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Cancer Genetics and Cytogenetics 122 (2000) 87–92

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Molecular and cytogenetic analysis of glioblastoma multiforme

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Received 6 March 2000; received in revised form 3 April 2000; accepted 17 April 2000

Abstract Glioblastoma multiforme (GBM) is the most common primary tumor occurring in the central nervous system of adults. Although progress has been made in clinical management of this tumor, little is known about the molecular defects underlying the initiation and progression of GBM. To address these issues, we have characterized five cases of GBM using cytogenetics, comparative genomic hybridization (CGH), fluorescence in situ hybridization (FISH), and direct sequencing. All of these tumors were observed to have clonal chromosome aberrations. Complicated chromosome translocations including der(18)t(2;4;12;18), der(X)t(X;10)(q27.1;p12.1) and der(10)t(10;15)(p11.23;q11.2), and der(1)(:1p31→1q44::7q11.3→7qter) were seen in three tumors. Loss of the *CDKN2* gene was noted in four tumors. A gain of copy number of the *Cathepsin L* gene was seen in two tumors. Amplification of the *CDK4, MDM2,* and GLI/CHOP genes was noted in two tumors, and amplification of the *PDGFR* gene was detected in one tumor. Mutation of exon 5 of the *TP53* gene was found in three tumors. No mutation of the *BCL10* gene was detected in five cases of GBM analyzed, although deletion of chromosome 1p was seen in two tumors. These results provide information for further investigation of GBM.

1. Introduction

Glioblastoma multiforme (GBM) is the most common primary neoplasm occurring in the central nervous system of adults. Although progress has been made in diagnosis and treatment of GBM, the prognosis of patients with GBM is still very poor [1]. This is because the carcinogenesis of GBM, particularly the molecular defects, which underline the initiation and progression of this tumor, is ill-defined [2].

Previous cytogenetics and molecular studies have shown that loss of chromosomes 1, 10, 11, 13, 17, 19, and 22, mutation of tumor suppressor genes such as *CDKN2, PTEN/MMAC1, DMBT1*, and *TP53*, and amplification and/or overexpression of genes such as *EGFR, MYCN, PDGFR, MDM2, GLI*, and *MET* are commonly observed in GBM [3,4]. These findings indicate that these genetic aberrations are likely to be associated with the development of GBM.

The *BLC10* gene was recently isolated from the breakpoint region of t(1;14)(p22;q32) in mucosa-associated lymphoid tissue (MALT) lymphomas [5,6]. *BCL10* is a cellular homolog of the equine herpes virus-2 *E10* gene. These genes contain an amino-terminal caspase recruitment domain (CARD) that is homologous to that found in several apoptotic molecules [5,6]. Mutation of *BCL10* has also been described in a wide range of other tumors [5]. As stated above, loss of chromosome 1p and mutation of *TP53*, another apoptosis-inducing gene, was frequently noted in GBM. It is intrinsic to determine whether or not mutation of *BCL10* exists in GBM.

To address these issues, we have characterized five cases of GMB using cytogenetics, comparative genomic hybridization (CGH), fluorescence in situ hybridization (FISH), and direct sequencing. Here, we present the findings of our study.

2. Materials and methods

2.1. Cytogenetics and FISH

Of the five GBMs studied, two tumors (nos. 4 and 5) have been reported [7,8]. The procedures of cytogenetic and FISH analyses of the three other GBMs (nos. 1, 2, and 3) were briefly described as follows. Tumor samples surgically removed before treatment were minced by using crossed scalpels and incubated in 2 mL Ham's F10 medium (GIBCO/BRL, UK) containing 10% fetal calf serum (FCS) and 2000

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0165-4608/00/$ – see front matter
PII: S0165-4608(00)00278-8

units/mL collagenase (Sigma C9891) at 37°C for 1 hour. The cell pellets were resuspended in 10 mL Ham's F10 containing 10% FCS, 100 units/mL penicillin and 100 mg/mL streptomycin, and incubated in 5% CO_2 at 37°C for 3–14 days. For cytogenetic analysis, cultured cells were harvested at passages 2–16, and metaphase chromosomes were prepared using standard techniques. At least 25 metaphase spreads were analyzed. Chromosome aberrations were described according to ISCN 1995 [9]. The FISH was performed on metaphase spreads and nuclei according to standard procedures using probes covering the entire human genome [7]. At least 50 metaphase spreads and 100 interphase nuclei were analyzed.

