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Laboratory Investigation

Alleletyping of an oligodendrocyte-type-2 astrocyte lineage derive from a human glioblastoma multiforme

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Summary

We have conducted alleletyping of two novel cell lines derived from glioblastoma multiforme, which appear to have arisen from different glial lineages, by using 76 fluorescently labeled oligonucleotide primers amplifying microsatellite loci covering the entire human genome. 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 cell line generated, *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. Hu-O-2A/Gb1 cells show allelic losses of loci on chromosomes 2, 5, 6, 7, 8, 9, 10, 11, 13, 15, 16, 17, 20 and 21. IN1434 cells are likely to have allelic losses of loci on chromosomes 1, 3, 8 and 10, although no control DNA is available for this cell line. These results, for the first time, provide a detailed information of the molecular genetic defects occurring in Hu-O-2A/Gb1 and IN1434.

Introduction

Gliomas are the most common primary neoplasms occurring in the central nervous system in adults. They are classified according to their cellular morphology as astrocytomas, oligodendrogliomas, or mixed in composition [1]. Traditionally, astrocytomas are subclassified into low-grade astrocytoma, anaplastic astrocytoma (AA) and glioblastoma multiforme (GBM) [1]. Currently they are graded to 0–4 criteria according to the presence or absence of four morphological criteria: nuclear atypia, mitoses, endothelial proliferation, and necrosis [2].

Recent studies on cells derived from gliomas have suggested that at least two distinct biological lineages may give rise to GBM [3–5], thus offering the opportunity to initiate comparison of genetic characteristics of tumors of differing biological origin. By growing glioma-derived cells in tissue culture conditions previously shown to promote *in vivo*-like development of glial precursor cells, it has been possible to isolate a human GBM cell line that is unambiguously derived from cells of the human oligodendrocyte-type-2 astrocyte (O-2A) lineage. Cells isolated from this GBM 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 ^{1}H-nuclear magnetic spectroscopic analysis) to primary O-2A progenitor cells, isolated from optic nerves of postnatal rats. In contrast, other GBMs that have been examined in this series differ from both O-2A progenitor cells and Hu-O-2A/Gb1 cells in most characteristics examined, and appear by all of these criteria to be derived from a separate glial lineage.

Much evidence for genetic aberrations linked to the initiation and progression of gliomas has been gathered [6–9]. For example, in astrocytic tumors, deletion or mutation of tumor suppressor genes such as the cyclin-dependent kinase-2 gene (CDKN2), the phosphatase and tensin homologue deleted on chromosome 10 (PTEN) and the mutated in multiple advanced cancers 1 (MMCA1) as well as amplification and/or

overexpression of genes such as epidermal growth factor receptor (EGFR), platelet-derived growth factor receptor (PDGFR), murine double minute 2 (MDM2), and gli have been noted in AA and GBM [10–12]. Loss of genetic materials from chromosomes 1, 10, 11, 13, 17, 19 and 22 have also been observed in all grades of astrocytomas [13–17], which suggest the presence of some other genes on these chromosomes that are likely to be associated with the development of astrocytic tumors. However, it is unclear whether there are specific genetic aberrations in Hu- O-2A/Gb1 cells and whether GBMs of apparently different cellular origins express similar genetic abnormalities. To investigate these subjects, we have conducted alleletyping of two tow-passage cell lines derived from GBM.

Materials and methods

Establishment of cell lines

Hu-O-2A/Gb1 cells (IN1789) were derived from a sporadic temporal GBM in a 59 year old male; IN1434 cells were derived from a sporadic frontal GBM in a 70 year old male. Both tumors were removed surgically before treatment. The specimens were minced using crossed scalpels and incubated in DMEM-CMF medium (ICRF) with 2000 units/ml collagenase (Sigma, UK) at 37°C for at least 1 h. IN1434 cells were grown in DMEM supplemented with 10% fetal calf serum. In contrast, Hu-O-2A/Gb1 cells were isolated by growth of cells in serum-free medium (DMEM-BS) mixed in a 50 : 50 ratio with astrocyte-conditioned medium. Astrocyte- conditioned medium was prepared from growing purified rat cortical astrocytes in DMEM-BS for 48 h. Cultures were grown in humidified incubators in 5% CO_2 at 37°C, and analyzed with cell-type specific markers after two passages of *in vitro* growth and at various subsequent passages up to passage twenty [3–5].

