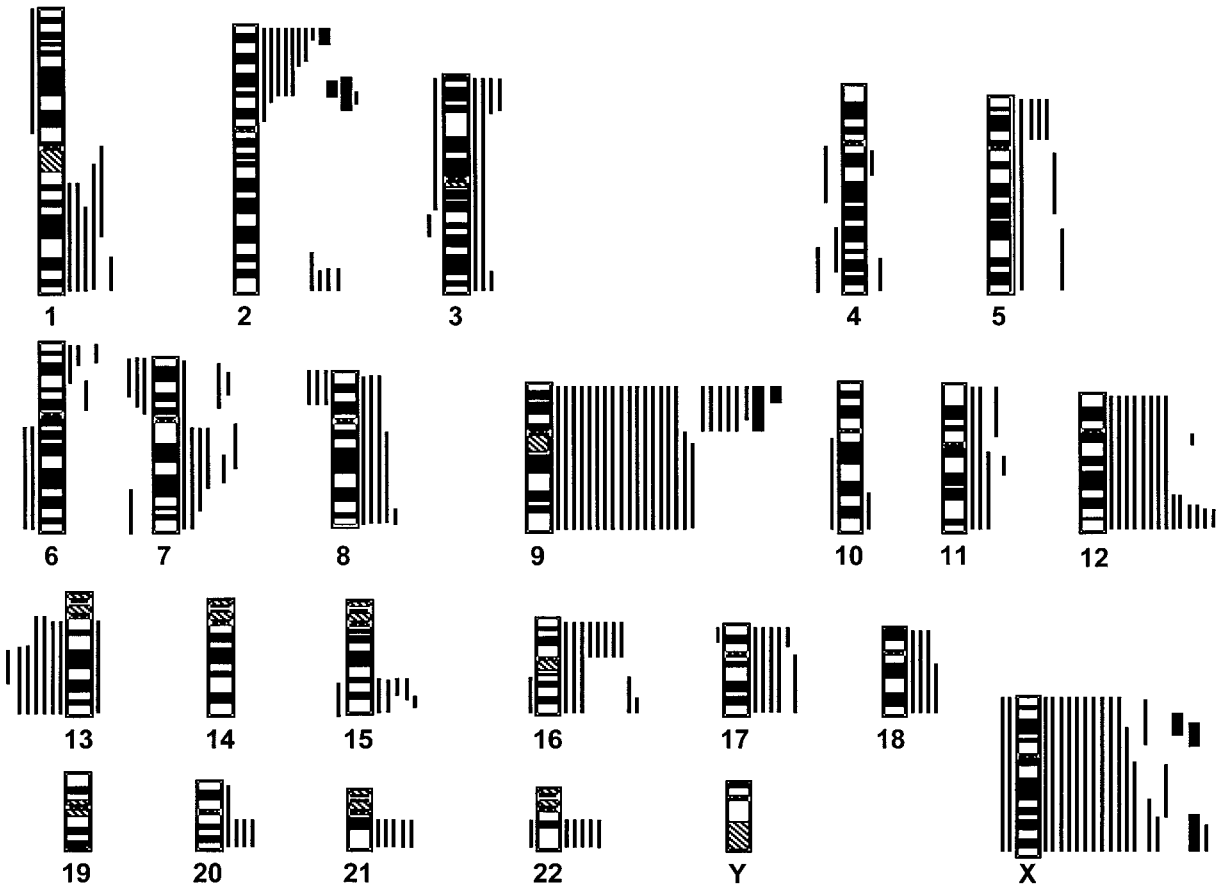

Figure 1. Summary of chromosomal imbalances detected by comparative genomic hybridization in 43 cases of primary mediastinal B-cell lymphoma. Lines to the left of the ideograms indicate losses of chromosomal material, and lines to the right indicate gains of chromosomal material. Black rectangles represent high-level DNA amplifications.

