Combined spectral karyotyping, comparative genomic hybridization, and *in vitro* apoptyping of a panel of Burkitt's lymphoma-derived B cell lines reveals an unexpected complexity of chromosomal aberrations and a recurrence of specific abnormalities in chemoresistant cell lines

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Abstract. The comprehensive cytogenetic profiles of a panel of 10 Burkitt's lymphoma (BL)-derived B cell lines, designated Akata, BL-28, BL-41, Daudi, DG-75, Mutu I, Mutu III, Namalwa, Rael, and Ramos, respectively, are reported herein. The unique origin of each cell line was established using multiplex quantitative fluorescence polymerase chain reaction (QF-PCR). Spectral karyotyping (SKY) revealed a large number of structural and numerical chromosomal aberrations, many of which had not been previously identified or resolved by conventional G-banding techniques. Notably, whereas all 10 cell lines harbored the hallmark translocation t(8;14)(q24;q32), no other common structural aberrations were identified, although translocations involving chromosomes 3, 13, and 17 were frequently seen. Moreover, analysis of chromosomal breakpoints by comparative genomic hybridization (CGH) revealed a number of recurring aberrations, such as gain of chromosomes 7 and 20, gains of regions at 2p, 3q, 13q and 16q, and losses at 3p, 4q and 17p. In addition, apoptyping (i.e. determination of *in vitro* responses to apoptosis stimulation) of the cell lines suggested specific association patterns between karyotypic changes (e.g. translocations involving 17p, and gains of portions of chromosomes 7 and 20) and resistance to the chemotherapeutic agent, etoposide. The current molecular cytogenetic characterization of 10 BL cell lines has thus identified several novel sites of rearrangements; moreover, the combined karyotyping and functional assessment (apoptyping) of these cell lines serves to enhance their utility in future studies aimed at gene discovery and gene function.

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Introduction

Burkitt's lymphoma (BL) is a highly aggressive non-Hodgkin's lymphoma (NHL) consisting of endemic, sporadic, and immunodeficiency-associated variants (1). All of these subtypes possess chromosomal rearrangements of the c-*myc* oncogene, the genetic hallmark of BL that is believed to contribute to lymphomagenesis through alterations in numerous aspects of cellular homeostasis, including proliferation, differentiation, telomere maintenance, and apoptosis (2). BL-derived cell lines are commonly used as model systems to study the biology and evolution of cancer (3). Indeed, Raji, the first continuous human cell line of hematopoietic origin, was established some 40 years ago from a Nigerian patient with BL (4). Shortly thereafter, BL cell lines were found to harbor the Epstein-Barr virus (EBV), leading to the discovery and isolation of this virus (5). The link between EBV and endemic BL proved consistent and became the first of a wide range of associations between this virus and cancer (6).

Recurrent chromosomal abnormalities in hematological malignancies and solid tumors are a powerful resource for the identification of genes with critical roles in cancer (7). Continuous cell lines provide an excellent source of material for the investigation of such genetic changes. However, the karyotypes of most BL-derived cell lines have been studied by conventional G-banding techniques, thus making it difficult to discern the origins of the chromosomes involved in rearrangements. Recent advancements in molecular cytogenetics, including the development of fluorescence *in situ* hybridization

(FISH) for gene localization, and protocols for global assessment of genomic imbalances and the simultaneous visualization of structural chromosomal aberrations (8,9), have propelled the analysis of cancer cells to an unprecedented level of resolution. The evolution of cancer cytogenetics is closely linked to the characterization of BL-derived cell lines. Hence, soon after the introduction of differential fluorescence banding (Q-banding) (10), chromosome G-banding and Q-banding studies of biopsies and B cell lines from BL revealed the hallmark translocation t(8;14), one of the first cancer-specific translocations (11,12). Moreover, our group recently performed a comprehensive characterization of the prototypic Raji cell line using the molecular cytogenetic techniques, SKY (spectral karyotyping, involving the simultaneous hybridization of 24 differentially labeled chromosome-painting probes), array-based CGH (comparative genomic hybridization), and FISH, and we demonstrated a number of novel genomic aberrations, concurrent with the t(8;14) translocation (13). However, for most BL-derived cell lines, a detailed picture of chromosomal abnormalities remains to be determined.

Resistance to apoptosis is one of the cardinal features of cancer cells (14,15). The role of the failure of apoptosis in causing cancer followed the discovery that the *bcl*-2 gene, which is frequently involved in chromosomal translocations in follicular lymphoma, encoded an anti-apoptotic protein (16). When *bcl*-2 was expressed *in vitro*, it not only protected cells from apoptosis due to growth factor withdrawal, it also prevented apoptosis following treatment with a diverse range of drugs, resulting in a multidrug-resistance phenotype. However, inhibition of apoptosis alone does not rapidly transform cells or cause cancer. In contrast, when inhibition of apoptosis is combined with activation of an oncogene, such as c-*myc*, cancer can develop very rapidly (17). Moreover, most conventional anti-cancer drugs target the apoptosis machinery of cancer cells, resulting in the activation of a caspase cascade and dismantling of the cell; conversely, alterations in apoptotic pathways in malignant cells contribute to their chemoresistance (18). The characterization of specific defects in apoptosis signaling pathways in cancer cells may thus explain the mechanism of chemoresistance and could also yield novel targets for treatment.

In the present study, we performed a comprehensive analysis of chromosomal aberrations in a panel of 10 BL-derived B cell lines, many of which are widely used in cancer research, based on spectral karyotyping (SKY) and comparative genomic hybridization (CGH). Furthermore, we aimed to assess whether specific patterns of karyotypic abnormalities were associated with the reaction of these cell lines to a common chemotherapeutic drug (etoposide) through *in vitro* cell-cycle/apoptosis profiling of the cell lines, a process we have termed 'apoptyping'.

Materials and methods

Cell lines and culture conditions. Burkitt's lymphoma (BL)-derived B cell lines, Akata, BL-28, BL-41, Daudi, DG-75, Mutu I, Mutu III, Namalwa, Rael, and Ramos, were obtained from the tissue culture collections of the Microbiology and Tumor Biology Center at Karolinska Institutet. The original references and Epstein-Barr virus (EBV) status of these cell lines are shown in Table I. Mutu I and Mutu III are both derived from the same patient, but are phenotypically distinct: Mutu I expresses only EBNA-1 but not the other EBV-encoded EBNAs whereas Mutu III expresses all 6 EBNAs and the 3 latent membrane proteins (LMPs) and has a lymphoblastoid phenotype (19). The human T leukemia cell line, Jurkat, was purchased from the European Collection of Cell Cultures (Salisbury, UK). All cell lines were cultured in RPMI-1640 medium (Gibco, Invitrogen, Carlsbad, CA, USA) supplemented with 10% fetal calf serum, 2 mM L-glutamine, and 1% penicillin/streptomycin.

Table I. BL-derived cell lines used in the present study.