2.2. CGH

Testing DNA extracted from the five GBMs and reference DNA were labeled with SpectrumGreen and SpectrumRed (Vysis Ltd., UK), respectively, and were hybridized on normal female metaphase spreads. The CGH was performed according to the standard protocols with minor modification, and digitized image and karyotyping analyses were conducted by using the standard software (Vysis). Thresholds for gains and losses were defined according to the criteria previously described [10]. At least ten cells per case were analyzed.

2.3. Direct sequencing

DNA from the five tumors was directly sequenced using five pairs of primers amplifying exons 1–3 of *BCL10* [5] and three pairs of primers amplifying exons 5, 9, and 15 of *TP53*. The nested PCR was performed in a mixture consisting of 2.5 μl 10 × buffer, 4 μl dNTP (1.25 mM), 1 μl primer, 0.5 μl Taq polymerase, 15 μl H_2O, and 2.5 μl DNA under standard conditions. The second PCR was then carried out in a mixture containing 8 μl sequencing reaction mix, 1 μl primer, 4 μl PCR products, and 7 μl H_2O under standard conditions. The purified PCR products were sequenced using dRhodamine dye terminators on an ABI 377 automated fluorescent DNA sequencer according to the supplier's instruction (Applied Biosystems, USA).

3. Results

Cytogenetic, CGH, and FISH analyses showed all five tumors to have chromosomal aberrations (Table 1). In tu-

Table 1
A summary of clinicopathology, cytogenetic, FISH, CGH, and direct sequencing analyses of five GBMs

						Sequencing analysis				
						BCL10			*TP53*	
Case	Age/Sex	Pathology	Cytogenetics	CGH	FISH	Exon 1	2	3	5	9
1	21/M	Astrocytoma IV	~92,XXXXY,+1,+del(1)(p11),+del(1)(q11)×3,+2,+4,+5,+6,−7,+8,+9,+10,+11,+11,+11, +12,+13,+dic(13;15)(p11; p11),+14,+16,+16,+17,+18,+18,+18,+19,+20,+21,+22,+22,+mar[cp25]	Gain: 1, 3p, 3q, 4p, 6, 7, 16 Loss: 10, 15	Loss of a copy of the *CDKN2* gene in 67% of cells, amplification of the genes of *CDK4, MDM2, GLI/CHOP* in 30% of cells.	—[a]	—	—	Deletion	—
2	52/M	Astrocytoma IV	90,XXXYYY,+1,+1,+1,+1,+2,+3,+3,+3,+5,+6,+7,+9,+10,+11,+11,+12,+13,+13,+14,+15,+15,+16,+18,+19,+19,+20,+21,+21,+21,+22,+mar[9]/57,XXY,+1,+6,+7,+7,+11,−12,+18,−20,+22,+mar[6]/51,XXYY,+1,−4,−5,+6,+7,+7,+8,−10,+11,−12,−14,+16,+18,+19,+21,−22,+mar[4]/50,XXX,−Y,+1,+3,−4,−5,+7,+8,−9,+10,+11,−12,−13,−14,−15,−17,+19,−20,−22,−22,+mar[3]/58,XX,−Y,+3,−4,−5,+7,−8,+9,+10,+13,+13,−14,−17,−17,+18,+20,+21,+mar[3]	Gain: 1, 3, 6, 7, 8, 9, 21 Loss: 2, 4, 5, 10, 13, 14, 15, 16, 17, 22	Loss of a copy of the *CDKN2* gene in 50% of cells.	—	—	—	TTG→CTG at codon 48	—
3	58/M	Astrocytoma IV	46,X,−Y,+7[13]/91,XXYY,+1,+1,+2,+der(2)t(2;18)(p22;q?),+3,+3,+4,+5,+5,+6,+6,+7,+7,+7,+8,+9,+9,+10,+11,+11,+12,+12,+13,+13,+14,+14,+15,+15,+16,+16,+17,+17,+18,+der(18)t(2;4;12;18)(q?;q?;q?;p11),+19,+19,+20,+20,+21,+21,+22,+22,+mar[12]	Gain: 4q12, 12q13→q15 Loss: 9pter→p12	Amplification of the *PDGFR* gene and co-amplification of the genes of *CDK4, MDM2, GLI/CHOP* in HD cells, loss of a copy of *CDKN2* in 44% of cells, translocation of the *DCC* gene to 2p22.	—	—	—	Deletion	—
4	59/M	Astrocytoma IV	~69,Y,+der(X)t(X;10)(q27.1;p12.1),+del(5)(p14),+7,+der(10)t(10;15)(p11.23;q11.2),−13,+dic(5;14)(q21;p11),del(15)(q11.2),+der(16)del(16)(p13)del(16)(q24)[cp25]	Gain: 7, 8, 21q Loss: 5p, 6, 10, 13q, 16p, 16q	Loss of a copy of *CDKN2* in 57% of cells, gain of copy number of the *Cathepsin L* gene in HD cells.	—	—	—	—	—
5	70/M	Astrocytoma IV	~69,XY,+der(1)(1;7)(:1p31→1q44::7q11.23→7qter)×2,+4,+4,+5,+6,+6,+7,+7,+7,+8,+9,+9,−10,+12,+del(13)(q12q21),+14,+15,+15,+16,+17,+17,+18,+18,+19,+20,add(20)(q13),+22,+mar[cp25]	Gain: 7p, 7q, 19, 20 Loss: 1p36→p32, 1q44, 10, 13q13→q21, 21	Translocation of the *LIMK1* gene to 1p31 and 1q44); gain of copy number of the *Cathepsin L* gene in HD cells.	—	—	—	—	—

