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Short communication

Analysis of pilocytic astrocytoma by comparative genomic hybridization

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Summary Very little is known about genetic abnormalities involved in the development of pilocytic astrocytoma, the most frequently occurring brain tumour of childhood. We have analysed 48 pilocytic astrocytoma specimens using comparative genomic hybridization. Only five of 41 tumours from children showed abnormalities detectable by comparative genomic hybridization, and in each case this represented gain of a single chromosome. Interestingly, two of seven tumours from adults showed abnormalities, which were multiple and relatively complex. Six of the seven tumours showing abnormalities were from female patients (two adults and four children). The most frequently detectable abnormality was gain of 9q34.1-qter, which was present in three cases (two adult and one paediatric). © 2000 Cancer Research Campaign

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Pilocytic astrocytoma (PA) is the most frequently occurring brain tumour in childhood. It is classified as grade I by the World Health Organization and does not tend to evolve into higher-grade tumours (Giannini and Scheithauer, 1997). Very little is currently known of the genetic abnormalities involved in the development of PA. More than 120 cases have been analysed cytogenetically, but no consistent abnormality has been identified. Of 119 paediatric cases examined, 38 showed detectable chromosomal abnormalities; eight adult cases have been examined and seven of these showed chromosomal abnormalities (Jenkins et al, 1989; Karnes et al, 1992; Ransom et al, 1992; Thiel et al, 1992; Ganju et al, 1994; Agamanolis and Malone, 1995; Debiec-Rychter et al, 1995; White et al, 1995; Bhattacharjee et al, 1997; Bigner et al, 1997; Zattara-Cannoni et al, 1998).

Only a small number of studies of molecular genetic abnormalities in PA has been undertaken to date. The cases included in these studies were either all paediatric or the patient age was not specified. Allelic loss has been reported on 17p, including at the *TP53* locus, although very few *TP53* mutations have been found (von Deimling et al, 1993; Lang et al, 1994; al-Sarraj et al, 1995; Phelan et al, 1995; Willert et al, 1995; Patt et al, 1996). Losses on 17q have also been found, and in some cases these encompassed the *NF1* locus (von Deimling et al, 1993; Platten et al, 1996). However, no *NF1* mutations were observed by single-strand conformation polymorphism analysis of 16 PA tumours (Scheurlen and Senf, 1995). A single case of PA was reported to have a non-sense mutation of *PTEN*, a gene that is frequently mutated in glioblastomas (Duerr et al, 1998). No other gene mutations have been reported and no loci have been shown to be consistently abnormal in PA.

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We have used comparative genomic hybridization (CGH) to screen for gross genomic copy number abnormalities in PAs from 48 patients. CGH enables the analysis of copy number imbalances across the genome in a single hybridization and avoids the need for cell culture (Kallioniemi et al, 1992).

MATERIALS AND METHODS

Tumour specimens

We studied archival frozen tumour tissue from patients operated at Karolinska Hospital Stockholm, Sahlgrenska Hospital Gothenburg and Addenbrooke's Hospital Cambridge. The clinical and histopathological data are summarized in Table 1. Of the 48 individual tumours studied, 41 were from patients less than 18 years of age and seven were from adults. We used the suffix 'a' to indicate tumour from primary operations and 'b' to indicate tumour from re-operations. Specimens PA8b, PA9b, PA45b, PA50b and PA53b were re-operated tumours for which tissue samples from the primary operation were not available.

DNA extraction

High molecular weight DNA was isolated from the frozen tumour tissue pieces by homogenization in 4 M guadinium isothiocyanate buffer followed by ultracentrifugation on a caesium chloride (CsCl) gradient. The DNA was purified by proteinase K digestion and phenol–chloroform extraction. Reference genomic DNA was prepared from blood of one healthy male and one healthy female donor.

Comparative genomic hybridization

CGH was carried out according to previously published protocols (Kallioniemi et al, 1992), with certain modifications. The reference and patient genomic DNA were differentially labelled