Cell line	Gender	EBV status	Author/(Refs.)
Akata	F	Pos.	Takada *et al* (63)
BL-28	M	Neg.	Lenoir *et al* (64)
BL-41	M	Neg.	Lenoir *et al* (64)
Daudi	M	Pos.	Klein *et al* (65)
DG-75	M	Neg.	Ben-Bassat *et al* (66)
Mutu I	M	Pos.	Gregory *et al* (27)
Mutu III	M	Pos.	Gregory *et al* (27)
Namalwa	n.a.	Pos.	Nadkarni *et al* (67)
Rael	n.a.	Pos.	Klein *et al* (68)
Ramos	M	Neg.	Klein *et al* (69)

EBV, Epstein-Barr virus; n.a., not available.

QF-PCR analysis. Genomic DNA was extracted by InstaGene Matrix (Bio-Rad Laboratories, Hercules, CA, USA) according to the manufacturer's protocol with slight modifications. Briefly, 50 μl of confluent cells (~300 cells) were collected and washed in PBS, then resuspended in 10 μl dH_2O. InstaGene Matrix (100 μl) was then added and samples were incubated at 56˚C for 30 min. Following incubation, samples were vortexed, maintained at 100˚C for 8 min, and centrifuged at 14,000 rpm for 2 min. Multiplex quantitative fluorescence polymerase chain reaction (QF-PCR) amplification of genomic DNA from each cell line (5 ng) was performed using the ChromoQuant kit (Cybergene AB, Stockholm, Sweden) according to the manufacturer's protocol. Samples were analyzed as previously described (20,21) using an ABI PRISM 3100 Avant genetic analyzer (Applied Biosystems, Foster City, CA, USA) operating with GeneMapper 3.5 software (Applied Biosystems).

Cytogenetics. Cells were harvested after mitotic arrest using colcemid (Invitrogen) and metaphase slides were prepared according to standard protocols. Twenty to fifty metaphases were analyzed for each cell line, and the karyotypes were described according to the International Conventional System for Human Cytogenetic Nomenclature (ISCN) (22).

SKY analysis. Spectral karyotyping was performed according to the protocol included in the Human Spectral Karyotyping Kit (Applied Spectral Imaging, Migdal Haemek, Israel).

Table II. QF-PCR analysis of the BL-derived cell lines.

Chromosome	Marker location	Cell line										
		Akata	BL-28	BL-41	Daudi	DG-75	Mutu I	Mutu III	Namalwa	Rael	Ramos	Jurkat
13	13q11-q21.1	260 282	266 290	266 282	258 276	244 272	258 288	258 288	260 274	260 264	270 274	270 284
	13q12.1-q14.1	450 453	446 461?	441	426 434	453 457	450 453	450 453	450	426 446	446	450
	13q14.3-q22	400 406	388 400	400 409?	390 396	396 420	403 424	403 421	396	396	400 409	396
	13q31-q32	428 430	428 454	456 462?	446 454	458 462	454 458	454 458	454 458	454 458	428 430	448 458
18	18pter-p11.22	159 163	159 163	163	163	159 163	154	154	150 166	154 163	154 166	159
	18q12.2-q12.3	482 486	478 482	478 490	486	476	476 482	476 482	470 482	478	482 490	466 480
	18q22.1-q22.2	350 354	358 392	358 380	354 358	364 380?	358 364	358 364	344	358	350 354	366 376
	18q22.3-q23	177	180 184	180	172 184	159 177?	180	180	188	184	180	177 180
21	21q21	248 257	260 275	254	264 268	242 254	242 252	242 252	235 239	242 252	252	257 264
	21q22.1	453 461	451	459	451 455	455 461	451 455	451 455	445 455?	445	453 461	459
	21q22.1	480 486	480 495	473 485	473	473 476	480 489	480 489	476 492	476 480	479 491	473 489
	21q22.2-qter	270 300	273 276	270 294	270 279	286 295	273	273	270	270	270 286	295
X	Xq26	101	105	105	105	109	93	93	101	113	99	113
	Xq26.1	290	266 282	290	294	290	286	286	294	284	290	290
X/Y (AMEL)	Xp22.1-p22.31 Yq	104 -	104 109	104 109	104 109	104 109	104 109	104 109	104 -	104 -	104 -	104 109
X/Y	Xq/Yq	218	202 220	200 214	204 224	-	200 224	200 224	200	224	200	204
Y	Yp11.3	-	205 207	203	203	203	203	203	-	-	-	201

QF-PCR markers were from the ChromoQuant kit (Cybergene AB) and the analysis was performed as detailed in Materials and methods. ? indicates possible trisomy.

Table III. Composite karyotypes of the BL-derived cell lines, as determined by SKY and CGH.

Cell line	Karyotype
Akata	47,X,-X,t(4;11)(p16.3;q13),+i(5)(p10),t(8;14)(q24;q32),der(13)t(2;13)(p12;p11), der(14;15)(q10;q10),+der(20)t(13;20)(q14;q13.3)x2[18]/47,idem,t(3;22)(q29;q11)[3]/ 47,idem,der(15)t(15;18)(q22?;q23?)[4]
BL-28	47,XY,t(8;14)(q24;q32),dup(12)(q22q24.1),del(13)(q21q22),+13,dup(16)(p11.2p13.1)[18]
BL-41	45,XY,der(2)t(2;16)(q35;q12),del(3)(q13.2q13.3),der(4)t(4;18)(q13;q?),+der(7)t(4;7)(q21;p21), der(8)t(8;10)(p23;q11.2),t(8;14)(q24;q32),-10,dup(13)(q14q31),der(15)t(3;15)(q21;p11.1), der(17)t(3;17)(q21;p11.2),-18[19]
Daudi	47,XY,+7,t(8;14)(q24;q32)[26]/47,idem,der(2)t(2;15)(q34?;q22?)[2]
DG-75	46,XY,der(4)t(4;18)(q31.3;q12),t(8;14)(q24;q32)[19]/92,idemx2[6]
Mutu I	46,XY,t(8;14)(q24;q32),+20
Mutu III	58~79,XXY,+Y,-1,+2,-2,+i(2)(p10),+i(3)(q10),-4,der(4)t(4;14)(q?;q?),der(4)(4;14;20)(q?;q?;q?), -5,+6,-6,der(6;13)(q?;q?),+7,dup(8)(p21q23),-8,t(8;14)t(q24;q32)x2,-9,+10,-10,+11,-11,-12,+del(12)(q?), -13,-14,+16,+der(16)t(15;16)(q1?;q24),-17,-18,+19,-19,+20,+21,-21,+22,-22[cp27]
Namalwa	47,X,-Y?,dup(1)(q12q25),t(2;8)(p11;q24.3),del(3)(p11.1p21),der(3)t(3;5)(q29;q22?),del(5)(q22?),+7, t(8;14)(q24;q32),der(10)t(3;10)(q26.2;p15.3),der(12)t(3;12)(q24;p13.3),del(14)(q13q22), +der(14)t(8;14)(q24;q32),der(15)t(15;15)(p11;q24),ins(17;1)(p11;p22p31)[6]/ 47,idem,-der(10)t(3;10)(q26.2;p15.3),+10,-der(15)t(15;15)(p11;q24),der(15)t(1;15)(p?;p11)[4]/ 47,idem,-t(2;8)(q11;q24.3),der(2)t(1;2)(p?p11),der(8)t(2;8)t(p11;q24.3)[2]
Rael	44,X,-Y?,del(9)(q21q22),der(9;17)(p10;p10),t(8;14)(q24;q32),der(9;15)(q10;q10),-17,i(17)(q10)[12]/45,idem,+20[9]
Ramos	45,X,-Y,del(3)(p14p14),t(8;14)(q24;q32), psu dic(7;16)(p11;p13),der(17)t(3;13;17)(q25;q31q14;p11.1)[15]/ 48,idem,+der(7)t(2;7)(p16;q32),der(13)t(3;13)(q25;q31),+17,+20[24]