Immunohistochemical analysis

Cultures were analyzed using monoclonal antibodies A2B5 [18], O1 and O4 [19], anti-galactocerebroside (GalC) [20] and glial fibrillary acidic protein (GFAP) [21]. A2B5 and O4 have been used previously to characterize rat O-2A progenitor cells [22–23]. O1 and GalC antibodies specifically label oligodendrocytes, while GFAP is a specific marker of astrocytes [3–5].

Primers and polymerase chain reaction (PCR)

DNA was extracted by standard methods from cells of passage 5 of Hu-O-2A/Gb1, control lymphocytes from same patient, and passage 16 of IN1434. Amplification of 76 microsatellite loci distributed throughout the entire human genome was conducted by using fluorescently labeled oligonucleotide primers in a omnigene thermal cycler (Hybaid) (GENSET) (Table 1). The reaction mixture (total 15 µl) consisted of 1.5 µl of 10x PCR buffer, 25 mM $MgCl_2$, 1.5 µl (2 mM each nucleotide) of dNTPs, 0.3 µl (5 OD U/ml) of each primer, 0.15 µl (10 mg/ml) of BSA, 0.1 µl of Taq polymerase, 9.25 µl of water, and 1 µl of DNA. PCR conditions were: 35–40 cycles of denaturation at 94°C for 1 min, annealing at the appropriate temperature (50–60°C) for 1 min, and extension at 72°C for 1 min, followed by a final extension for 5 min at 72°C.

Electrophoresis and data analysis

The PCR products were analyzed on 4.5% polyacrylamide denaturing gels (Anachem, Leciester) in 1xTBE (89 mM Tris-borate, 2 mM EDTA pH 8.0) buffer using an ABI 377 automated fluorescent DNA sequencer (Applied Biosystems, Foster City, California, USA), which has a four-color detection system. Two µl of each PCR reaction was combined with 2 µl formamide and 0.5 µl of a TAMRA fluorescent size marker (GS500, Applied Biosystems, Foster City, California, USA). This mix was denatured for 6 min at 94°C after which 1.5 µl was loaded into each well on a prewarmed gel. Gels were run for 2.5 h at 200 watts power, 60 amps current, 2900 volts voltage, 51°C temperature, and scan rate of 2400 scans/hour. Whilst the samples were undergoing electrophoresis the fluorescence was detected in the laser scanning region using filter set C and was collected and stored using the GeneScan Collection Software 1.1 (Applied Biosystems, Foster City, California, USA). The fluorescent gel data collected during the run was analyzed using GeneScan Analysis Software 2.0.2 (Applied Biosystems, Foster City, California, USA) at the end of the run. Each fluorescent peak was quantitated in terms of allele, peak height and peak area. The results were then imported into Genotyper (version 1.1)

