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Genetic aberrations in glioblastoma multiforme: translocation of chromosome 10 in an O-2A-like cell line

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Summary We have examined the genetic aberrations in two near-diploid glioblastoma multiforme cell lines that appear to have arisen from different glial lineages. One cell line, Hu-O-2A/Gb1, expresses antigens and metabolic profiles characteristic of the oligodendrocyte-type-2 astrocyte (0-2A) lineage of the rat central nervous system. This line generates, in vitro, cells with characteristics of 0-2A progenitor cells, oligodendrocytes and astrocytes. The second cell line, IN1434, is derived from an astrocyte or a precursor cell restricted to astrocytic differentiation. In Hu-O-2A/Gb1 the sole homologue of chromosome 10 is disrupted at band 10p11–12.1 by translocation with chromosomes X and 15. The translocation breakpoint is localized between genetic markers D10S2103 and [D10S637, D10S1962, D10S355]. Other aberrations include a 5;14 translocation, deletion of the long and short arms of chromosome 16 and loss of one copy of the *CDKN2* gene. IN1434 cells share some cytogenetic abnormalities with Hu-O-2A/Gb1 cells, despite their apparent derivation from a different biological origin, but also have translocations involving the long and short arms of chromosome 1 and the long arm of chromosome 7, and deletion of chromosome 13 at bands 13q12–21.

Keywords: cytogenetics; FISH; molecular genetics; glioblastoma multiforme

Recent studies have suggested that at least two distinct biological lineages may give rise to glioblastoma multiforme (GBM; Noble et al, 1995). By growing glioma-derived cells in tissue culture conditions previously shown to promote in vivo-like development of glial precursor cells, a human GBM cell line that is unambiguously derived from cells of the human oligodendrocyte-type-2 astrocyte (O-2A) lineage has been isolated. Cells from this line (termed Human O-2A/GlioBlastoma 1, or Hu-O-2A/Gb1) express similar antigens, responsiveness to cytokines and small metabolite profiles (as detected by 1H-nuclear magnetic spectroscopic analysis) to primary O-2A progenitor cells isolated from optic nerves of postnatal rats. In contrast, a second new GBM cell line, designated IN1434, differs from both O-2A progenitor cells and Hu-O-2A/Gb1 cells in most characteristics examined and appears to be derived from an astrocytic lineage.

Consistent genetic aberrations found in GBM include deletion or inactivation of the *CDKN2* gene, amplification and/or overexpression of genes such as *EGFR*, *PDGFR* and *GLI*, as well as loss of heterozygosity (LOH) from chromosomes 13, 17 and 22 (Furnari et al, 1995). A critical step in the development of GBM appears to be LOH of part or an entire homologue of chromosome 10, which occurs almost invariably in GBM but not in lower grade tumors. Mutations have been found in GBM in two novel genes, *PTEN*/*MMAC1* at band 10q23.3 (Li et al, 1997; Steck et al, 1997) and *DMBT1* at 10q25.3–26.1 (Mollenhauer et al, 1997). However, none of the other relevant genes on chromosome 10 have been identified. In order to characterize some of the significant genetic aberrations in GBM, and to determine whether GBMs of different lineages have similar aberrations, we have conducted a detailed genetic analysis of the cell lines Hu-O-2A/Gb1 and IN1434.

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MATERIALS AND METHODS

Establishment of cell lines

Hu-O-2A/Gb1 was derived from a sporadic temporal GBM in a 59-year-old male; IN1434 was derived from a sporadic frontal GBM in a 70-year-old male. Both tumours were removed surgically before treatment. The specimens were minced using crossed scalpels and incubated in calcium and magnesium Dulbecco's modified Eagle medium–(DMEM–CMF) medium (ICRF) with 2000 units/ml collagenase (Sigma, UK) at 37°C for at least 1 h. For each tumour, parallel cultures were set up with different conditions: DMEM supplemented with 10% fetal calf serum (FCS) and 25 μg ml^{-1} of gentamicin, or serum-free medium (DMEM–BS; Bottenstein and Sato, 1979) mixed in a 50:50 ratio with astrocyte-conditioned medium (ACM). ACM was prepared from growing purified rat cortical astrocytes in DMEM–BS for 48 h (Noble and Murray, 1984; Noble et al, 1984). Cultures were grown in humidified incubators in 5% carbon dioxide at 37°C, and analysed with cell-type specific markers after two passages of in vitro growth and at various subsequent passages up to passage twenty.

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Immunohistochemical analysis

Cultures were analysed using monoclonal antibodies A2B5 (Eisenbarth et al, 1979), O1 and O4 (Sommer and Schachner, 1981), anti-galactocerebroside antibody (GalC; Ranscht et al, 1982) and anti-glial fibrillary acidic protein (GFAP; Bignami et al, 1972). A2B5 and O4 have been used previously to characterize rat O-2A progenitor cells (Raff et al, 1983; Barnett et al, 1993). O1 and GalC antibodies specifically label oligodendrocytes, while GFAP is a specific marker of astrocytes.