Briefly, cocktail probe was hybridized on 1-day old pepsin-treated metaphase slides. Slides were then incubated with Cy5 and Cy5.5 detection reagents (Applied Spectral Imaging) and counterstained with 4,6-diamino-2-phenylindole (DAPI) dissolved in an anti-fade solution (Vectashield, Vector Inc., Burlingame, CA, USA). Images were acquired using SD200 Spectral Imaging System version 1.41, consisting of a Spectral cube and CCD camera (Applied Spectral Imaging) mounted on a Zeiss Axioscope2 microscope (Zeiss, Jena, Germany). Image analysis was performed using SkyView 2.1 and Case Data Manager EXPO 2.0 software (Applied Spectral Imaging).

CGH analysis. CGH was performed essentially as described previously (23). Genomic DNA from cell lines and reference DNA (Promega, Mannheim, Germany) was digested into fragments of 100-2,000 bp using DpnII (New England Biolabs, Beverly, MA, USA). The resulting DNA fragments were purified and the DNA was directly labeled by Universal Linkage System ULS (Q-BIOgene Molecular Cytogenetics, Illkirch, France) according to the manufacturer's instructions. Reference DNA was labeled with rhodamine (Q-BIOgene) and sample DNA was labeled with dGreen (Q-BIOgene). Samples were then mixed and re-purified using the PCR purification kit (Qiagen, Hilden, Germany), Cot-1 DNA (Invitrogen) was added prior to ethanol precipitation, and the pellet was resuspended in Hybrisol VI solution (Q-BIOgene). Labeled DNA was then applied onto metaphase slides from normal lymphocyte cultures. After overnight hybridization, the slides were washed and counterstained with DAPI. Non-overlapping chromosomes were analyzed on a Zeiss Axioplan2 fluorescence microscope and images were captured with a cooled CCD camera (SenSys Photometrics, München, Germany) operating with SmartCapture software (Digital Scientific, Cambridge, UK). Data analysis was performed using the Quips software from Vysis (Downers Grove, IL, USA).

Apoptosis/cell-cycle analysis. For induction of apoptosis, BL-derived cell lines or Jurkat T cells in logarithmic growth phase were incubated for 24 h in the presence or absence of etoposide (6 μg/ml) (Bristol-Myers Squibb, Stockholm, Sweden). Simultaneous assessment of apoptosis and cell-cycle distribution was then performed according to standard procedures (24). Briefly, cells were harvested, fixed in ethanol for 12 h, and stained with propidium iodide/RNase ready-made solution (Becton-Dickinson, San Jose, CA, USA). Cells were then analysed on a FACScan (Becton-Dickinson) equipped with a 488-nm argon laser using CellQuest software (Becton-Dickinson). Data were depicted as histograms and

the percentages of cells displaying hypodiploid DNA content (sub-G1 phase) versus cells contained in the G1 and G2-M phase of the cell cycle were determined.

Results

Cross-contamination analysis by QF-PCR of 10 human BL-derived B cell lines. Quantitative fluorescence polymerase chain reaction (QF-PCR) is a standard method in prenatal diagnostics and is employed for the rapid identification of aneuploid disorders and aberrations involving sex chromosomes (25). We decided to use QF-PCR for the analysis of genomic DNA from our panel of BL-derived cell lines, in order to establish the unique origin of each cell line prior to embarking on laborious karyotyping studies. The Jurkat cell line, a classic leukemia cell line that accounts for numerous cases of cross-contaminations among other leukemia-lymphoma cell lines (26), was included for comparison. With this rapid method, sex determination of each of the 10 BL cell lines was easily performed, and the results were found to be in concordance with the original references (Table I). Furthermore, QF-PCR confirmed the relationship between the Mutu I and Mutu III cell lines (27), whereas all other cell lines were shown not to be related (Table II). Hence, cross-contamination of the BL-derived cell lines was excluded.

Identification of hidden chromosomal aberrations by spectral karyotyping (SKY) of BL-derived cell lines. Numerical and structural chromosomal aberrations were identified by SKY in each of the BL-derived cell lines; the majority of these aberrations have not been reported previously. A full description of the karyotypes is presented in Table III. Hence, we identified a total of 41 numerical and 63 structural aberrations (45 translocations, 8 deletions, 9 duplications, and 1 insertion). All cell lines harbored the hallmark translocation, t(8;14)(q24;q32), and at least one additional aberration (range: 2-11 aberrations per cell line); the highest number of chromosomal aberrations was observed in the Namalwa cell line. None of the non-t(8;14) aberrations were common to all BL cell lines. However, several frequently recurring abnormalities were observed, including trisomy 7, trisomy 20, and translocations involving chromosomes 3, 13, and 17. Even for cell lines that appeared to be similar upon QF-PCR analysis, the spectral karyotypes were found to be markedly different. The special case of the Mutu cell lines (derived from the same patient) is of particular interest, and indicates that variations in the program of EBV latency may have a dramatic impact on the genetic constitution of a cell line (Table III). Furthermore, the complexity of some of the observed translocations [such as der(3;13;17) in the Ramos cell line, or der(4;14;20) in the Mutu III cell line] suggests how they could have been overlooked by conventional G-banding techniques. SKY analysis, on the other hand, unequivocally characterized all abnormal chromosomes, leaving no markers of unknown origin. SKY derivative chromosomes, i.e. those chromosomes displaying translocations, insertions, or numerical aberrations, are shown for each cell line in Fig. 1. Since chromosomal breakpoints of all translocations cannot always be determined by this method, additional CGH analysis, and a comparison of inverted DAPI banding with the CGH profiles, was performed.

Table IV. Chromosomal imbalances and breakpoints detected by CGH.

Cell line	Losses	Gains
Akata	-	2p12-pter, **5pter-q11.2, 13q14-qter, 20**
BL-28	-	12q22-24.1, 13q12-q14, 13q31-qter, 16p11.2-p13.1
BL-41	2q35-qter, 3q13.2-q13.3, 4q13, 10pter-q11.2, 17pter-p12	**3q21-qter,** 7p15-qter, 13q14-q31, 16q12.1-q23, 18q22-qter
Daudi	-	7
DG-75	4q32-qter	18q12-qter
Mutu I	-	20
Mutu III	-	2p, 3q, 8p22-pter, 16p13.1-q21
Namalwa	3p11.1-p21, 14q14-q22, 17p	1q12-q25, 1p31-p32, 1p35-pter, 3q24-qter, 7, 8q23-qter, 14q11-q13, 14q31-qter, 15q24-qter
Rael	9q21-q22, 17p11.2-pter	-
Ramos	3p14, 13q32-qter	2p16-p24, **3q25-qter, 7 (7q10-q32),** 13q14-q31, 17p11.1-17qter, 20

Designations in bold denote areas of amplifications (defined as fluorescence intensity values ≥2.0; see Fig. 2 for representative ratio profiles).