Table 1. A summary of alleletyping of two GBM cell lines

Marker	Genetic (cM)	Cytogenetic	LOH[a] in Hu-O-2A	Allele[b] in IN1434	Marker	Genetic (cM)	Cytogenetic	LOH[a] in Hu-O-2A	Allele[b] in IN1434
D1S214	16.4	1p36.31-36.21	—	18	D10S189	17.3	10p15-13	1	ND
D1S197	78.3	1p33	—	6	D10S197	50.5	10p13-12.1	1	ND
D1S2691	197	1q23.3-24.2	—	4	D10S220	72.5	10q11-21	1	ND
D1S2800	256.1	1q41-42.13	—	2,8	D10S539	75.4	10q21.1	N	3
D2S162	21.3	2p25.1	—	13	D10S210	89.4	10q21.1-22.1	1	ND
D2S391	73.8	2p16.3-14	N	6,7	D10S201	105.9	10q23.2-23.3	1	ND
D2S112	145.8	2q21.1	10[c]	3,7	D10S574	124.4	10q23.31-25.1	12	9
D2S206	248.3	2q37.2-37.3	—	11	D10S192	131.2	10q24.2-25.1	1	ND
D3S1597	24.1	3p24.3-24.1	—	11	D10S190	147.2	10q25.3-26.13	1	ND
D3S1309	157.4	3q22-23	—	7	D10S186	165.6	10q26.2-26.3	N	ND
D3S1262	207.2	3q26.3-27	N	3	D10S212	180.7	10q26.3	3	ND
D4S1599	22	4p16.1-15.32	—	6,7	D11S902	24.7	11p15.3-15.2	7	7,8
D4S1551	38.3	4p15.32-15.1	—	4,6	D11S1328	128.4	11q23.2-23.3	8	4,5
D4S1586	146.4	4q28.3-31.21	—	2,6	D12S94	1.1	12p13	N	8,9
D5S432	21.4	5p15.31-14.2	—	13	D12S1635	66.8	12q11-13	—	6,7
D5S1962	82.8	5q13.2-14.1	—	6,7	D12S105	118.9	12q22	N	2,5
D5S394	179.8	5q35.1-35.2	6[c]	3,6	D13S291	43.7	13q14.12-14.13	2	2
D6S260	29.6	6p23-22.3	14	16,22	D13S158	86.9	13q22.3-32.1	5	2,5
D6S273	44.9	6p22.1-21.31	8	1,5	D14S274	53.8	14q22.1-23.3	N	8,11
D6S468	108	6q21	5	8	D14S277	68	14q23.3-24.1	—	9
D6S264	179.1	6q27	N	4,9	D15S144	25.3	15q13.3	N	8
D7S517	7.8	7p22.3	—	6,10	D15S158	106	15q26.2-26.3	5	2,9
D7S516	42.1	7p15.2-14.3	—	3	D16S423	8.4	16p13.3	N	ND
D7S492	100.5	7q21.11	—	6	D16S519	19.7	16p13.3-13.13	—	2
D7S489	101	7q21.11	—	1,6	D16S420	43.2	16p12.3-12.1	—	ND
D7S669	105	7q21.13	—	3,5	D16S419	65.8	16q12.2-22.1	2[c]	2,7
D7S440	108–112	7q21.12-21.3	9[c]	8,9	D16S496	84.4	16q22.1-23.1	N	ND
D7S518	112.9	7q21.13-21.3	10	6,8	D16S516	108	16q23.1-24.1	4	ND
D7S657	120	7q21.3-22.1	—	1,2	D17S786	18.1	17p13.2-13.1	—	9
D7S496	120.7	7q21.3-22.1	N	4,7	D17S802	108.2	17q24.3-25.3	5[c]	8,10
D7S466	131.46	7q22.1-22.2	—	3	D18S53	40.4	18p11.23-11.22	—	11
D7S471	142–143	7q31.31-31.32	N	9,19	D18S64	83	18q21.2-21.32	—	10,11
D7S530	157	7q31.33-34	N	5,6	D19S424	10.8	19p13.3	—	9,10
D8S298	42.7	8p21.2-12	4[c]	4	D19S418	97.5	19q13.43	—	4,6
D8S281	122.6	8q23.3-24.12	7	6	D20S95	16.4	20p12.3-12.2	—	5,9
D9S259	45.1	9p13.3-13.2	2	8	D20S109	73.6	20q12-13.12	12[c]	10,12
D9S290	141.1	9q34.12-34.3	—	4,9	D21S1260	51.6	21q22.3-qter	4[c]	9,12
D10S249	0	10p15.3	2	4	D22S315	16.2	22q11.2	—	10,13

[a] LOH of allele 1 to n; N: non-informative; '—': informative but no LOH. [b] No matched control DNA available in this cell line, only each allele is shown; ND: not done. [c] AI of allele 1 to n.

(Applied Biosystems, Foster City, California, USA) for further analysis and printing.