Cytogenetic analysis

The cell lines were harvested at passages 14–16 and metaphase chromosomes prepared using standard techniques. Chromosome aberrations were described according to ISCN (Mitelman, 1995). A structural chromosome rearrangement or chromosome gain had to be detected in at least two metaphase cells, and loss of a chromosome in at least three such cells, to be defined as a clonal aberration.

Fluorescence in situ hybridization

Fluorescence in situ hybridization (FISH) was performed on metaphase spreads according to standard procedures (Senger et al, 1993) using probes shown in Table 1. Chromosome banding was produced by staining with DAPI (diamidino-2-phenylindole-dihydrochloride 0.06 μg ml^{-1})/propidium iodide (0.5 μg ml^{-1}) dissolved in Cityfluor. Preparations were examined with a Zeiss Axioskop microscope equipped with a cooled charged coupled device (CCD) camera (Photometrics), and digitized images analysed using SmartCapture software (Digital Scientific, Cambridge, UK). At least 50 metaphase spreads and 100 interphase nuclei were analysed for each experiment.

Microsatellite analysis

Microsatellite analysis of 74 loci derived from the Généthon set (Gyapay et al, 1994) and covering all chromosomes, was performed on the Hu-O-2A/Gb1 cell line only, as control lymphocytes were unavailable for IN1434. A subset of these loci is shown in Table 2. All experiments were conducted 'blind'. Forty nanograms DNA from cells of passage 5 of Hu-O-2A/Gb1 and control lymphocytes was amplified in 50 μl of reaction solution containing 2.5 μl 1 × standard PCR buffer (Promega), 1.5 mM magnesium chloride, 0.4 μM deoxynucleoside triphosphates (dNTPs), 0.4 μM of each primer, and 0.5 u *Taq* polymerase. The thermal cycling protocol was 94°C (4 min) × 1, 94°C/55°C (1 min each) × 35, and 72°C (10 min) × 1. Products were electrophoresed for 4 h on 8% non-denaturing polyacrylamide gels, and detected by silver staining using standard methods. Allele loss was scored by eye at informative loci if the intensity of one allelic band in the Hu-O-2A/Gb1 was diminished in comparison with the other allele, allowing for the relative intensity of the alleles in the lymphocyte DNA. Novel alleles were scored as replication errors (RERs).

Protein truncation test

For mutation analysis of the DNA mismatch repair genes, total mRNA was extracted from cells of passages 5 and 15 of Hu-O-2A/Gb1 using the Quick Prep Micro kit (Pharmacia), and total cDNA made using the First Strand kit (Pharmacia). Pairs of

Table 1 Probes used for FISH analysis

Probe[a]	Location	Source (Ref.)
CHR...B	Specific paints for all chromosomes	Cambio
Midi	1p36.1	E Volpi
pUC1.77	1cen	HT Cooke and J Hindley
pA3.5	3cen	HF Willard
Coatasome 5	5 paint	Oncor
p4A16	5+9cen	T Hulsebos
YN548	5q21	WF Bodmer
Coatasome 7	7 paint	Oncor
p7E1	7cen	EW Jabs
LIMK1	7q11.23	X Mao (Mao et al, 1997)
4.4	8 cen	A Baldini
pM292	9 cen	A Baldini
CDKN2	9p21.3–22.3	A Kamb
Cathepsin L	9q13	X Mao
cos50A5	9q13	S Chamberlain
AF10[b]	10p12	T Chaplin (Chaplin et al, 1995)
14A7[b](D10S2103)	10p12.1	J Mao (Ma et al, 1996)
JC2139 (D10S637)	10p11.2 (50 cM)	J Mao (Zheng et al, 1994)
6B2[b] (D10S1962)	10p11.2	J Mao (Ma et al, 1996)
JC2075 (D10S355)	10p11.2 (52 cM)	J Mao (Zheng et al, 1994)
pA10RR8	10 cen	P Devilee
sJRH-2b	10 cen	E Volpi
FGF8	10q23	C Dickson
EIF5AP1	10q23.3	A Steinkasserer (Steinkasserer et al, 1995)
Oligo[c]	12 cen	E Volpi
GLI/CHOP	12q13.3q14.1	R Anand
CDK4	12q14	R Anand
MDM2	12q14.3q15	R Anand
66G11	13 cen	N Jankovsky
127B12[d]	13q12	N Jankovsky
104H12	13q12	N Jankovsky
43E12	13q13	N Jankovsky
47A12	13q14	N Jankovsky
97G2	13q14	N Jankovsky
98G5	13q21	N Jankovsky
170D10	13q22	N Jankovsky
87F7	13q32	N Jankovsky
47Q12	13q33	N Jankovsky
44BO6	13q34	N Jankovsky
54CO2	13q34	N Jankovsky
36CO5	13q34	N Jankovsky
pLC11A	11 + 14 cen	HF Willard
cos11	14q24.2	D Bennett
pTRA	15 cen	KH Choo
pSE16	16 cen	HF Willard
CN2.3	16p13.1–13.3	H Durbin
CMAR	16q24.1	H Durbin
D20Z1	20 cen	Cambio
AP2-g	20q13.2	H Hurst
p141/CH22	22 cen	HF Willard
E289	22q11.1	P Scambler
C614	22q11.2	P Scambler
N14C6	22q11.2	P Scambler
N14A2	22q12.1	P Scambler
LIF	22q12.3	P Scambler
KI831	22q12.3	P Scambler
N78F11	22q13.1	P Scambler
N119A3	22q13.2	P Scambler
N14C3	22q13.2–13.3	P Scambler
N66C3	22q13.3	P Scambler
Coatasome X	X paint	Oncor
DXZ1	Xcen	Oncor
YAC 8B7	Xq27.1	R Vatcheva
27D2	Xq28	R Vatcheva