TABLE 2. Interphase Cytogenetic Data in 31 Cases of Primary Mediastinal B-Cell Lymphoma

DNA probe mapping to[a]	Cutoff value (%)[b]	No. of cases (total $n = 31$)	%
Xq28 (+)	6 (f) 10.8 (m)	27	87
9p21 (+)	2.1	30/40	75
12q14 (+)	8.6	11	35
11q23 (+)	3.9	7	23
3q26 (+)	6.3	6	19
13q14 (−)	12	3	10
17p13 (−)	6	2	6
6q21 (−)	3.3	0	0

[a]In this column, the chromosomal localization of the DNA probes is given; (+) indicates that the respective region was overrepresented, and (−) indicates that the respective region was deleted.
[b]Cutoff values for the respective aberrations were calculated according to standard procedures; f, female; m, male.

The other three cases had primary extranodal manifestations (testis, tonsils, and gastrointestinal tract).

By FISH analysis using a DNA probe spanning the *CDKN2A* gene, 9p gains were identified in 30 of 40 MBLs in percentages ranging from 8.5% to 77% of interphase nuclei (mean, 39.6%). By CGH, the consensus region was mapped further telomeric to chromosomal bands 9p23–24. Depending on the availability of further material, eight of the 10 MBL cases without 9p gains detected by FISH were analyzed using the YAC probe 776a11. In one of these cases, three hybridization signals were found in 68.5% of nuclei. In this case, the CGH profile exhibited an overrepresentation of the terminal part of 9p. In the other seven cases with normal CGH findings, the FISH data with the probe 776a11 were in complete concordance with the data obtained with cos p16. In these eight cases, three hybridization spots for YAC 776a11 were identified in 0.5–6% (median, 1%) of interphase nuclei. These data were compared with data from interphase studies of three other types of B-cell neoplasms. None of 31 mantle cell lymphomas and

TABLE 3. Gains of Chromosome Arm 9p in Various B-Cell Lymphomas*

Lymphoma type	9p+ detected by CGH	%	9p+ detected by FISH	%
Primary mediastinal B-cell lymphoma	24/43	56	30/40	75
Follicular lymphoma	0/78	0	ND	
B-cell chronic lymphocytic leukemia	0/60	0	0/50	0
Mantle cell lymphoma	0/27	0	0/31	0
Diffuse large-cell lymphoma	3/72	4	ND	
Burkitt lymphoma	0/27	0	ND	
Diffuse large-cell lymphoma of the GI tract	1/31	3	3/29	10

*CGH, comparative genomic hybridization; FISH, fluorescence in situ hybridization using a DNA probe mapping to chromosome arm 9p; ND, not done; GI, gastrointestinal tract.

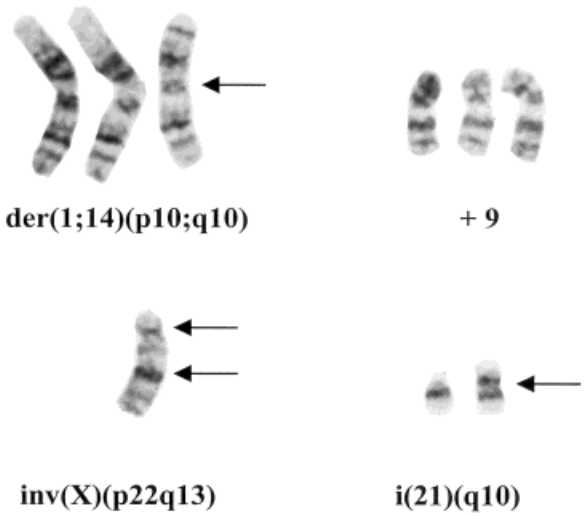

Figure 2. Partial karyotype of the cell line MedB-1. Chromosomal aberrations are indicated by arrows.

50 chronic B-cell leukemias exhibited a 9p gain (Schröder et al., 1995; Bentz et al., 2000). In diffuse large-cell lymphomas of the gastrointestinal tract, such 9p gains were identified in three of 29 cases (percentages of cells with ≥ 3 hybridization signals, 16%, 42.5%, and 77%, respectively; Barth et al., 1998). These interphase cytogenetic data confirm the high specificity of 9p gains in MBL ($P < 10^{-11}$, Fisher's exact test). The CGH and FISH data for chromosome 9 in various lymphoma entities are listed in Table 3.