Assignment of chromosomal breakpoints and imbalances in BL cell lines by CGH analysis. To determine the breakpoints of chromosomal rearrangements, and to detect genomic losses and gains, the 10 BL cell lines were subjected to metaphase CGH analysis. We identified a total of 49 breakpoints in rearranged chromosomes. Partial CGH profiles for the Akata, BL-41, and Mutu III cell lines, illustrating the criteria for gains and losses, are depicted in Fig. 2. In general, there were no whole chromosome losses in our panel of cell lines, and more gains than losses of genomic material were observed. Recurring breaks (two or more) were noted at 13q14, 13q31, and 16p13.1 (Fig. 3). Whole chromosome gains were found to affect chromosomes 7 and 20, whereas regional gains often occurred on 3q and 13q, less often on 2p and 16q, and in a few cases on 1q, 1p, 5p, 8q, 12q, 14q, 15q, 17q, and 18q. Regional losses most often occurred on 3p, 4q, and 17p, and were also noted on 2q, 3q, 9q, 10p, 13q, 14q, and 18q. Regions of high-level gain (amplifications) were detected at six sites: 3q21-qter (BL-41), 3q25-qter (Ramos), 5pter-q11.2 (Akata), 7q10-q32 (Ramos), 13q14-qter (Akata), and chromosome 20 (Akata). Combined CGH results from all 10 cell lines revealed several common regions of gains and losses of genomic material, such as 2p16-pter, 3q25-qter, 7q11.2-q32, 16q12.1-q21, 13q14, and 13q31 (gains), and 3p14 and 17p (losses). All abnormalities detected by CGH are listed in Table IV,

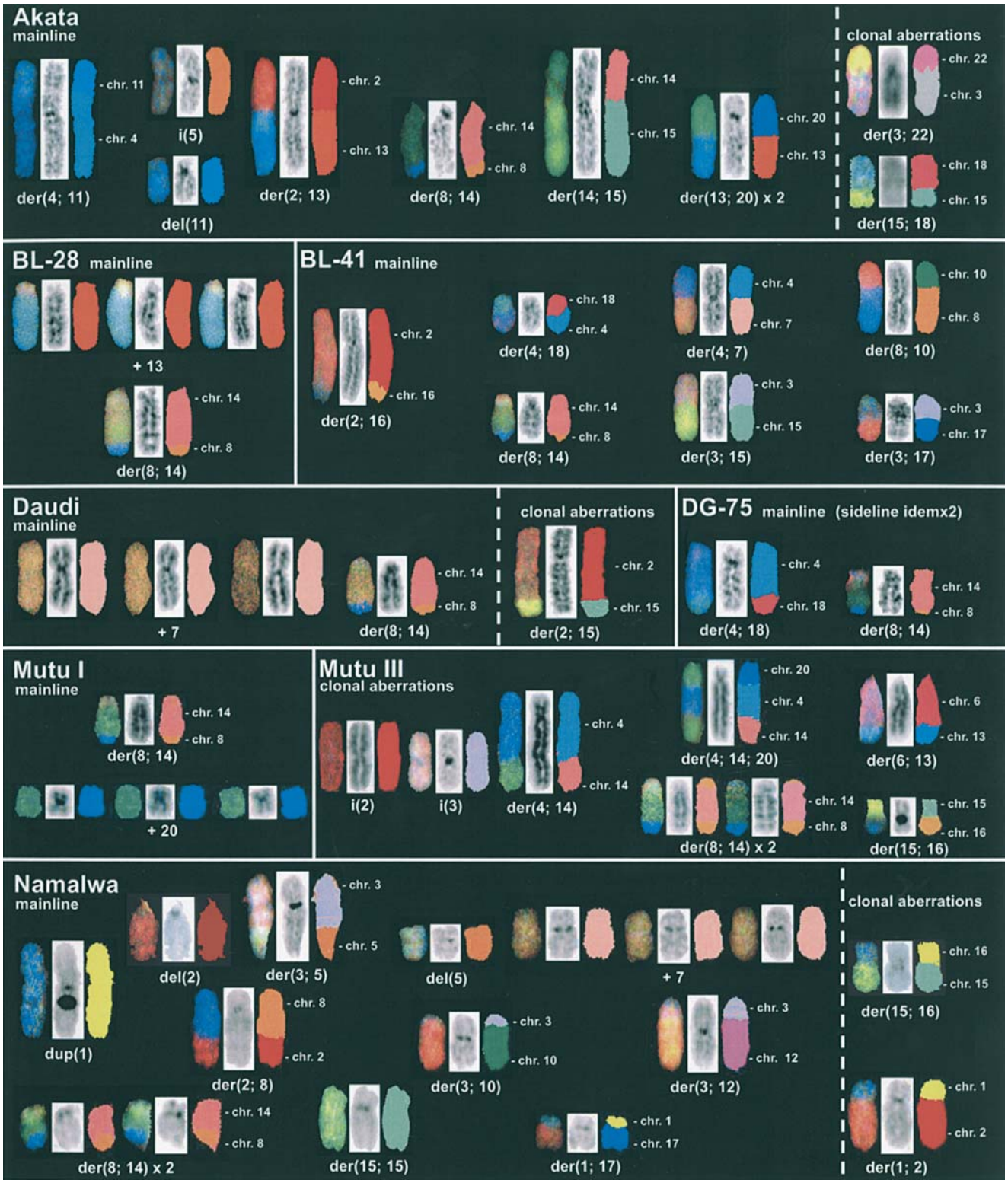

Figure 1. Spectral karyotyping of 10 BL-derived BL cell lines. Shown from left to right for each derivative chromosome are hybridization display colours, gray-scale inverted 4,6-diamino-2-phenylindole (DAPI) images, and SKY classification colours. Clonal aberrations are also depicted. Note the presence of the characteristic t(8;14) translocation in all 10 cell lines.

and the combined CGH profiles of all 10 BL cell lines are presented in Fig. 3. Some breakpoints in rearranged chromosomes were not identified (for instance, aberrations present in sublines may not be detected by standard metaphase CGH). Nonetheless, there was, overall, a remarkable correspondence between imbalances in the CGH profiles and the numerical alterations detected by SKY analysis, and these studies thus underscore the usefulness of a combined analysis in detecting genomic changes in cancer cells.