[a]All probes are cosmids except for chromosome paints, centromeres, and probes designated YAC. [b]The exact order of these probes on chromosome 10 is not known. [c]Primers of PRINS. [d]All the probes on chromosome 13 were mapped during the course of this work.

Table 2 Cytogenetic and genetic maps[a] of selected microsatellite markers used in analysis of Hu-O-2A/Gb1

Marker	Cytogenetic	Genetic(cM)
LOH at *APC* gene		
D5S409	5q13q23	116
D5S421	5q13q23	129
D5S404	5q13q23	135
LOH in chromosome 10		
D10S189	10p13	18
D10S211[b]	10p11.2	48
D10S197	10p11	53
D10S220	10q11q21	77
D10S210	10q21q22	95
D10S201	10q22	116
D10S192	10q23q24	142
D10S190	10q25q26	161
D10S186	10q26qter	180
LOH at *MSH2* gene		
D2S119	2p16	75
D2S391	2p16	81
D2S288	2p16	82
D2S123	2p16	85
LOH at *MLH1* gene		
D3S1561	3p23p21	59
D3S1277	3p23p21	60
D3S1611	3p23p21	60
D3S1612	3p23p21	60
RER status		
D1S216	1p21	123
6D6S434	6q21q23	113
D8S255	8p11	69
D11S29	11q23	?
D11S904	11p14p13	40
D11S1313	11p12	68
D11S901	11q12q13	94
D11S968	11q22	165
D17S787	17q24	83
D20S100	20q13	95

[a]Mapping information from Whitehead Institute/MIT and Généthon. [b]Mapping from Trybus et al, 1996. LOH, loss of heterozygosity; RER, replication error.

oligonucleotides were used to amplify specifically cDNA from the *hMSH2*, *hMLH1*, *hPMS2* and *GTBP* genes in two parts and the *hPMS1* gene in three parts (Liu et al, 1996). For the *APC* gene, genomic DNA samples from passages 5 and 15 of Hu-O-2A/Gb1 and passage 16 of IN1434 were used as templates. Exon 15, which comprises the greatest part of the coding region of the *APC* gene, was amplified specifically in four parts. The protein truncation test (PTT) was performed using standard procedures (van der Luijt et al, 1997). Protein products were visualized by eye on the autoradiograph and their lengths compared with molecular weight markers and samples of known wild-type genotypes.

RESULTS

Immunohistochemical phenotypes

Phenotypes were stable during the entire period tested, over 20 passages. Hu-O-2A/Gb1 cells grew in both sets of culture conditions. In medium with FCS, approximately 80% of cells were positive for the astrocytic marker GFAP, but not for any of the other markers tested. When the cells were grown in serum-free medium with ACM, approximately 30–40% of cells were GFAP-positive alone and up to 60% were O4-positive. Approximately 20–25% of the O4-positive cells were also O1- and GalC-positive. Approximately 1% of cells were A2B5-positive alone while 1–2% were both GFAP- and A2B5-positive. Thus, the Hu-O-2A/Gb1 cultures contained the cell types that together comprise the O-2A lineage.

IN1434 cells grew only in culture medium with FCS. Cells expressed GFAP but did not react with A2B5, 04, 01 or GalC antibodies, indicating that they were committed solely to astrocytic differentiation. In addition, the metabolite composition of IN1434 cells did not resemble that of O-2A progenitor cells, as analysed by proton nuclear magnetic resonance (^{1}H-NMR) spectroscopy (M Noble et al, unpublished observations). Therefore, all analyses conducted thus far are most consistent with the view that Hu-O-2A/Gb1 cells and IN1434 cells are derived from different glial lineages.