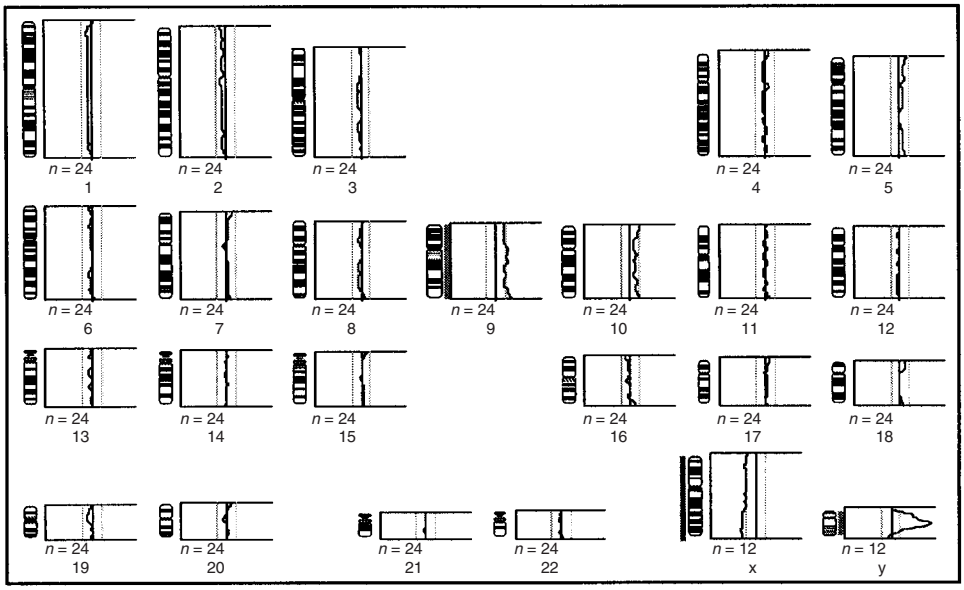

Figure 1 CGH interpretation profile of case PA25a. The ration profile was determined from analysis of 12 separate metaphases. As the tumour arose in a male patient, we utilised control DNA from a female. There is evidence of gain of all of chromosome 9 in the tumour specimen.

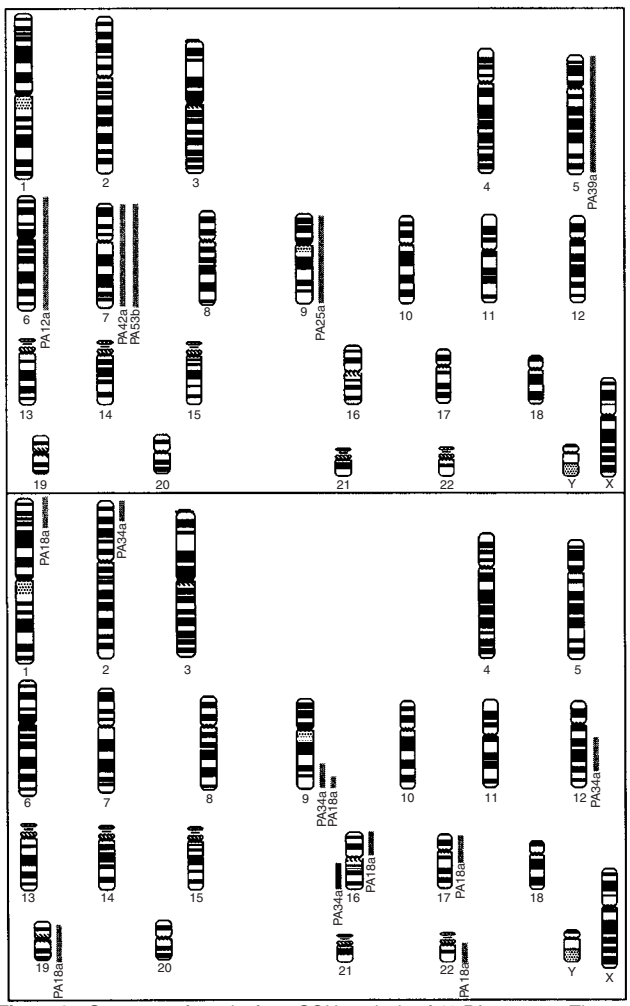

Figure 2 Summary of results from CGH analysis of 48 PA tumours. The top and bottom boxes show the results from paediatric and adult cases respectively. Lines to the right of each chromosome in the ideogram depict regions of DNA gain, whilst lines to the left of each chromosome depict regions of DNA loss.

by DOP-PCR (Telenius et al, 1992) using biotin-16-dUTP and fluorescein isothiocyanate (FITC)-dUTP respectively. They were then co-hybridized onto normal human metaphases. The biotin label was detected with avidin-Cy3. Twelve to 15 metaphases were analysed for each specimen using the Vysis Quips CGH program. The thresholds used for the CGH ratio profiles were 1.2 for gain and 0.8 for loss. Each specimen was analysed at least twice.