Comparison between molecular karyotypes of BL cell lines and G-banding of cell lines and primary BL tumors. We performed a detailed comparison of the karyotypes of BL cell lines obtained with molecular cytogenetic techniques and

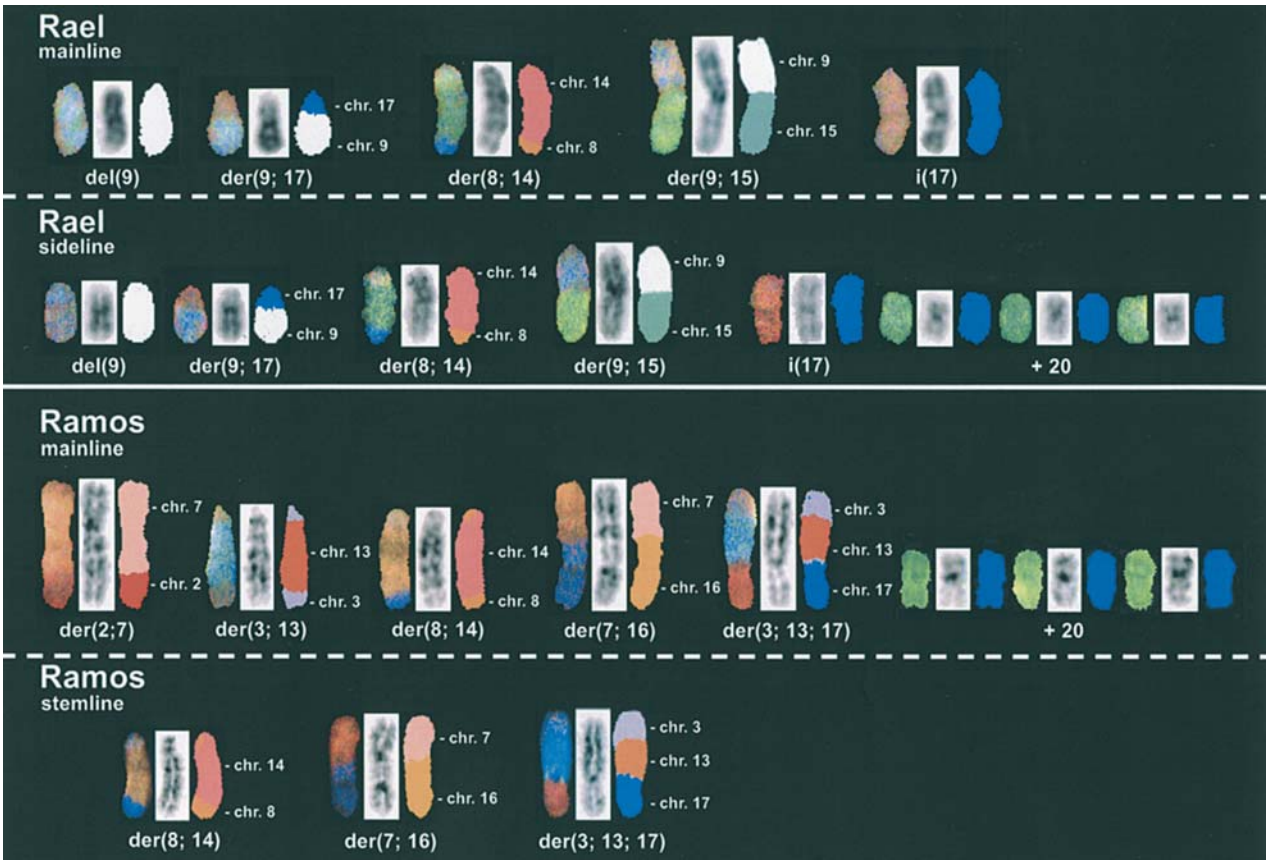

Figure 1. Continued.

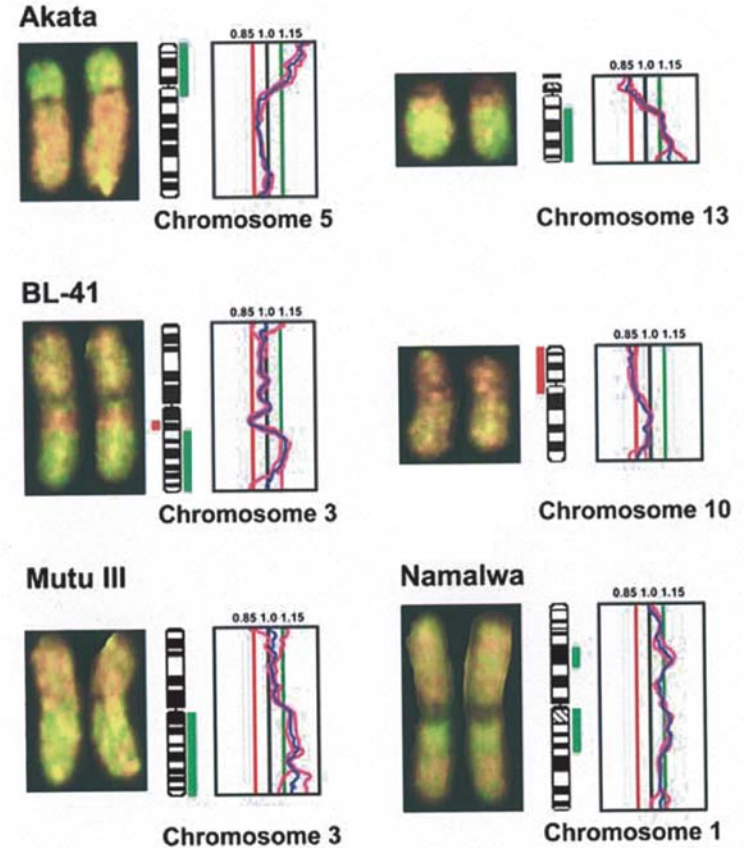

Figure 2. Partial CGH profiles of 3 representative BL cell lines illustrating the criteria for gains and losses of genomic material. The blue line in the ratio profile (right) represents the mean of the two chromosomes depicted to the left. Sample-to-reference ratios of ≥0.8 and ≤1.2 were considered normal; the vertical green and red bars on the right and left of the ideogram designate gains and losses, respectively.