Assessment of loss of heterozygosity (LOH) and allelic imbalance (AI) by calculating allele ratios

The comparison of the ratios between Hu-O-2A/Gb1 and its control was done using two formulas for calculation: (1) T1 : T2/N1 : N2, and (2) T2 : T1/N2 : N1. In these formulas T1 and N1 are the area under peak (AUP) of the smaller allele, and T2 and N2 are AUP of the larger allele. Formula 1 was used to calculate the ratio of the smaller allele, while formula 2 was used to calculate the ratio of the larger allele. For ratios greater than 1, the reciprocal of ratio is calculated to give a value between 0.00 and 1.00. A value of 0.5 or less was assigned as indicative of LOH, and a value between 0.5 and 1.0 was assigned as indicative of AI.

Results

Immunohistochemical phenotypes

Phenotypes were stable during the entire period tested. However, Hu-O-2A/Gb1 varied according to the culture conditions. In medium with fetal calf serum, approximately 80% of cells were GFAP positive, but none of the other antibodies gave a positive reaction. When the cells were grown in serum free medium as described previously, 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-0-2A/Gb1 cultures contained the several cell types that together comprise the O-2A lineage [4–5].

In contrast, cultures of IN1434 cells expressed GFAP but did not react with A2BS, O4, O1 or GalC antibodies, indicating that these cells were committed solely to astrocytic lineage. The astrocytic phenotype of IN1434 cells was maintained when these cells were grown in the same conditions as the Hu-O-2A/Gb1 cells, indicating that their antigenic phenotype was not solely a reflection of their original isolation in serum-containing medium. In addition, the metabolite composition of IN1434 cells did not resemble that of O-2A progenitor cells, as analyzed by ^{1}H-NMR spectroscopy. Therefore, all analyses conducted thus far are consistent with the view that Hu-O-2A/Gb1 cells and IN1434 cells are derived from different glial lineages [4–5].

Genotypes

Genetic and cytogenetic maps of 76 microsatellite loci used in this study are shown in Table 1, which is based on published data [24–29]. In Hu-O-2A/Gb1 cells, 62 of 76 markers were found to be heterozygous (informative). Of 62 informative markers, allelic losses including LOH and AI were shown on loci of chromosomes 2, 5, 6, 7, 8, 9, 10, 11, 13, 15, 16, 17, 20, and 21 (Table 1) (Figures 1 and 2). IN IN1434, of 67 markers used, 24 were homozygous and 43 were heterozygous. Allelic losses appeared to present on loci of chromosomes 1, 3, 8, and 10 (Table 1) (Figure 2).

Discussion

We have conducted alleletyping of two near-diploid GBM cell lines of different cellular origins.

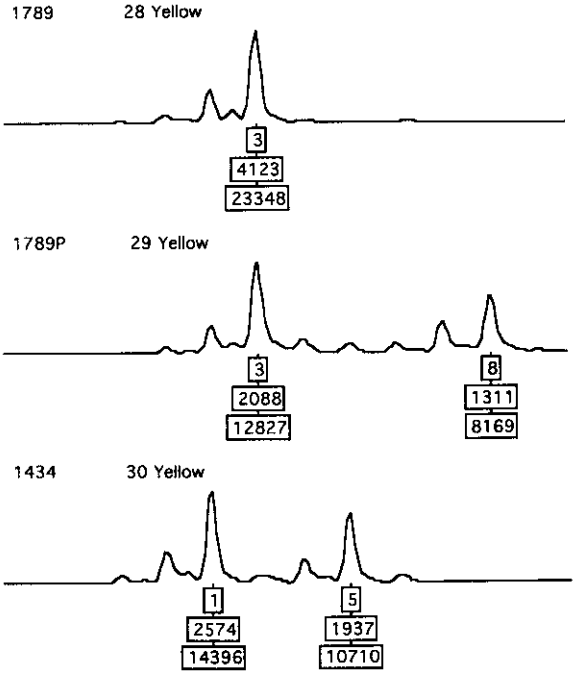

Figure 1. Fluorescent peaks of the fluorescent gel of Hu-O-2A/Gb1 cells (1789, 28 yellow), control lymphocytes (1789P, 29 yellow), and IN1434 cells (1434, 30 yellow) at locus D6S273. Each peak was quantitated in terms of allele (3, 8 or 1, 5), peak height (4123, 2088, 1311, 2574, 1937) and peak area (23348, 12827, 8169, 14395, 10710). Hu-O-2A/Gb1 cells show LOH of allele 8 of the locus.