Genetic aberrations

Hu-O-2A/Gb1

Cultures of Hu-O-2A/Gb1 grown in serum-free medium with ACM were used for all subsequent studies. The chromosomes in 50 banded metaphase spreads were analysed and 11 karyotyped. A chromosome number of 42–78, with a mode of 45, was present. Clonal numerical aberrations were loss of chromosomes 6, 10, 13, 15, 16, 19 and 21, and gain of chromosomes 7 and 8. Every cell had lost one homologue of chromosome 10, and the remaining homologue had additional material on the short arm. Other structural aberrations include 14p+, del(5)(p15), and several markers (Figure 1). Seventy-eight per cent of cells showed trisomy 7 and 22% showed tetrasomy 7. FISH and microsatellite analysis were then used to characterize these aberrations in detail (see below). Each of these approaches gave results that were consistent with each other and with the cytogenetic analysis.

Translocation of chromosome 10

Nine microsatellite loci mapping to chromosome 10 (Table 2) showed allele loss along the entire chromosome (Figure 2 A–C). The sole chromosome 10 was translocated at band 10p11–12 to chromosomes 15 and X (Figure 2 D–G, Figure 3). FISH showed that probes AF10 and 14A7 (at 10p12) were translocated to a derivative X chromosome at band Xq27–28, while probes 6B2, JC2139, and JC2075 (at 10p11.23), and pA10RR8 (10cen), sJRH-2b (10cen), FGF8 (10q23) and EIF5AP1 (10q23.3) remained on the derivative chromosome 10. The region of chromosome 15 from band 15q11.2–15qter was shown by FISH to translocate to band 10p11–12 of the derivative chromosome 10. Probe pTRA-20 (15cen) was absent from this chromosome. Probes YAC 8B7 (Xq27.1) and 27D2 (Xq28) were not present on the derivative X chromosome or any other chromosome. The X chromosome paint hybridized only to the derivative X chromosome. The region Xq27-qter thus appears to be entirely lost from the genome.

dic(5;14)

In addition to an apparently normal homologue of chromosome 5 and a chromosome 5 with a deletion of band 5p15, most cells (92%) contained a dicentric chromosome with material from bands 5pter–5q11–21 translocated onto the short arm of chromosome 14 (Figure 2H). Chromosome 5 material was not detected by FISH on any other chromosome. Probe YN548 (5q21) was not present on this chromosome. No mutation was detected in exon 15 of the APC gene using the PTT (data not shown).

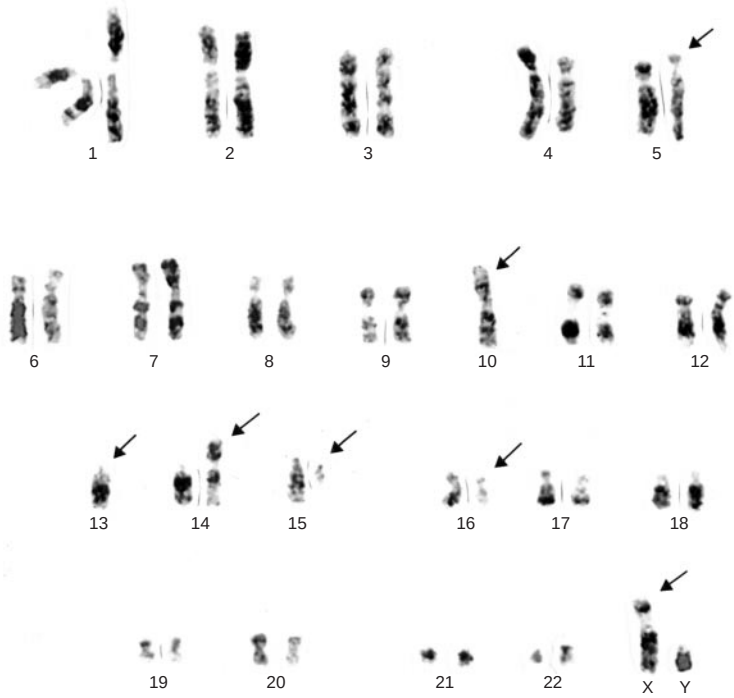

Figure 1 G-banded karyotype of Hu-O-2A/Gb1. Arrows indicate chromosomes discussed in the text