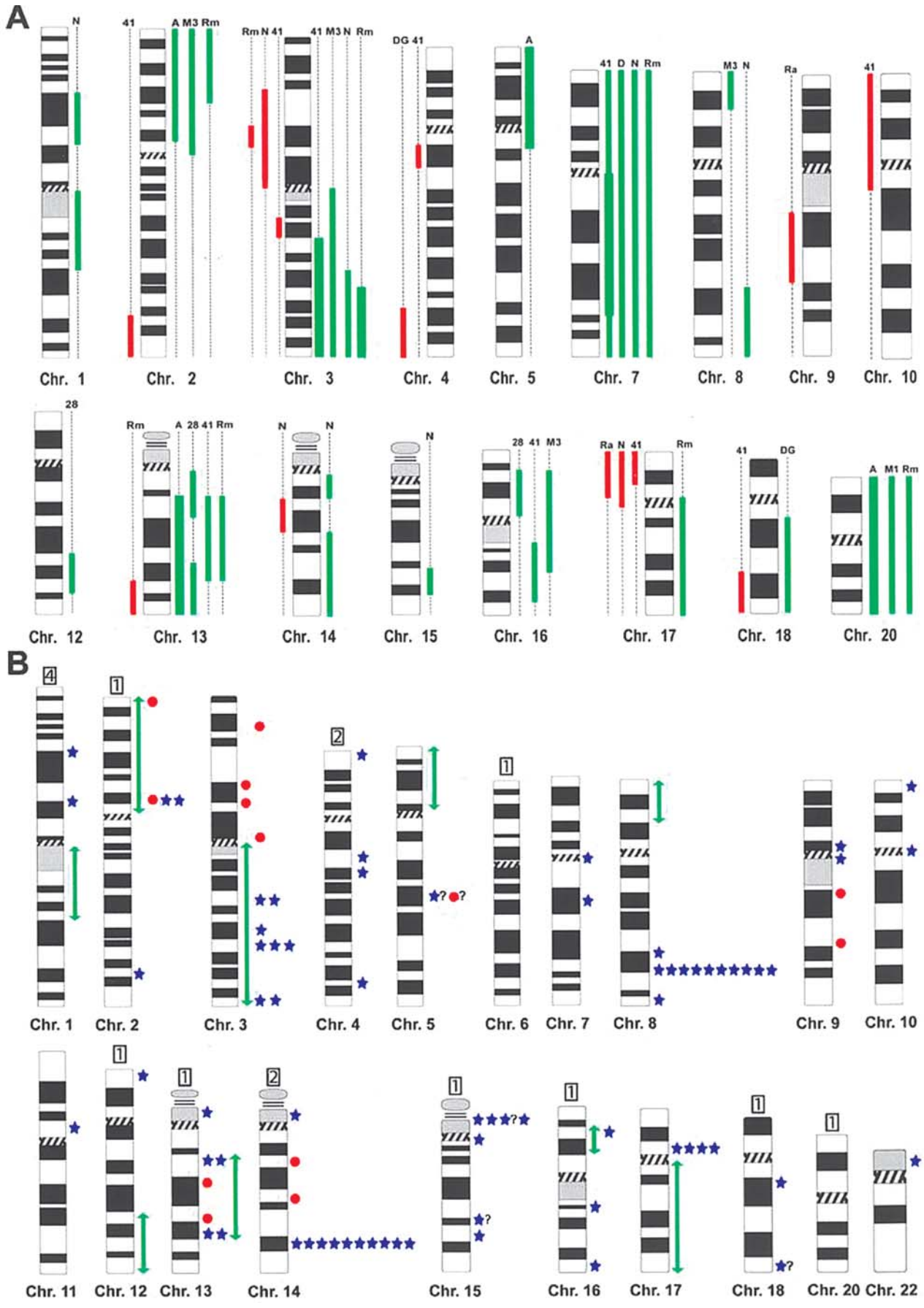

Figure 3. Ideograms showing DNA copy number changes identified by CGH in the 10 BL cell lines. (A) The vertical lines on either side of the ideogram indicate losses (left) and gains (right) of the chromosomal region. Thick green lines indicate regions with high-level gains (amplifications). (B) Ideograms showing all of the chromosomal breakpoints noted in the BL cell lines using SKY (inverted DAPI images) in combination with CGH. Blue stars indicate breakpoints of translocations, and stars with a question mark indicate a breakpoint for which the localization remains uncertain. Red circles indicate breakpoints for deletions, green arrows depict duplications. Numbers above chromosomes indicate how many unidentified breakpoints are assigned to the chromosome.

Table V. G-banded karyotypes and p53 status of the BL cell lines.

Cell line	TP53 status	Previous karyotypes	Author/(Refs.)
Akata	Mutant[a]	46,X0,t(8q-,14q+),+20	Takada *et al* (63)
BL-28	WT[b]	46,XX,t(8;14),dup(1)(q23-q25),19q+	Berger and Bernheim (72)
BL-41	Mutant[c]	48(42-49,XY,+7,-13,+2mar,add(8)(q24),t(8;14)(q24;q32), der(15)t(13;15)(q13;p11),add(17)(p12)	Drexler *et al* (73)
Daudi	Mutant[d] (nonsense)	48XY,-5,+7,t(8;14),+11p-q-,15q-,+ marker 46-48,XY,t(8;14),+6,+7,del(15)(q13-q15) 46(45-48)XY/XXY,+7,-9,t(8;14)(q24;q32)	Steel *et al* (74) Berger and Bernheim (72) Drexler *et al* (73)
DG75	WT[b]	46,XY,14q+ 46XY,t(8;14)(q24;q32)	Berger and Bernheim (72) Drexler *et al* (73)
Mutu I	Mutant[b]	'Carried the characteristic translocation' t(8;14)	Gregory *et al* (27)
Mutu III	Mutant[b]	'Carried the characteristic translocation' t(8;14)	Gregory *et al* (27)
Namalwa	Mutant[d] (missense)	'Grossly abnormal' XX 1p+q+ (dupl 1q),3q+,6q-,+7,t(8;14),inv10,13q+,-15,16q+,-21,-22, + up to 6 markers 48,X,+7,+18,1q+,3q+,del(3)(p11),del(4)(q21),inv(10),del(15)(q21-q24),46,X,t(8;14), +7,+18,-1, dup(1)(q22-q25),dup(3)(q13-q24), variations 44(43-46),X,-Y,-5,-6,+7,-10,-13,-21,+3mar,ins(1;?)(p11;?),ins(3)(q11q29),der(5)t(1;5)(q25;q35), del(8)(p22),der(14)t(14;?)(p11;?), der(15)t(15;21)(p11;q11), + 'Namalwa' markers	Steel *et al* (74) Berger and Bernheim (72) Drexler *et al* (73)
Rael	Mutant[b]	n.a.	
Ramos	Mutant[d] (missense)	49XY,t(6,17),t(7;16),t(8;14),+7,+2 markers	Steel *et al* (74)

[a]Farrell *et al* (41); [b]Wiman *et al* (70); [c]Ramqvist *et al* (71); [d]Gaidano *et al* (42). n.a., not available; WT, wild-type.

the previously published karyotypes based on conventional cytogenetics of the same cells. Karyotype analysis based on chromosome banding often results in marker chromosomes that are comprised of rearranged fragments whose origin remains ambiguous. By contrast, the origins of all complex rearrangements previously unidentified in G-banding studies were resolved using SKY and CGH (Table V). In addition, several new or hidden aberrations were revealed. However, a correspondence in chromosome numbers and characteristic markers was seen, which allowed us to conclude that the same cell lines have been used. A matter of considerable importance is the relationship between cell lines and the primary tumors from which these cell lines arose. Previous studies show that HeLa (the first human cell line of epithelial origin) (28) and Raji (the first human cell line of hematopoietic origin) (4,29) have remained stable despite many decades of *in vitro* cultivation (13,30). We conducted a search of the public Mitelman Database to investigate which genetic changes are common for patients with BL, and this list of structural aberrations is presented in Table VI. Several aberrations in our panel of BL cell lines, in addition to t(8;14)(q24;q32), were either identical to aberrations listed in the patient database or occurred on the same chromosome arm affected in BL patients. Hence, the concordance of our findings suggests that the cell lines characterized herein are relevant models of BL tumors, at least from a cytogenetic perspective.