Hu-O-2A/Gb1 cells express antigens, have differentiation potential and have a 1H-NMR profile characteristic of the oligodendrocyte-type 2 astrocyte (O-2A) progenitor cell lineage of the rat. IN1434 cells, in contrast, appear to derive from a lineage committed solely to astrocytic lineage [3–5].

Cytogenetic analysis of the Hu-O-2A/Gb1 has shown that this line had a chromosome number ranging from 42 to 78 with a mode of 45. The typical karyotype was 45, Y, +der(X)t(X;10)(q27.1; p12.1), −5, +der(5)del(5)(p), +7, −10, −10, +der(10)t(10;15)(p11.23; q11.2), −13, −14, +derder(14)dic(5;14), −15, +der(15)del(15)(q), −16, +der(16)del(16)(q; p). Fluorescence *in situ* hybridisation (FISH) study has revealed that in Hu-O-2A/Gb1, 100% of cells were monosomy X, 95% were monosomy 10, 41% monosomy 18, 28% monosomy 15, 23% monosomy 1, 21% monosomy 17, and 21% monosomy 14. Seventy-eight percent of cells were trisomy 7 and 22% tetrasomy 7, 79% trisomy 8, 34% trisomy 5 [30]. IN1434 cells had a chromosome number ranging from 41 to 94 with a mode of 46. All

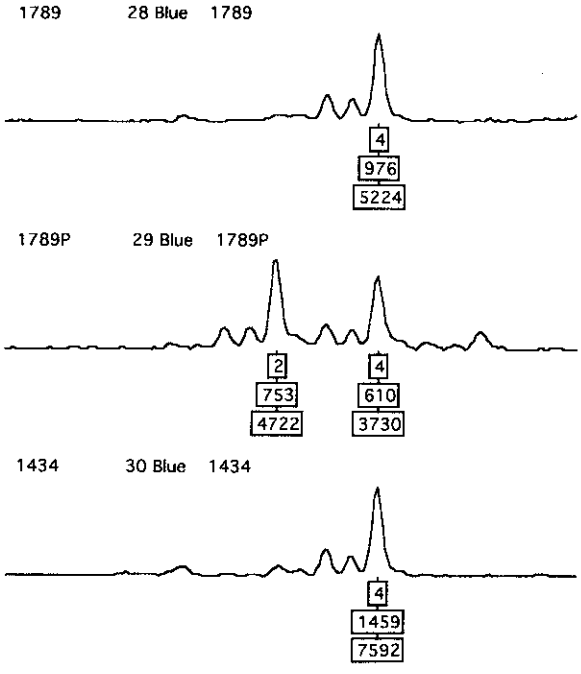

Figure 2. Fluorescent peaks of the fluorescent gel of Hu-O-2A/Gb1 cells (1789, 28 blue), control lymphocytes (1789P, 29 blue), and IN1434 cells (1434, 30 blue) at locus D10S249. Hu-O-2A/Gb1 cells show LOH of allele 2, and 1N1434 cells show homozygosity of allele 4 of the locus, respectively.

IN1434 cells were monosomy X, 71% monosomy 8, 67% monosomy 22, 67% monosomy 4, 60% monosomy 10, 57% monosomy 6, 17% monosomy 18, 14% monosomy 2, 14% monosomy 15. All cells were trisomy 20, 81% trisomy 7 and 11% tetrasomy 7, 66% trisomy 18 and 17% tetrasomy 18, 60% trisomy 5 and 20% tetrasomy 5, 75% trisomy 19, 40% tetrasomy 9, 40% tetrasomy 14, 33% tetrasomy 16, 14% trisomy 2, 14% tetrasomy 15 [30]. Most cytogenetic aberrations in these lines are consistent with those noted in other studies of GBM [6–9].