[a] Wild-type.

mor numbers 4 and 5, the majority of cells were near-diploid (ND). Tumor numbers 1 and 2 had a hyperdiploid (HD) cell population, and tumor number 3 had 52% ND cells and 48% HD cells. Three tumors, numbers 3, 4, and 5, expressed complicated translocations including der(18)t(2;4;12;18). (Figs. 1 and 2), der(X)t(X;10)(q27.1;p12.1), and der(10)t(10;15)(p11.23;q11.2), as well as der(1)(:1p31→1q44::7q11.23→7qter) with a translocation of the *LIMK1* gene to 1p31 and 1q44. Three tumors, numbers 1, 4, and 5, demonstrated deletions involving chromosomes 1, 5, 13, 15, and 16 (Fig. 3). Loss of the *CDKN2* gene was observed in four tumors. A gain of copy number of the *Cathepsin L* gene was seen in two tumors (nos. 4 and 5). Amplification of *CDK4, MDM2, GLI/CHOP* genes was noted in two tumors (nos. 1 and 3), and amplification of the *PDGFR* gene was detected in tumor number 3. Although deletion of chromosome 1p was seen in two tumors (nos. 1 and 5), no mutation of *BCL10* mapping to 1p22 was noted in the five GBMs (Table 1). Numerical aberration of chromosome 17 was observed in four tumors, and mutation of exon 5 of *TP53* was found in three tumors (nos. 1, 2, and 3) (Table 1).

4. Discussion

Although multicolor-FISH was not available when this study was initiated, cytogenetic characterization of the five GBMs was paralleled by an intensive FISH analysis in which whole chromosome paints and centromere-specific probes spanning the entire human genome, as well as unique sequence probes, were used [7]. All numerical and structural chromosome abnormalities detected by G-banding analysis were confirmed by FISH (Figs. 1 and 2). The CGH also revealed a similar gain and loss of chromosomes and chromosome regions (Fig. 3) (Table 1). All of these results show that overall cytogenetics, CGH, and FISH findings were consistent with each other.

In this study, the immunophenotypes of two tumors, numbers 4 and 5, have been described as the oligodendrocyte-type-2 astrocyte and the astrocyte and/or its precursor, respectively [11]. Several genetic aberrations observed in these tumors, such as +7, −10, −13, −22, and loss of *CDKN2* are consistent with those noted in other studies [2–4]. Like many other epithelial carcinomas, deletion and gain of chromosomes are thought to be common genetic aberrations in GBM [12]. However, three of the five GBMs showed complicated translocations: der(18)t(2;4;12;18) (no. 3), der(X)t(X;10)(q27.1;p12.1) and der(10)t(10;15)(p11.23;q11.2) (no. 4), and der(1)(:1p31→1q44::7q11.23→7qter) (no. 5), which have rarely been reported by others. Furthermore, we found that the homogeneous staining region (hsr) of the long arm of der(18)t(2;4;12;18), containing coamplified genes of *PDGFR*, *CDK4*, *MDM2*, and *GLI/CHOP* (Figs. 1 and 2), defined the region of breakpoint at 10p11–12.1 of der(X)t(X;10)(q27.1;p12.1) and der(10)t(10;15)(p11.23;q11.2) between genetic markers D10S2103 and (D10S637, D10S1962, D10S355), and identified *LIMK1* as one of the genes linked to der(1)(:1p31→1q44::7q11.23→7qter) [7].