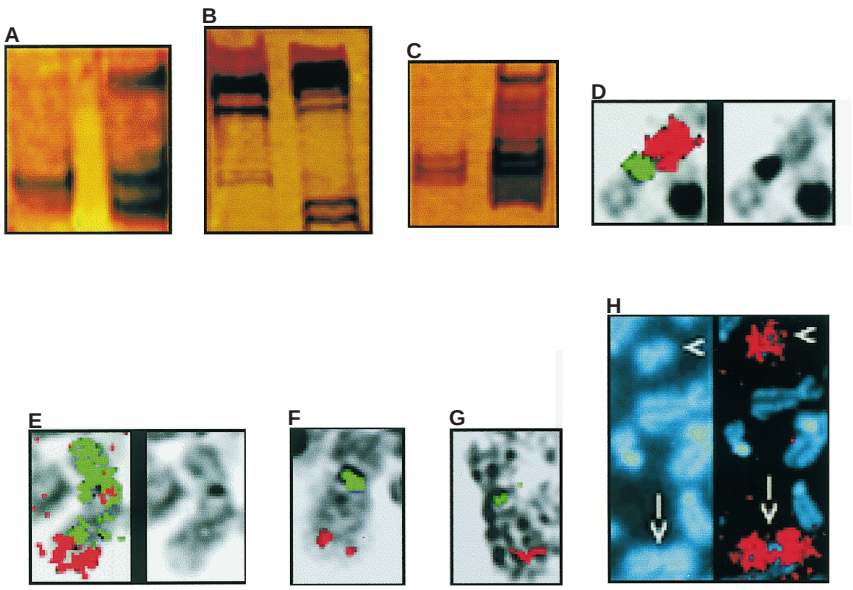

Figure 2 (**A–C**) Microsatellite analysis of chromosome 10 in Hu-O-2A/Gb1. Left: tumour DNA. Right: normal DNA. (**A**) D10S189 (10pter–p13). (**B**) D10S210 (10q21–22). (**C**) D10S192 (10q23–24). (**D–K**) FISH analysis of Hu-O-2A/Gb1. (**D**) Probes pA10RR8 (10 cen, FITC) and CHR15B (15 paint, Texas red) on the der(10) chromosome. (**E**) Coatasome X (X paint, FITC) and CHR10B (10 paint, Texas red) on the der(X) chromosome. (**F**) Cosmid 14A7 (10p12.1, Texas red) and DXZI (X cen, FITC) on the der(X) chromosome. (**G**) YAC8B7 (Xq27.1, Texas red) and DXZ1 (X cen, FITC) on the der(X) chromosome. (**H**) CHR16B (16 paint, Texas red) on partial metaphase spread stained with DAPI, showing normal and deleted homologues of chromosome 16

del(16)(p;q)

One apparently normal homologue of chromosome 16 was present in every cell, as well as a homologue with both arms deleted (Figure 2I). Probes CN2.3 (16p13.1–13.3) and CMAR (16q24.1) were absent from this chromosome. Microsatellite analysis of eight loci mapping to chromosome bands 16p13.3–q21 showed retention of both alleles at each locus.

Other chromosomes

FISH analysis showed that 57% of cells had lost a copy of the *CDKN2* (p16) gene (9p21–22). FISH analysis using other probes shown in Table 1 failed to detect any abnormalities. Analysis of microsatellite loci (Table 2) in Hu-O-2A/Gb1 cells at passage 5 showed that the tumour was RER–. No allele loss occurred at microsatellite markers close to the *hMSH2* and *hMLH1* genes.

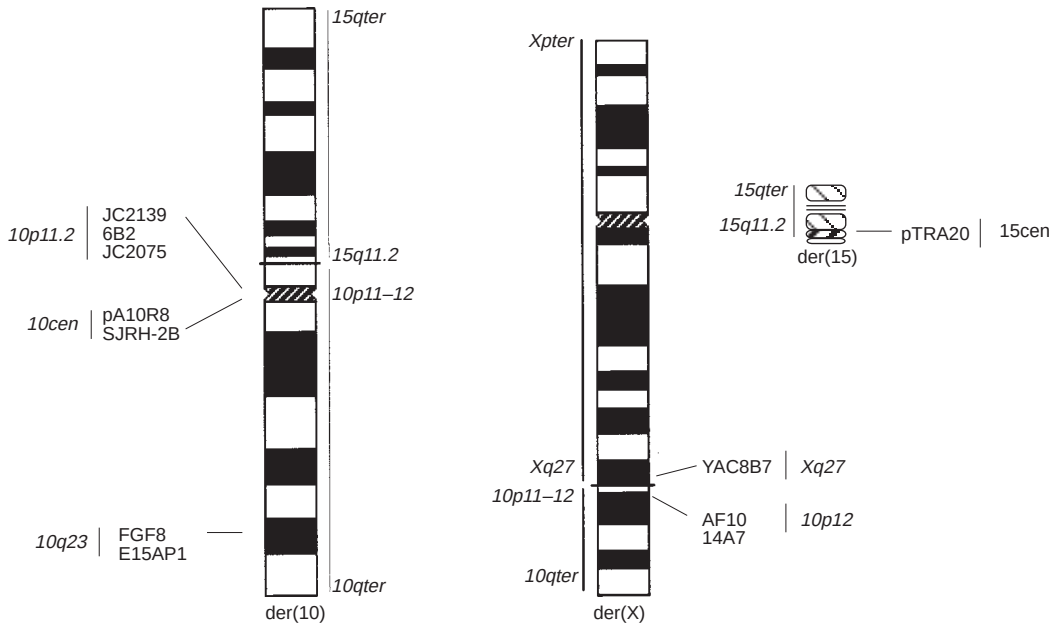

Figure 3 Diagram of translocations involving chromosome 10 in Hu-O-2A/Gb1. FISH results are shown on the derivative chromosomes

Subsequently, the PTT was used to search for truncating mutations at loci *hMSH2*, *hMLH1*, *hPMS1*, *hPMS2* and *GTBP*. All products amplified from the mRNA of the tumour cells at passage 15 were of wild-type length at these five loci. PTT analysis showed that Hu-O-2A/Gb1 cells did not contain a truncated protein which would indicate a nonsense or frameshift mutation. No abnormalities were detected in the remaining microsatellite loci.