Most cancer susceptibility genes conform to the model of tumor suppressor genes in which both alleles need to be inactivated in order to contribute to oncogenesis [31]. In tumors arising in susceptible individuals, loss of the wild-type allele, or LOH, is frequently observed at the appropriate locus. LOH may arise by any of several mechanisms. It may be a consequence of a multilocus chromosomal event, such as deletion, mitotic recombination, or nondisjunctional chromosome loss with or without reduplication; or a consequence of a locus-restricted event, such as gene conversion or point mutation [32]. To carry out LOH studies, DNA samples extracted from both tumor and normal tissues of the same patient as well as polymorphic DNA markers normally have to be obtained. In this study, although no control DNA was available for IN1434, homozygosity of more than 3 markers was only seen on chromosomes 1, 3, 8, and 10 that also showed cytogenetic aberrations (Table 1) [30]. The phenomenon of non-random distribution of homozygosity of DNA markers revealed in this study might open an alternative way to investigate allelic losses in tumor samples without the matched control DNA, provided more than 2 polymorphic markers on each chromosome are tested.

Previous studies suggested the presence on chromosome 10 of tumor suppressor genes besides PTEN/MMCA1 [11–12,17]. In this study both cell lines had allelic losses of loci throughout chromosome 10 (Table 1), which were consistent with our cytogenetics findings that each line had lost one homologue of chromosome 10 [30]. The remaining homologue in Hu-O-2A/Gb1 was translocated at band 10p11.2 to chromosome 15 and X [30]. The breakpoint of the rearrangement was defined in a cosmid contig mapped on 10p12.1-11.2, namely, 10pter-14A7-breakpoint-JC2139, 6B2, JC2075-cen [30]. The same region also shows LOH in glioma and prostate cancer [17,33]. In addition, Hu-O-2A/Gb1 cells had an allelic loss of locus D15S158 that franks the breakpoint region on chromosome 15 of the 10; 15 translocation (Table 1). The X; 10 translocation also results deletion of the region Xq27-qter from the genome. Although loss of an entire sex chromosome is common in glioma, we are unaware of nullisomy for this region of X-chromosome being described previously in GBM [30].

The majority of cells of both Hu-O-2A/Gb1 and IN1434 had trisomy 7 and tetrasomy 7. Trisomy 7 and tetrasomy 7 are one of the most common aberrations in gliomas and other malignancies [34]. An investigation using CGH has found that gain of 7q in particular is the most frequent event detected in adult low-grade astrocytomas [35], suggesting that genes on 7q play an early role in tumour development. This has been confirmed in our CGH study of IN1434 as sites of genomic gain at bands 7q11.23 and 7q35 were observed [30]. We cloned a human LIM-kinase gene (LIMK1) from a cDNA library of Hu-O-2A/Gb1 cells and mapped it to chromosome 7q11.23 [36]. The LIMK gene encodes a protein that contains two LIM domains and a novel kinase domain, and is thought to play a regulatory role

in differentiation and proliferation [37]. We found a deletion of the LIMK gene in the Williams syndrome, a contiguous gene deletion syndrome showing vascular, connective tissue and nervous system defects [38]. Both Hu-O-2A/Gb1 and IN1434 cells showed amplification and overexpression of LIMK1 [30]. Moreover, each cell in IN1434 had a complex rearrangement in which chromosome 7 material, including LIMK1, was translocated to a derivative chromosome 1 at both the long and short arms [30]. These results imply that alteration of the LIMK gene may contribute to tumorigenesis of GBM. On the other hand, Hu-O-2A/Gb1 cells also expressed allelic losses of loci D7S440 and D7S5 18 (7q21.12-21.3) (Table 1). Allelic losses of loci on 7q21-32 have been found in leiomyomas, prostate, breast, colon and ovarian carcinomas [39–43]. Furthermore, a marked increase in deletion at 7q22 has been noted in neuroglial tumors [9]. These studies also suggest the presence on this region of 7q of tumor suppressor genes that may play a role in the development of these malignancies.