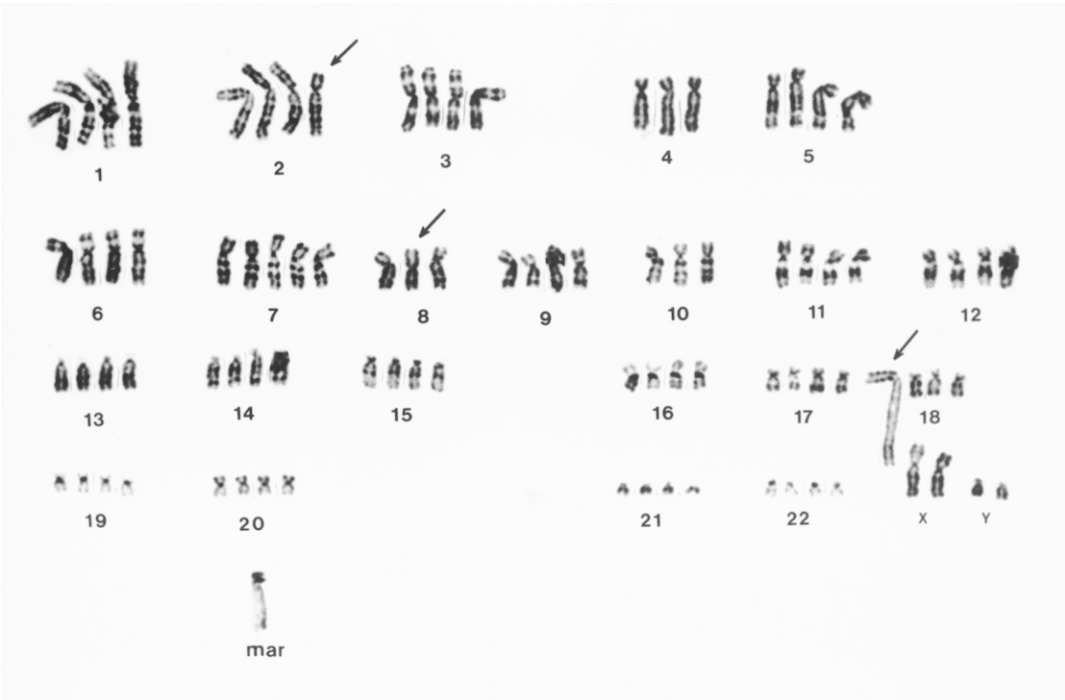

Fig. 1. Illustration of a karyotype of a hyperdiploid cell of tumor number 3 with der(2)t(2;18)(p22q),i(8q), der(18)t(2;4;12;18), and a marker chromosome.