IN1434

Forty metaphase spreads had a chromosome number of 41–94, with a modal chromosome number of 46 (Figure 4). Eighty-eight per cent of cells were near-diploid cells and 12% were hyperdiploid. Cytogenetic and FISH analysis revealed clonal gains of chromosomes 5, 7, 18, 19 and 20, and clonal losses of chromosomes 6, 8, 10 and 22. FISH and microsatellite analysis were again used to characterize these aberrations in detail, and gave consistent results.

t(1;7)

Each cell had one normal chromosome 1 and a rearranged chromosome 1 with chromosome 7 material translocated to both the long and short arms (Figure 5 A–D). FISH analysis showed that probe p7E1 (7cen) localized to the telomeric region of the long arm of the rearranged chromosome 1. A cosmid probe for the *LIMK1* gene (7q11.23) was found on the telomeric regions of both long and short arms of chromosome 1. This chromosome is thus described as der(1)t(1;7)(p31;q11.23)/(q44;q11).

del(13)

One normal chromosome 13 and a rearranged chromosome 13 were present. Two-colour FISH was performed with 13 cosmid probes covering the entire chromosome 13 (Figure 5 E–G). Cosmids 127B12 (13q12), 43E12 (13q13), 47A12 and 97Q (13q14) were found to be deleted from the rearranged chromosome, indicating an interstitial deletion.

Other loci

FISH analysis with all other probes listed in Table 1 showed normal patterns. No *APC* mutation was detected using the PTT. All other genomic regions tested appeared normal.

DISCUSSION

We have characterized genetic aberrations in two near-diploid GBM cell lines of different cellular origins. The Hu-O-2A/Gb1 cells grown in serum-free medium with ACM express antigens and have a differentiation potential and ^{1}H-NMR profile characteristic of the oligodendrocyte-type 2 astrocyte (O-2A) progenitor cell lineage of the rat (Noble et al, 1995). IN1434 cells, in contrast, appear to derive from a lineage committed solely to astrocytic differentiation. Several cytogenetic aberrations in these lines have been noted in other studies of GBM (Mitelman, 1994; Debiec-Rychter et al, 1995). Of particular interest, however, are a translocation of the sole copy of chromosome 10 in Hu-O-2A/Gb1 and a complex rearrangement involving chromosomes 1 and 7 in IN1434.

Previous studies suggest the presence on chromosome 10 of tumour suppressor genes besides *PTEN*/*MMCA1* which are inactivated during progression to GBM (Karlbom et al, 1993; Ichimura et al, 1998). Both cell lines examined here had lost one homologue of chromosome 10. The remaining homologue in Hu-O-2A/Gb1 is translocated at band 10p11.2 to chromosomes 15 and X. The rearrangement is defined as follows: 10pter–[AF10, 14A7] – breakpoint – [JC2139, 6B2, JC2075] – cen. The *AF10* gene which is disrupted in acute leukemia (Chaplin et al, 1995) appears unaffected in Hu-O-2A/Gb1. It is not yet clear whether genetic material has been lost or a gene disrupted by this translocation. If so, the cell line may be useful for cloning a tumour suppressor gene. The same region also shows LOH in prostate cancer (Trybus et al, 1996).

The X;10 translocation in Hu-O-2A/Gb1 also results in deletion of the region Xq27–qter from the genome. Although loss of an