Characterization of the Cell Line MedB-1

Both the parental lymphoma and the cell line MedB-1 express a number of B-cell-restricted (e.g., CD19, CD20, and CD22) surface antigens (data not shown). Since 1991, banding analyses have been performed repeatedly on the cell line. The karyotype is as follows: 47,Y,inv(X)(p22q13),+der(1;14)(q10;q10),+9,−14,i(21)(q10) (Fig. 2). The karyotype has proved stable for the past 9 years. For comparison of the cell line with the original tumor, CGH analysis was done. The average ratio profile of this tumor clearly shows an overrepresentation of 1q, 9, and 21 in the original tumor (Fig. 3). These findings correspond exactly to the chromosomal imbalances diagnosed in the cell line MedB-1 by chromosomal banding analysis. Because the CGH data fit exactly to the cytogenetic data of MedB-1, this cell line is derived from a cell clone that is predominant in the parental tumor tissue.

DISCUSSION

In this comprehensive molecular cytogenetic analysis of MBL, a characteristic pattern of genetic aberrations was identified. Two aberrations, that is, gains of 9p and gains of Xq, were found in more than 70% of cases (75% and 87%, respectively). Whereas Xq gains were present in approximately 10% of other B-cell lymphomas, gains of the short arm of chromosome 9 were rare in B-cell lymphomas other than MBL (six of 308 cases). Five of these lymphomas with 9p gains were primary extranodal lymphomas.

These findings are in line with data based on chromosome banding studies: in a compilation of chromosome aberrations in non-Hodgkin's lymphoma, gains of chromosome 9 or its short arm were identified in two of 245 cases with a t(8;14), in none of 91 cases with a t(11;14), and in four of 355 cases with a t(14;18) (Johansson et al., 1995). Recently, gain of 9p was also found in four of 12 cases of Hodgkin's disease (Joos et al., 2000). The minimal commonly overrepresented region both in MBL and Hodgkin's disease mapped to bands 9p23–24. Candidate genes in this chromosomal region are the Janus kinase 2 gene (*JAK2*) and the gene encoding the nuclear factor I/B (*NFIB*). *JAK2* is a tyrosine kinase, which is capable of activating several members of the STAT family of transcription factors and thus affecting cell growth and differentiation (Weber-Nordt et al., 1998). Recently, the involvement of *JAK2* in chromosomal translo-