Apoptyping reveals differences in chromosomal abnormalities between chemosensitive and chemoresistant BL cell lines. Next, we measured DNA content in our panel of BL cell lines incubated in the presence or absence of the chemotherapeutic agent, etoposide. Jurkat cells were included as a positive control. Analysis of flow cytometric profiles focused on three distinct populations of cells: a) a sub-G1 or hypodiploid population, corresponding to cells undergoing apoptosis; b) a G1 population, corresponding to cells in the G1 phase of the cell cycle; and c) a G2-M population, corresponding to dividing cells in the G2 and M phases of the cell cycle (24). Based on this analysis, 3 of the BL cell lines were classified as sensitive (Akata, BL-28, Namalwa) and 5 as resistant (BL-41, Daudi, DG-75, Mutu I, and Mutu III); 2 cell lines (Rael and Ramos) were determined to be intermediate-to-resistant, in comparison to the Jurkat cell line (Fig. 4). The maximal response to etoposide (35% of sub-G1 cells) was observed in the Namalwa cell line. Furthermore, cell lines classified as sensitive or intermediate displayed either: a) apoptosis + G2-M accumulation (Akata, BL-28, Rael, Ramos), or b) apoptosis + G1 restriction of progression (Namalwa), whereas resistant cell lines showed c) G2-M accumulation (BL-41, DG-75, Mutu I, Mutu III), or d) G1 restriction of progression (Daudi). We also asked whether any specific associations between chromosomal aberrations and apoptosis/cell-cycle profiles of the BL cell lines were evident. Interestingly, a recurrence of specific

Table VI. Structural aberrations detected in primary BL tumors.

Chromosome arm	Aberrations	No. of cases
	Balanced translocations	
2p	**t(2;8)(p11;q24);** t(2;8)(p12;q23); t(2;8)(p12;q24)	18
8q	**t(8;14)(q24;q32)**	189
18q	t(14;18)(q32;q21)	6
22q	t(8;22)(q23;q12); t(8;22)(q24;q11)	32
	Unbalanced aberrations	
1p	add(1)(p36)	2
1q	i(1)(q10); dup(1)(q12q32); dup(1)(q21q31); dup(1)(q21q32); dup(1)(q21q44); dup(1)(q23q32); del(1)(q25)	24
2q	del(2)(q32)	3
3p	del(3)(p24)	2
3q	del(3)(q21)	2
6q	del(6)(q21)	3
7q	add(7)(q22)	2
8p	der(8)t(1;8)(q21;p11)	2
9q	**del(9)(q22)**	2
13q	add(13)(q34)	7
14q	**add(14)(q32)**	15
15p	**add(15)(p11)**	2
17q	**i(17)(q10)**	2
18q	add(18)(q21)	2
22q	der(13;22)(q10;q10)	2

Data were obtained from the Mitelman Database at http://cgap.nci.nih.gov/Chromosomes/Mitel_Search. Designations in bold indicate aberrations that also occurred in the BL cell lines investigated herein (these aberrations were either identical to those listed in the database, or occurred on the chromosome arm also affected in patient specimens).

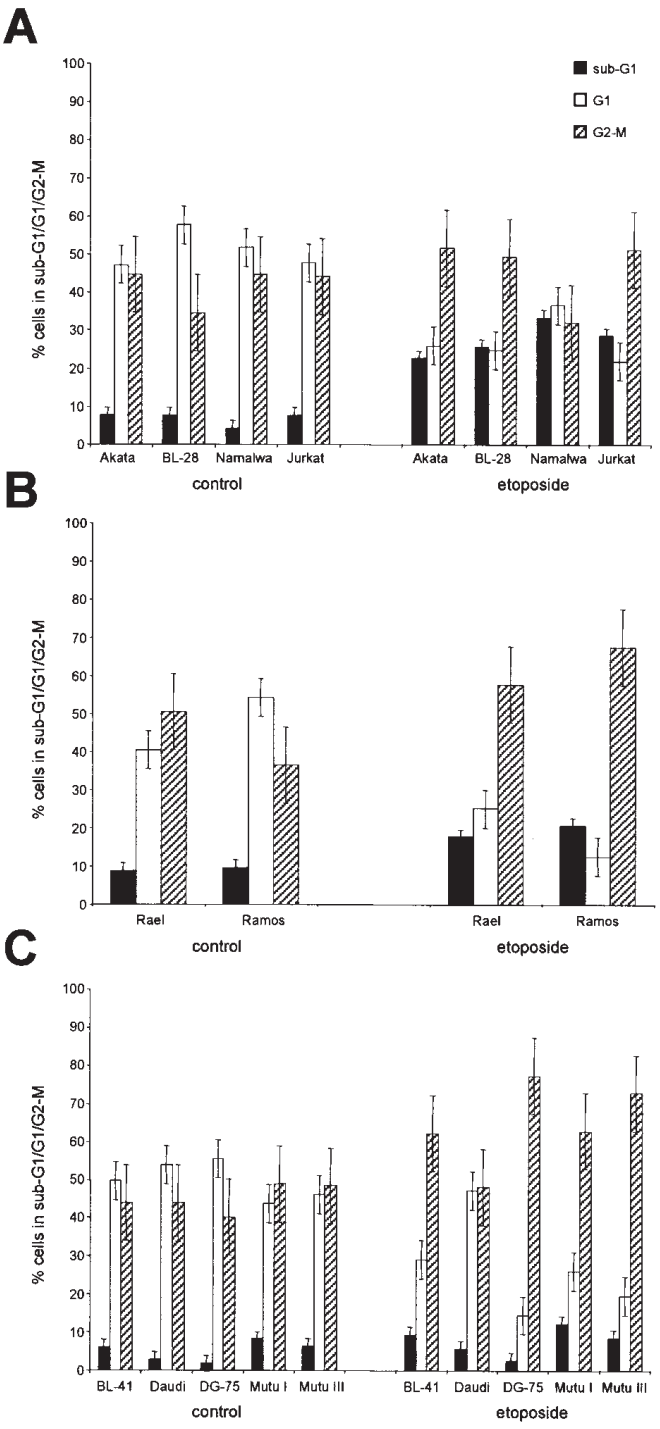

Figure 4. Apoptyping of BL-derived cell lines. Quantification of apoptosis/cell-cycle responses of sensitive (A), intermediately sensitive (B), and (C) resistant BL cell lines. The Jurkat T cell line was included as a positive control. Data are derived from DNA histograms of propidium iodide-stained cells. In sensitive cell lines, a hypodiploid (sub-G1) peak emerges upon treatment, indicative of apoptosis-specific DNA fragmentation. Cell-cycle arrest is reflected as an accumulation of cells in the G1 or G2-M phase of the cell cycle (consult text for details). The percentage of cells in the sub-G1 (black bars), G1 (white bars), and G2-M phases (cross-hatched bars) of the cell cycle in the presence or absence of etoposide (6 μg/ml) is depicted for each cell line. Data are shown as mean ± SD (n=3-6). For comparison, the p53 status of each cell line is shown in Table V.

abnormalities [apart from the t(8;14) translocation] was observed. Hence, among the 7 BL cell lines classified as resistant or intermediate, translocations involving 17p, gain of most of chromosome 7, and gain of chromosome 20 occurred three times. Moreover, translocations involving the q arms of chromosomes 4 and 18, and translocations involving chromosomes 2, 3, and 16 were noted in at least two of the chemoresistant cell lines (Table III).