RESULTS

Seven of the 48 tumours showed chromosomal abnormalities detectable by CGH. A representative copy number karyogram is shown in Figure 1. In paediatric cases the abnormalities were gains and involved chromosomes 5, 6, 7, 9 (see Figure 2 for frequency). No loss of chromosomal regions was detected. Only five of the 41 paediatric tumours (12%) were abnormal. Of the 18 female and 23 male paediatric cases analysed, four female patients and only one male patient had detectable chromosomal abnormalities. In each of these only one aberration was seen and this involved the gain of a single chromosome. Two of the tumours gained a chromosome 7 while the other three gained a chromosome 5, a chromosome 6 or a chromosome 9.

Of the seven tumours from adult patients two (29%) showed multiple abnormalities, which included regional copy number changes as well as gains of whole chromosomes. Of the six female and one male adult cases only two female patients had detect-able aberrations. PA 18a had gains of 1p33-pter, 9q34.1-qter, 17q21.3-pter and whole gains of 19 and 22. PA 34a showed gains of 2p22-pter, 9q31-qter, 12q13.2-q23, together with loss of 16q.

There was no association between the presence of abnormalities detectable by CGH and the anatomical location of the tumours. The five abnormal paediatric cases were located in the posterior fossa (two cases), optic chiasm, left frontal lobe and hypothalamus (one case each), while the two abnormal adult cases were located

Table 1 Clinical details relating to PA specimens analysed

Case No	Age	Sex	%tum Tissue	Tumour location	CGH results
Paediatric cases with normal CGH results					
PA2a	5	M	90	Not known	normal
PA5a	3	F	65	cerebellum	normal
PA7a	3	F	85	Optic nerve	normal
PA8b	8	F	75	Cerebellum, ve	normal
PA9b	13	M	85	Hypothalamus	normal
PA14a	5	M	>90	Posterior fossa	normal
PA15a	14	F	Not known	Vermis	normal
PA16a	1	M	85	Hypothalamus	normal
PA17a	10	F	80	Posterior fossa	normal
PA17b	13	F	75	Posterior fossa	normal
PA19a	13	M	80	3rd ventricle	normal
PA20a	6	M	90	Posterior fossa	normal
PA23a	5	F	90	4th ventricle	normal
PA24a	18	M	90	4th ventricle	normal
PA28a	Not known	M	>80	Right tempora	normal
PA28b	Not known	M	>85	Not known	normal
PA29a	15	M	90	Posterior fossa	normal
PA30a	16	F	85	Right cerebella	normal
PA31a	5	F	90	Brainstem	normal
PA32a	4	M	Not known	4th ventricle	normal
PA33a	2	M	90	3rd ventricle	normal
PA37a	16	M	>85	Posterior fossa	normal
PA40a	5	M	85	Optic nerve	normal
PA43a	6 m	M	90	Hypothalmus	normal
PA44a	1	M	85	Optic nerve	normal
PA45b	9	M	>90	Posterior fossa	normal
PA47a	8	F	90	Posterior fossa	normal
PA48a	5	F	85	Cerebellum	normal
PA49a	3	M	Not known	Vermis	normal
PA50b	Not known	M	60–70	4th ventricle	normal
PA51a	8	M	Not known	Right cerebella	normal
PA52a	6	F	Not known	Posterior fossa	normal
PA54a	6	F	100	Cerebellum	normal
PA55a	8	F	75	Cerebellum	normal
PA56a	18	M	80	Posterior fossa	normal
PA57a	10	M	80	Posterior fossa	normal
PA57b	13	M	100	Posterior fossa	normal
PA58a	10	M	90	Not known	normal
PA60a	7	F	80	Posterior fossa	normal
Paediatric cases with abnormal CGH results					
PA12a	17	F	85	Posterior fossa	+6
PA25a	10	M	85	Optic chiasm	+9
PA39a	11	F	90	Posterior fossa	+5
PA42a	12	F	85	Left frontal lobe	+7
PA53b	7	F	100	Supra sellar	+7
Adult cases with normal CGH results					
PA1a	33	F	85	Optic nerve	normal
PA3a	32	M	80	Cerebellum	normal
PA10a	20	F	85	Cerebellum	normal
PA35a	Not known	F	80	Midline	normal
PA41a	23	F	85	4th ventricle	normal
Adult cases with abnormal CGH results					
PA18a	33	F	85	Pineal region	+1p33-pter, +9q34.1-qter, +16p, +17q21.3-pter, +19, +22
PA34a	48	F	90	Vermis	+2p22-pter, +9q31-qter, +12q13.2-q23, −16q

in the pineal region and posterior fossa. Two parts of specimen PA39a were analysed; interestingly, whereas one of these was normal, the other showed gain of chromosome 5. In all other tumours where two specimens were available no abnormalities were seen in the profiles of either tumour.