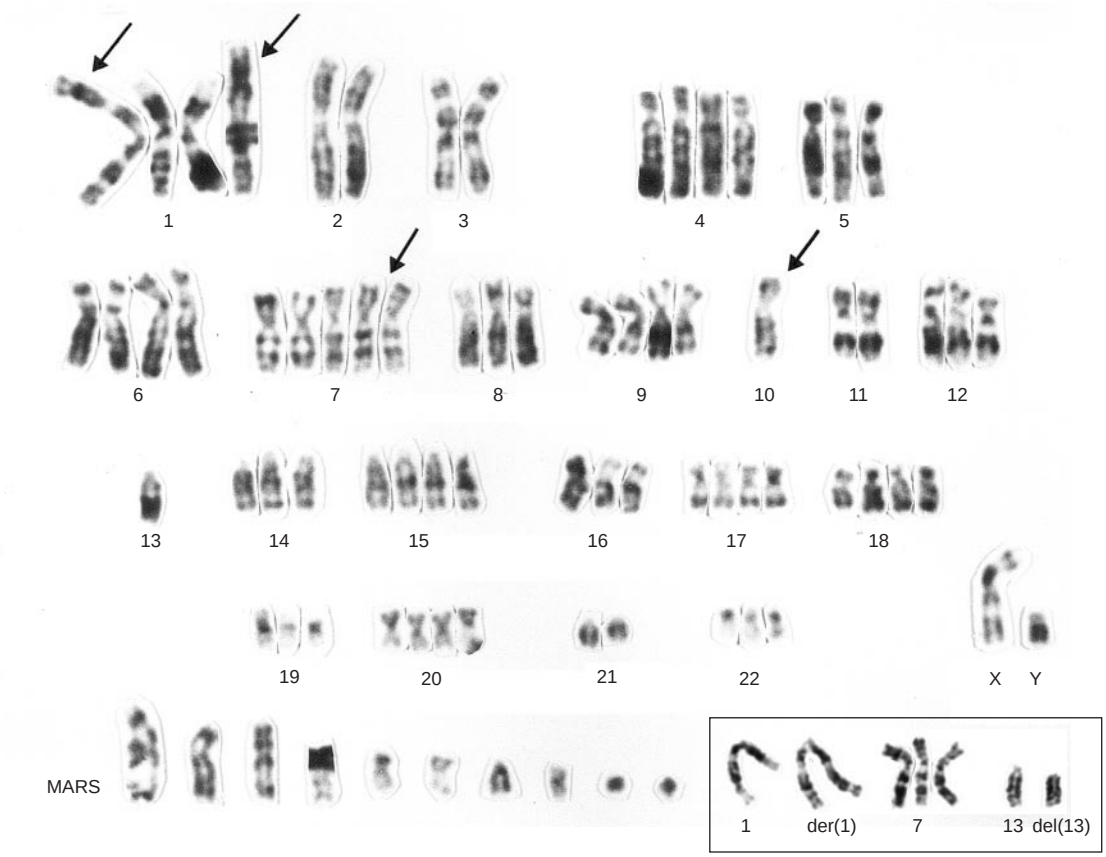

Figure 4 G-banded karyotype of IN1434. Arrows indicate chromosomes discussed in the text. Inset: partial karyotype showing chromosomes 1, der(1), 7, 13 and del(13)

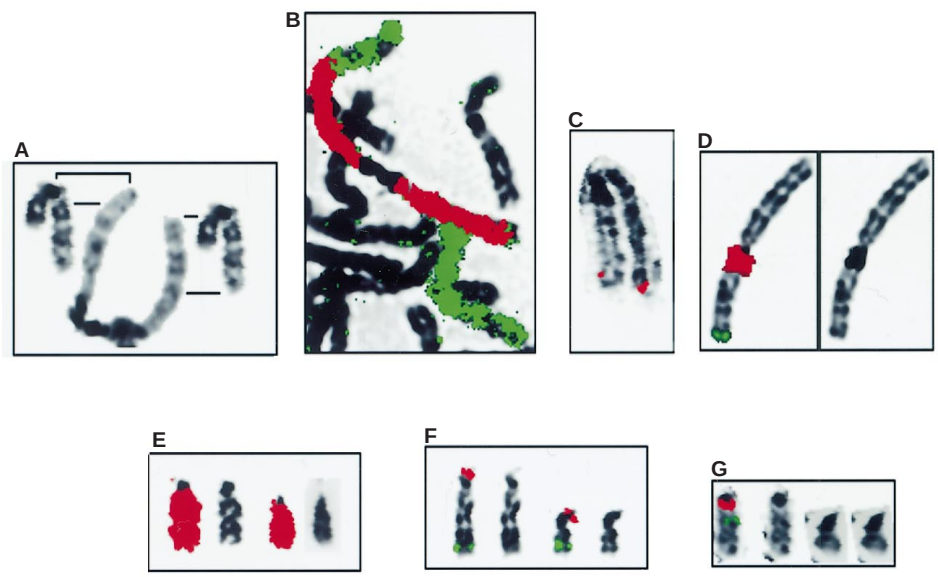

Figure 5 (**A–D**) FISH analysis of the der(1) in IN1434. (**A**) G-banded der(1) chromosome and chromosome 7, showing regions of chromosome 7 translocated to the der(1). (**B**) CHR1B (1 paint, rhodamine) and CHR7B (7 paint, FITC) showing translocation of chromosome 7 to 1p and 1q. (**C**) Cosmid LIMK1 (7q11.23, rhodamine) hybridizing to both short and long arms of the der(1). (**D**) Probes p7E1 (7 cen, FITC) and pUC1.77 (1 cen, rhodamine) showing 7 cen sequences at the distal long arm region of the der(1). (**E–G**) FISH analysis of del(13) in IN1434. (**E**) CHR13B (13 paint, rhodamine) showing one smaller chromosome 13. (**F**) Cosmids 66G11 (13 cen, rhodamine) and 37Q12 (13q33, FITC) present on both homologues of chromosome 13. (**G**) Cosmids 127B12 (13q12, Texas red) and 14A12 (13q14, FITC) present on the normal chromosome 13 but not on the del(13)

entire sex chromosome is common in gliomas, we are unaware of nullisomy for this region of the X chromosome being described previously in GBM.