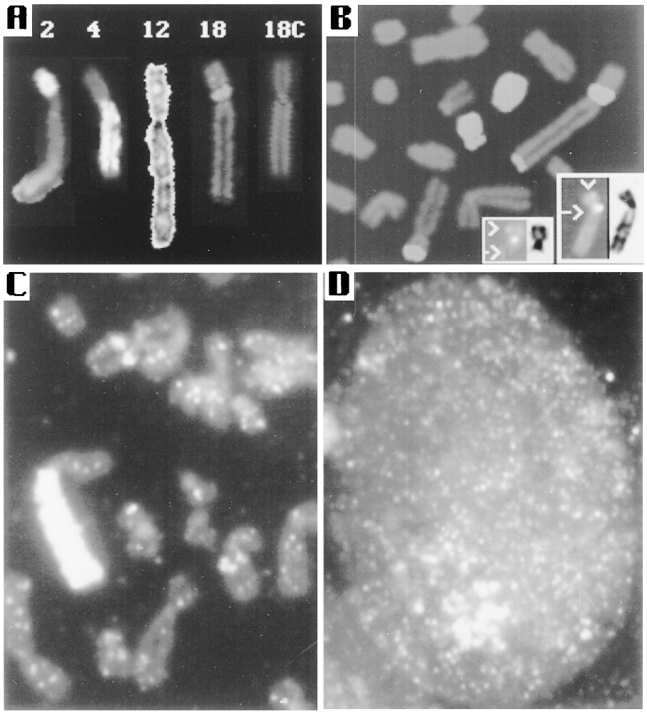

Fig. 2. FISH analysis of the hyperdiploid cells of tumor number 3. (A) A FISH partial karyotype illustrating the chromosomal origin of der(18)t(2;4;12;18) by using whole chromosome paints 2 (light green), 4 (yellow), 12 (dark green), 18 (red), and chromosome 18 centromere-specific probe (red). (B) A FISH partial karyotype demonstrating translocations of genetic materials from chromosome 18q to der(18)t(2;4;12;18) and the p arm of der(2)del(2)(p22q21) and der(2)t(2;18)(p22;q) by means of a whole chromosome paint 18 (FITC detection), chromosome 2 centromere-specific probe (*pBS4D*, arrow, FITC detection), and a unique sequence probe of *DCC* (*40DH1*, arrow head, rhodamine detection). (C, D) Illustration of the amplification domain of the *CDK4* gene (FITC detection) on hsr of der(18)t(2;4;12;18) and on a nucleus, respectively.

These results therefore suggest that there might be other genes involved in these translocations. It would be interesting to use microdissection and molecular cloning to further identify and characterize these genes in our GBM samples.

Chromosome 1 aberrations are seen in a wide range of tumors, including GBM [12]. Frequent losses of alleles from 1p have been found in GBM, meningioma, and neuroblastoma [2–4], suggesting that gene(s) on 1p may be important for the pathogenesis of these tumors. *BCL10* originally isolated from MALT B-cell lymphomas with t(1;14)(p22;q32) is a cellular homolog of the equine herpes virus-2 *E10* gene. These two genes contain an amino-terminal caspase recruitment domain homologous to that found in several apoptotic molecules. *BCL10* and *E10* activated NF-kappaB, but caused apoptosis of 293 cells. Truncated *BCL10* activated NF-kappaB, but did not induce apoptosis. Wild-type *BCL10* suppressed transformation, whereas mutant forms had lost this activity and displayed gain-of-function transforming activity. Mutations of *BCL10* were also detected in other tumor types [5, 6]. On the other hand, *TP53* is also an apoptosis-inducing gene. Mutations of *TP53* have been seen in a wide range of tumors, including GBM [4,13]. In this study, chromosome 17 aberrations were observed in four tumors, mutation of exon 5 of *TP53* was seen in three tumors (Table 1) and overexpression of the mutant *TP53* proteins was noted in tumor numbers 1 and 2 (data not shown). Although deletion of 1p was detected in two GBMs, no mutation of *BCL10* was found in five GBMs (Table 1). These results indicate that *TP53* rather than *BCL10* is involved in the carcinogenic pathways of GBM.

Cathepsin L is a lysosomal cysteine protease whose expression and secretion is induced by malignant transformation, growth factors, and tumor promoters [14]. Many human tumors express high levels of *Cathepsin L*, which is a broad spectrum protease with potent elastase and collagenase activities [15]. The human *Cathepsin L* gene has been cloned and mapped to human chromosome 9q21–q22 [16]. It has been suggested that *Cathepsin L* may be associated with invasion and metastasis of human malignancy. In our study, tumor numbers 4 and 5 showed a gain of copy number of the *Cathepsin L* gene (Table 1). Further studies are necessary to determine if gain or amplification of the *Cathepsin L* gene is clinically significant in GBM.

It is known that phenotypic and karyotypic heterogeneities exist among GBMs at the same stage within the same tumor. Cytogenetic heterogeneity may have some potentially clinical significance, as GBMs with only ND cells are resistant to chemotherapeutic agents such as BCNU and carmustine, while GBMs with HD cells are much more sensitive to these agents [17,18]. In our study, tumor number 3 showed approximately an equal number of ND and HD cells. We isolated both ND and HD cells using a fluorescence-activated-cell sorting vantage, and cultured them separately (data not shown). It would be interesting to determine the biological behavior of these two types of cells by measuring their chemosensitivity to BCNU and carmustine in vitro.