This study showed that Hu-O-2A/Gb1 had allelic losses of loci D13S291 (13q14.21-13) and D13158 (13q22.3-32.1) (Table 1). This result has been confirmed by our cytogenetic study [30]. IN1434 had an interstitial deletion of chromosome 13 involving bands 13q12-14 [30]. The retinoblastoma susceptibility gene 1 (RB1) at band 13q14 is a prime candidate target for deletion, as it is commonly mutated in astrocytic gliomas. Interestingly, mutation of RB1 has been found to correlate inversely with the mutations of the CDKN2 gene in gliomas [44]. This may not be the case with Hu-O-2A/Gb1 as this line had an allelic loss of locus D9S259 franking the CDKN2 gene (Table 1). Furthermore, 57% of Hu-O-2A/Gb1 cells had also lost one homologue of the CDKN2 gene [30]. Thus, further examination of the RB1 and CDKN2 genes in the two cell lines here will allow us to know the likely mechanism of the cell cycle regulatory pathway in glioma development.

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 [30]. Most cases of Turcot's syndrome, which includes gastrointestinal tumors and GBM, result from germ-line mutations of the APC tumor suppressor gene at band 5q21 [45]. APC is therefore a prime candidate gene for GBM. This study showed that Hu-O-2A/Gb1 only had an allelic loss of locus D5S394 which lies outside the region of APC (Table 1). No APC mutation was detected in Hu-O-2A/Gb1, IN1434 and several other GBMs by a protein truncation test [30]. We have no evidence, therefore, that somatic APC mutation was involved in the pathogenesis. We tested Hu-O-2A/Gb1 cells for microsatellite instability and for mutations of five DNA mismatch repair (MMR) genes, since a subset of patients with Turcot's syndrome have germ-line mismatch repair mutations. Neither microsatellite instability nor MMR mutations were detected [30]. This study only showed that Hu-O-2A/Gb1 had an allelic loss of locus D2S112 which lies outside the region of the mismatch repair genes (Table 1). Recently other studies failed to detect microsatellite instability in gliomas [46,47], suggesting that microsatellite instability and MMR mutations are unlikely to play an important role in glioma development.

This study also showed that Hu-O-2A/Gb1 had allelic losses of loci on chromosome 6, 16, and 17 (Table 1) (Figure 1) that were consistent with our cytogenetics and FISH findings [30]. As DNA was extracted from low passage Hu-O-2A/Gb1 cells, the impact of selection in *in vitro* culture on this line might be less significant. These results provide a detailed information of genetic changes occurring in Hu-O-2A/Gb1, and indicate that the allelic losses might be the major genetic events underlying the development of the GBM from which this cell line derived.

Previous studies on the alterations associated with neoplastic transformation of human glial cells have demonstrated that particular genetic abnormalities occur with great frequency in specific categories of gliomas, as assigned by cytological evaluation. For example, while most GBMs demonstrate a number of the abnormalities described in the introduction section of this paper, tumours morphologically classified as oligodendrogliomas and oligo-astrocytomas tend to express a different constellation of abnormalities, such as allelic losses on 19q and 1p [47–49], and do not express the characteristic genetic changes seen in GBMs Tumors identified as meningiomas, pilocytic astrocytomas or medulloblastomas tend to exhibit still different collections of genetic alterations. Thus, there has been some reason to anticipate a correlation between the constellation of abnormalities expressed and the biological origin of a given tumor. The results of our study demonstrate that Hu-O-2A/Gb1 and IN1434 cells expressed not only genetic abnormalities observed with great frequencies in other adult GBMs [6–9, 13–17,34], but also specific aberrations. Thus, in this study, it is still unclear whether there exists a

relationship between the tumor cell lineage and the constellation of aberrations that presumably contribute to the generation of malignancy. Answers to these questions will require genetic studies on further gliomas which have been unambiguously assigned to particular lineages.

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