In Hu-O-2A/Gb1, a region of chromosome 5 from bands 5pter-5q11–21 was translocated to the short arm of chromosome 14, resulting in a dicentric chromosome. Two intact copies of chromosome 5 were also present. Most cases of Turcot's syndrome, which includes gastrointestinal tumours and GBM, result from germ-line mutations of the APC tumour suppressor gene at band 5q21 (Hamilton et al, 1995). APC is therefore a prime candidate tumour suppressor gene for GBM. Microsatellite analysis did not detect allele loss at markers on chromosome 5, and no *APC* mutation was detected by PTT. We have no evidence, therefore, that somatic *APC* mutations were involved in the pathogenesis of Hu-O-2A/Gb1.

We tested Hu-O-2A/Gb1 cells for mutations of five DNA mismatch repair (MMR) genes and for microsatellite instability, since a subset of patients with Turcot's syndrome have germ-line MMR mutations. Neither MMR mutations nor microsatellite instability were detected. Recently other studies failed to detect microsatellite instability in gliomas (Amariglio et al, 1995; Ritland et al, 1995), suggesting that the tumours of Turcot's syndrome may be unrepresentative of sporadic cases.

FISH analysis showed that 57% of Hu-O-2A/Gb1 cells had lost one homologue of a cdk4 inhibitor, CDKN2A, which negatively regulates cell cycle progression. Homozygous deletion, mutation and loss of expression of the *CDKN2* gene are among the most common genetic aberrations in AA and GBM (Kamb et al, 1994; Nobori et al, 1994).

Both Hu-O-2A/Gb1 and IN1434 had trisomy 7 in the majority of cells and tetrasomy 7 in the remaining cells. Trisomy 7 is another common aberration in gliomas. An investigation using comparative genomic hybridization has found that gain of 7q in particular is the most frequent event detected in adult low-grade astrocytomas, suggesting that genes on 7q play an early role in tumour development (Schröck et al, 1996). However, since trisomy 7 is also found in non-neoplastic brain cells, its relevance to glioma development is still debatable (Johansson et al, 1993). No amplification of the *EGFR* gene (7p12) was found by FISH analysis in Hu-O-2A/Gb1 or IN1434 (data not shown). However, high expression of another gene on chromosome 7, *LIMK1* (7q11.2), was observed in paraffin-embedded tumour tissue from which Hu-O-2A/Gb1 was derived (Gutowski et al, in preparation).

Each cell in IN1434 had a complex rearrangement in which chromosome 7 material, including the *LIMK1* gene, was translocated to a derivative chromosome 1 at both the long and short arms. This rearrangement can be described as der(1)t(1;7)(p31;q11.23)/(q44;q11). High expression of *LIMK1* mRNA was also observed in this line (Gutowski et al, in preparation). The expression profiles of *LIMK1* in the two cell lines are consistent with a generally high expression of the gene in normal brain tissue (Proschel et al, 1995).

Cytogenetic and FISH analysis showed that IN1434 had an interstitial deletion of chromosome 13 involving bands 13q12–14. These findings confirm previous studies showing deletion of chromosome 13 in AA and GBM (Kim et al, 1995). The *RB1* gene at band 13q14 is a prime candidate target for the deletion, as it is commonly mutated in astrocytic gliomas. Interestingly, mutation of *RB1* has been found to correlate inversely with mutations of the *CDKN2* gene in gliomas, confirming that perturbation of the cell cycle regulatory pathway that includes *RB1*, *CDKN2* and *CDK4* is a critical step in glioma development (Ueki et al, 1996).

The findings presented here raise intriguing questions concerning the relationship between genetic aberrations and the cellular lineages of tumors. Hu-O2A/Gb1 cells, which by a variety of stringent biological criteria appear to be derived from oligodendrocyte precursors, do not show the cytogenetic aberrations characteristically observed in oligodendrogliomas or oligoastrocytomas such as LOH on 19q and 1p (Kraus, 1995), but rather those seen with great frequencies in other GBMs. Moreover, GBM-associated aberrations also were found in IN1434 cells that appear to be derived from an astrocytic lineage. Answers to these questions will require genetic studies on further gliomas which have been unambiguously assigned to particular lineages.

The extent to which the O-2A lineage contributes to glioma formation itself remains to be determined. However, reports that such tumors frequently express sulfatide and/or GalC (Jennemann et al, 1990; Singh et al 1994), and that some glioma-derived cell lines can be induced to express GalC or mRNA for proteolipid protein (Gillaspy et al, 1993; Kashima et al, 1993), are consistent with the view that the Hu-O-2A/Gb1 cell line is not a unique example of an O-2A tumour.

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