DISCUSSION

The majority of cases analysed in this study show no chromosomal abnormality. Some aberrations, however, are detectable in a number of different chromosomes. The detectable chromosomal

aberrations the paediatric tumours always involved gain of whole chromosomes. In adults gain of whole chromosomes or subchromosomal regions was seen, as well as regional loss. The difference in frequency and the types of aberration seen in the adult cases is interesting. A higher incidence of malignant progression has been reported in adult PAs compared to paediatric PAs (Giannini and Scheithauer, 1997).

The male to female ratio in our series was equal; there were 24 males and 24 females. However, four of the five paediatric tumours and both of the adult tumours with detectable abnormalities were from females. This finding is interesting and merits further investigation. There was no association between the presence of abnormalities detectable by CGH and the anatomical location of the tumours. The finding that one region of specimen PA39a was normal, whereas another showed gain of chromosome 5 is also of interest, and is consistent with the notion of clonal evolution in the neoplasm.

Our CGH results are consistent with data from previous cytogenetic studies. In the approximately 120 paediatric cases analysed cytogenetically to date, gains have been seen on chromosomes 5 (four cases), 6 (two cases), 7 (nine cases), 12 (two cases), 17 (two cases), 19 (three cases) and 22 (three cases) (Jenkins et al, 1989; Karnes et al, 1992; Agamanolis and Malone, 1995; Debiec-Rychter et al, 1995; White et al, 1995; Bhattacharjee et al, 1997; Bigner et al, 1997; Zattara-Cannoni et al, 1998). In the approximately eight adult cases analysed cytogenetically, gains have been seen for chromosomes 5 (two cases), 6 (three cases), 7 (two cases) and 12 (one case). Gain of chromosome 7 is one of the most common abnormalities in PA and has also been described as one of the most characteristic aberrations in glioblastoma multiforme (grade IV astrocytoma) (Liu et al, 1998). However, trisomy 7 has also been reported in non-neoplastic tissue (including brain tissue), both in vitro and in vivo, and the significance of its role in oncogenesis remains controversial (Johansson et al, 1993).

We detected several abnormalities that have not previously been reported in PA. These included gain of regions of chromosome 1p and chromosome 2p, in cases PA18a and PA34a respectively. Our most frequent finding (in three cases) was of gain on 9q, which was detected in both adult tumours with abnormalities and in one paediatric tumour (which showed gain of all of chromosome 9). The minimal region of gain is 9q34.1-qter. A variety of genes are located in this region including *ABL* (Abelson murine leukaemia), *VAV2* (Rho-family guanine-nucleotide exchange factor) and *PBX3* (pre B-cell leukaemia transcription factor).

In those cases of PA in which no abnormalities are detectable with CGH, it is not necessarily the case that no chromosomal aberrations are present. As CGH demonstrates loss or gain of DNA sequences, balanced translocations remain undetected. In addition, CGH will not detect polyploidy and will only detect DNA sequence copy number changes if they differ from the average copy number of chromosomes in the entire tumour specimen. It should be noted however that polyploidy in PA has been reported only in those rare cases which have undergone malignant transformation (Tomlinson et al, 1994; Mathew et al, 1996).

Abnormalities present in a small percentage of tumour cells may also not be detectable by CGH. In a recent cytogenetic study 12 out of 24 cases of paediatric PA were reported to be mosaic, and six of these cases showed random chromosomal gains and losses (Zattara-Cannoni et al, 1998). In addition, PA tumours may contain regions of DNA gain or loss that are beyond the resolution of CGH. With metaphase chromosome targets the technique can detect amplifications of approximately 2 MB (representing a product of amplicon size and copy number increase) and regions of DNA loss of 10 MB or greater (Kallioniemi et al, 1994).

In conclusion, we have demonstrated that major cytogenetic abnormalities are relatively rare in PA. Interestingly, however, the specimens from adults in our study showed more complex aberrations than those from paediatric patients. In the small number of abnormal cases no frequently occurring aberration is detectable, although there is some consistency between our findings and those from previous cytogenetic studies. Determination of mechanisms underlying the development of PA may require detailed genotypic analysis of tumour tissue, for example using developing DNA microarray technology.

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