Discussion

We have conducted a comprehensive molecular cytogenetic characterization of 10 widely used BL-derived B cell lines using SKY and CGH. QF-PCR analysis was performed to exclude cross-contamination of the cell lines prior to karyotyping, and to ascertain the common origin of the Mutu cell lines. In addition, functional assessment (apoptyping) served to classify these cell lines as sensitive or resistant to the chemotherapeutic drug, etoposide, and allowed us to identify specific

patterns of chromosomal aberrations in the chemoresistant cell lines. Overall, our results suggest an unexpected degree of karyotypic complexity among BL cell lines. A number of previously hidden aberrations were detected, including balanced and unbalanced translocations, deletions, duplications, and insertions. The origins of all complex rearrangements were resolved, thus testifying to the usefulness of the molecular cytogenetic techniques utilized herein.

Spectral karyotyping (SKY) has been shown to reveal hidden structural aberrations in hematological malignancies (31). Similarly, our previous SKY analyses of childhood acute lymphoblastic leukemia (ALL) samples have disclosed numerous abnormalities not identified by G-banding, and served to provide prognostically important information (32,33). The cell lines included herein were established before the advent of molecular cytogenetics, and the previous karyotypes were based on chromosome banding methods. The current study has thus served to refine and resolve the karyotypes of these cell lines. Moreover, we identified corresponding abnormalities in our panel of BL-derived cell lines and primary BL tumors, suggesting that these cell lines represent a faithful model of BL, at least from a cytogenetic point of view. Similarly, in their study of the so-called NCI-60 panel of cancer cell lines, Roschke *et al* (34) showed that the spectrum of aberrations in each cell line correlated with the spectrum of aberrations seen in the corresponding primary tumor. On the other hand, recent studies of the NCI-60 cell lines have shown that the expression of tumor- and tissue-specific genes can vary between cell lines originating from the same type of tumor (35). Future gene expression profiling studies of the panel of BL cell lines presented herein may serve to complement the current cytogenetic findings.

Previous studies have indicated that cancer cell lines may remain karyotypically stable under steady-state conditions, despite decades of continuous cultivation (13,30). However, when a change in culture conditions is introduced, such as a selection pressure toward certain phenotypic features (e.g. drug resistance), it may cause changes in the cytogenetic constitution of a cell line (36,37). Our characterization of the Mutu I and Mutu III cell lines provides evidence that variations in the expression of EBV genes also may exert a dramatic influence on the degree and complexity of karyotypic aberrations. Kiss *et al* (38) have shown that re-introduction of EBV into an EBV-negative subline of the BL cell line, Akata, activates the expression of *TCL*-1, a cellular proto-oncogene, and they suggested that high expression of *TCL*-1 is necessary for the development of the BL phenotype. Further exploration of the chromosomal abnormalities in the Mutu III cell line, and a detailed comparison between Mutu I and Mutu III, may provide additional clues regarding the contribution of EBV to the growth and/or tumorigenicity of EBV-positive BL.

Chromosomal translocations involving c-*myc*, the genetic hallmark of BL, were among the earliest molecular alterations found in human cancer (39,40). Since then, only a few secondary lesions have been identified in BL, including anomalies involving chromosome 17p, correlating with deletions and mutations of tumor suppressor gene p53 (41,42), and mutations or translocations of the *BCL*6 gene located on chromosome 3q (43,44). Alterations involving the long arm of chromosome 1 were also reported in several cases of BL and 'Burkitt-like' ALL (45,46), and Lenoir and colleagues found that aberrations of chromosome 1 were more frequent in BL cell lines with high and moderate tumorigenicity in nude mice (47). In line with these findings, recent molecular cytogenetic characterization of two cell lines derived from sporadic BL (CA46 and ST486) disclosed that only the highly tumorigenic CA46 cell line harbored a 1q23-q24 duplication (48). In the current survey, only Namalwa, the most complex of the 10 cell lines, displayed aberrations of chromosome 1q (gain of genomic material at 1q12-q25). On the other hand, we observed a spectrum of other recurring aberrations, including trisomies of chromosomes 7 and 20, and translocations involving chromosomes 3, 13, and 17. Moreover, common regions of copy number changes included gains at 2p, 3q, 13q, and 16q, and losses at 3p, 4q, and 17p. All of these aberrations have been observed previously in cancer cell lines or primary tumors. For instance, Mehra *et al* (49) have reported on the cytogenetic features of 10 NHL cell lines, and noted frequent gains on 3q, 7p and 7q, 13q, and loss of genomic material on 17p. Furthermore, in a recent study of BL and BL-like lymphomas in children, chromosomal abnormalities that involved 13q32 and partial duplication of 1q were shown to be associated with poor prognosis (50).

Resistance to apoptosis is one of the cardinal features of cancer cells, and contributes to their chemoresistance (18). Around half of all human tumors carry mutations in the tumor suppressor, p53, and tumors that express wild-type p53 frequently display other alterations in the p53 pathway that ablate the p53 response (51). For instance, recent observations suggest that deregulated *BCL*6 expression may functionally inactivate p53, even in the absence of p53 mutations (52). Moreover, metastatic melanomas often show silencing of Apaf-1, an adaptor molecule that acts together with cytochrome c and pro-caspase-9 to mediate p53-dependent apoptosis (53). Our recent studies of the Raji BL cell line have revealed a novel mechanism of chemoresistance: plasma membrane sequestration of Apaf-1, also resulting in defective cytochrome c-dependent activation of caspases (54). Of note, 8 of the 10 BL cell lines included in the present study were previously reported to harbor mutations in p53. Furthermore, as shown herein, 3 of these cell lines displayed deletions of 17p, rendering the mutant copy of p53 hemizygous. A majority of the BL cell lines tested were resistant to the chemotherapeutic drug, etoposide (55; and the present study); moreover, several cell lines displayed cell-cycle deregulation, a common finding in high-growth fraction lymphomas (56). However, since not all of the BL cell lines express mutant p53, and not all cell lines are defective for Apaf-1 (54), other underlying defects may determine the outcome of treatment. Indeed, we observed a recurrence of specific chromosomal abnormalities in the chemoresistant cell lines. Additional studies are needed to determine the putative role of these anomalies in the unresponsiveness to chemotherapeutic agents. Interestingly, at the time of writing of the present report, Roschke *et al* (57) provided evidence that the karyotypic 'state' of a cancer cell line is a potential determinant for anticancer drug discovery targeting the most aggressive and intractable tumors.

In conclusion, our studies have provided a detailed cytogenetic overview of 10 human BL-derived cell lines. Properly

authenticated cancer cell lines are an indispensable research tool and have yielded seminal insights into the biology of human cancer (58,59); a thorough characterization of cell lines should thus be a priority task for the laboratories which use them. Detailed information on chromosomal abnormalities in hematological malignancies is important because specific aberrations have a strong prognostic value (60,61). Moreover, precise analysis of certain rearrangements is a crucial step in the identification of genes that play a role in the pathogenesis of human cancer (62). Hence, the cytogenetic data presented here should serve as a valuable resource in future studies aimed at gene discovery and functional analysis.

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