Acknowledgments

We would like to thank Drs. M. Noble and J. L. Darling for providing GBM samples; Drs. T. G. Willis, H. Peng, M. Q. Du and M. Dyer for providing information on the *BCL10* gene; Mrs. P. A. Gorman, Ms. J. A. Williamson, Ms. T. A. Jones, and Ms. S. McGuire for technical assistance; and Dr. D. Sheer for commenting on the early draft of this paper. The initial part of this work was supported by the Royal Society and the Imperial Cancer Research Fund.

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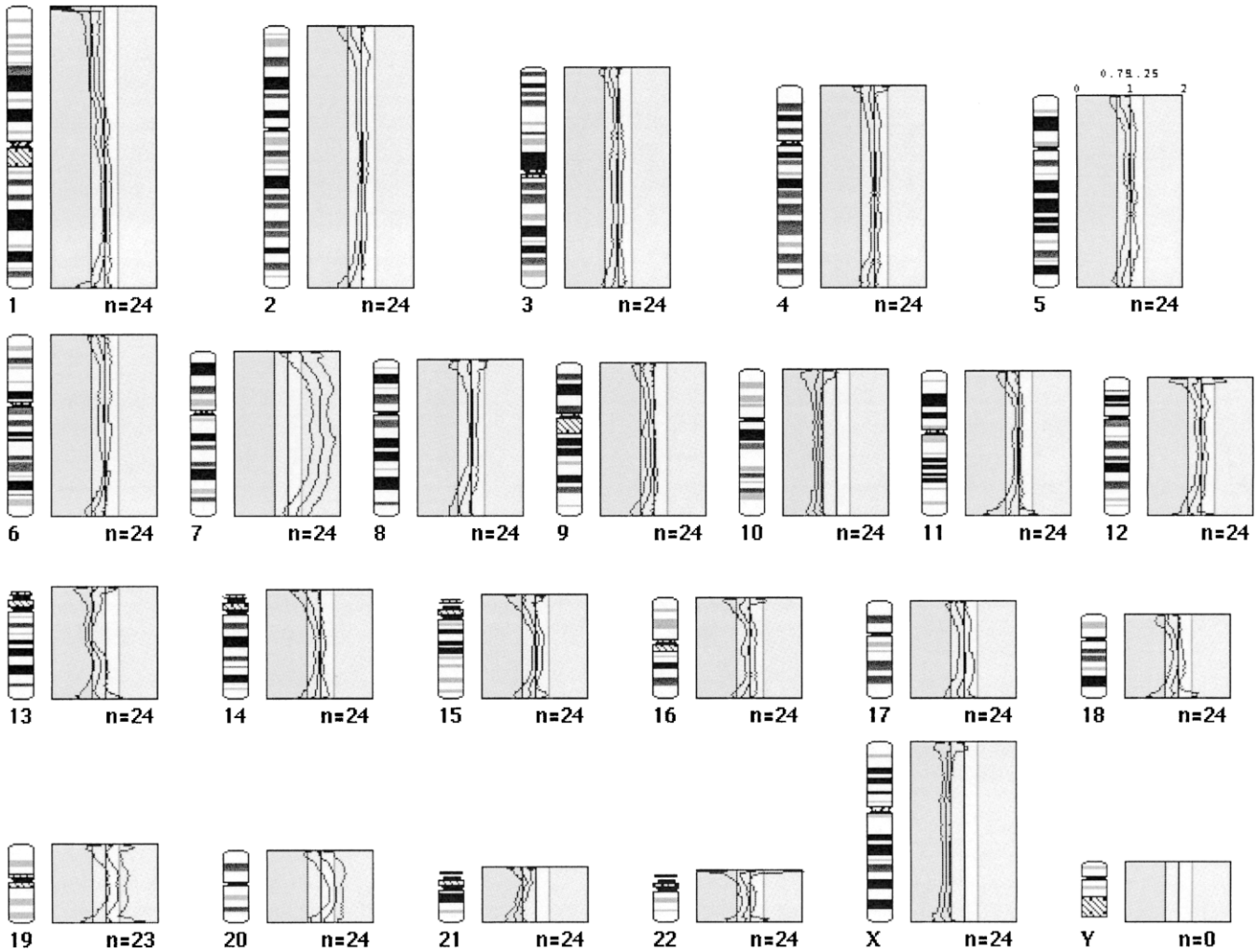

Fig. 3. Illustration of a CGH ratio karyotype of tumor number 5 with genetic imbalance of gains of 7p, 7q, 19, and 20, as well as losses of 1p36–p32, 1q44, 10, 13q13–q21, and 21.

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