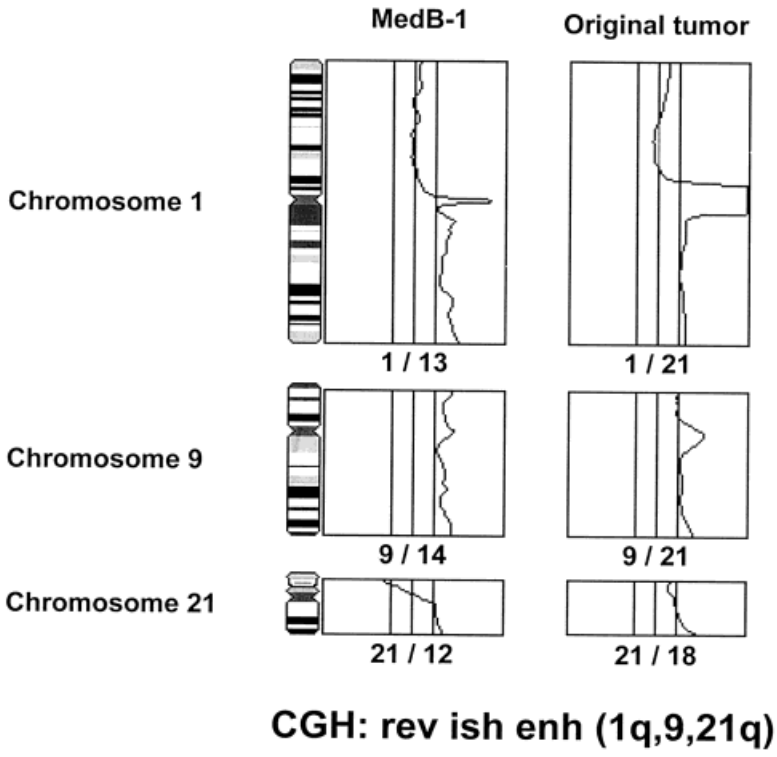

Figure 3. Comparison of chromosomal aberrations in the cell line MedB-1 (left) and in the parental tumor of patient no. 14 (right). The ratios of fluorescein isothiocyanate to rhodamine fluorescence are plotted along the chromosomes. The central line indicates a ratio value of 1.0. The lines to the right indicate ratio values of 1.25, and the lines to the left indicate values of 0.8. Identical aberrations were found in Med-B1 and the parental tumor. CGH, comparative genomic hybridization.

cations in lymphoid and myeloid neoplasms has been established (Lacronique et al., 1997; Peeters et al., 1997). In one of the Hodgkin's disease cases, as well as in MBL 16, an amplification of the *JAK2* gene was confirmed (Joos et al., 2000). The other candidate gene, *NFIB*, belongs to the family of nuclear-factor-1 transcription factors. *NFIB* has been found to be involved in chromosomal translocations in pleomorphic adenomas of the salivary glands (Geurts et al., 1998). In addition, proteins of the nuclear-factor-1 family are involved in the pathogenesis in certain types of virus-induced murine lymphomas (Ethelberg et al., 1997).

More detailed molecular and functional analyses of MBL are facilitated by the cell line MedB-1. This is the first cell line derived from a well-characterized MBL. MedB-1 retained the characteristic immunophenotypic features of the original tumor (data not shown). By CGH, the close clonal relationship between the cell line and the original tumor was shown (Fig. 3). In addition, the cell line carries the most pertinent chromosomal aberrations of MBL, that is, 9p and Xq abnormalities. Whereas the whole chromosome 9 is overrepresented, the aberration of chromosome X is an inversion involving chromosome bands Xp22 and Xq13. Potential pathogenetically relevant genes on band Xp22 include *AGMX2* and *FIGF*. *AGMX2* alterations are the molecular basis of a rare form of agammaglobulinemia (Sitz et al., 1990). The *FIGF* gene encodes a protein that induces angiogenesis in vivo and in vitro and exhibits mitogenic properties (Marconcini et al., 1999). The gene encoding the gamma receptor for interleukin 2 (IL2RG) and the *TAF2A* gene are located on chromosomal band Xq13. The *IL2RG* gene is associated with severe combined immunodeficiency (Puck et al., 1993), and the *TAF2A* gene encodes a protein with a possible role in the initiation of gene transcription (Pennisi, 1997).

Another published cell line (Karpas 1106) derives from a lymphoma with a predominantly mediastinal manifestation and genetic aberrations involving chromosomes 9 and X (Nacheva et al., 1994). This lymphoma was classified as lymphoblastic lymphoma. In a later publication, however, the same cell line was regarded as deriving from a marginal zone lymphoma (Tamura et al., 1998). Considering the clinical presentation of this case, the immunophenotype (CD5−, CD10−, and CD23−), and the genetic aberrations, Karpas 1106 may also derive from an MBL. This appears all the more likely in light of the fact that MBLs display a broad histomorphologic spectrum, including a medium-size variant (Paulli et al., 1999).

In conclusion, our data show that MBL exhibits a characteristic pattern of genetic aberrations with gains of 9p and Xq in the majority of cases. Gains of 9p are highly characteristic of this lymphoma type. These findings further support the view that MBL is a specific disease entity among diffuse large B-cell lymphomas.

ACKNOWLEDGMENTS

We gratefully acknowledge Christian Manegold (Heidelberg, Germany) for providing the clinical data for most of the cases of MBL. We are also grateful for the excellent technical assistance of Sabine Gantner and Magdalena Schlotter.

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