Prognostic implications of p53 protein, epidermal growth factor receptor, and Ki-67 labelling in brain tumours

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Summary The expression of p53 protein, epidermal growth factor receptor (EGFR), and Ki-67 nuclear antigen was examined by immunohistochemistry in biopsies of 16 types of human brain tumours, including 43 astrocytomas. P53 protein, almost certainly its mutant form, was expressed in seven of the 16, and EGFR in 11 of the 16 types of tumours. In astrocytomas both the proportion of tumours which expressed p53 or EGFR increased with grade of malignancy as did the mean Ki-67 labelling index (LI; p53–0% in grade 1, 17% in grade 2, 38% in grade 3, 65% in grade 4; EGFR~0% in grade 1, 33% in grade 2, 85% in grade 3, 95% in grade 4; mean Ki-67 LI=1.1% in grades 1 and 2, 8.3% in grade 3, and 13.4% in grade 4. Astrocytomas which expressed p53 or EGFR had a significantly higher Ki-67 LI at P<0.05 (11.8% and 10.7%, respectively) than those that did not (6.2% or 4.1%, respectively). Patients with astrocytomas expressing p53 or EGFR had a significantly reduced survival (P=0.035 and P=0.007, respectively). Only 11% of the p53 +ve and 13% of the EGFR +ve patients were alive at 100 weeks. Following diagnosis compared to 36% of p53-ve or 60% of EGFR-ve patients. Patients with Ki-67 LI >5% had a reduced survival (P<0.0001) – none survived beyond 8 weeks following diagnosis, whilst 63% of patients with <5% positive cells were still alive at 100 weeks. The univariate analysis showed that in astrocytomas expression of p53 mutants, EGFR protein, and Ki-67 >5% are associated with malignant progression and poor prognosis. The multivariate analysis revealed that only tumour grade and Ki-67LI were independent prognostic factors for survival.

Glial tumours are the most common primary tumours of the CNS (Russell & Rubinstein, 1989). Almost all types of glial tumours can recur and display malignant progression to some degree depending on the histopathological type of tumour, grade of malignancy, its location, the patient’s age, and the extent of surgical resection (Russell & Rubinstein, 1989). However, the onset of the malignant process is highly variable, and prognostic predictions cannot be made in individual patients. In both low and high grade astrocytomas loss of heterozygosity for alleles on chromosome 17p has recently been found, suggesting that during early stages of tumorigenesis mutation and acquisition of homozygosity has occurred in a recessive oncogene on that chromosome (James et al., 1989; El-Azouzi et al., 1989). Also, several high grade astrocytomas have been found to contain point mutations in gene p53, which is localised on chromosome 17p (Nigro et al., 1989). Malignant gliomas have also been shown to have abnormal chromosomes 1, 6, 9, 10, 13, 22, sex chromosomes, and an extra chromosome 7 (Bigner et al., 1984; James et al., 1988). The epidermal growth factor receptor (EGFR) gene which is located on chromosome 7 (Shimizu et al., 1985) has been shown to be amplified and rearranged (Liberman et al., 1984; Liberman et al., 1985; Wong et al., 1987; Sugawa et al., 1990), and the EGFR protein found overexpressed in the most malignant gliomas, especially the glioblastoma multiforme (Azri et al., 1989; Reifenberger et al., 1989). On the basis of these findings Bigner and Vogelstein (1980) have proposed a model for malignant progression of gliomas in which losses of chromosomes 17p, 13, or 22 occur in low grade gliomas, and loss of chromosome 10 represents a critical step in transition from grade 3 (anaplastic astrocytomas) to grade 4 (glioblastoma multiforme), whilst abnormalities of 9p and EGFR amplification stimulate further progression. In Primitive Neuroectodermal Tumours (PNETs) chromosomes 1 and 17 have been implicated in tumour development by cytogenetic studies (Bigner et al., 1988; Griffin et al., 1988), and subsequently allele loss has been found on chromosomes 17p, 6q, and 16q (Thomas & Raffel, 1991). The genes affected by putative mutations on these chromosomes have not yet been identified in PNETs but gene p53 on chromosome 17p is a candidate.

Normal p53 gene behaves as a tumour suppressor gene. It encodes a 53kD nuclear phosphoprotein, which is thought to be involved in regulation of cell growth (Finlay et al., 1989; Stanbridge, 1990). The normal p53 protein is undetectable by standard immunohistochemistry because of its low cellular levels and a very short half-life, about 20 min (Finlay et al., 1989). Point mutations in the gene lead to expression of nonfunctional mutant forms with substantially longer half-lives (up to about 24 h), and an elevation of cellular levels to 10–100 fold above normal values (Finlay et al., 1989) which can be detected by immunohistochemistry (Cattoretti et al., 1988; Iggo et al., 1990; Rodrigues et al., 1990). An association between expression of p53 mutants, epidermal growth factor receptor (EGFR), and poor prognosis has recently been reported to occur in human breast carcinomas (Harris et al., 1990) but has not yet been examined in astrocytomas. EGFR is a 170 kD transmembrane glycoprotein with an extracellular ligand-binding domain, a transmembrane region and an intracellular portion with tyrosine kinase activity (Hunter, 1984). Binding of EGFR or transforming growth factor alpha (TGF-alpha) to EGFR results in activation of tyrosine kinase activity and stimulation of DNA synthesis, leading to mitosis (Stoscheck & Carpenter, 1987). The aim of this study was to increase our understanding of basic biological mechanisms in central nervous system (CNS) tumours and to correlate expression of mutant p53 protein and EGFR with tumour proliferative activity and the patients’ progress. 78 CNS tumours were examined, including 43 astrocytomas and 6 PNETs using immunohistochemistry and monoclonal antibodies to p53 and EGFR proteins. Monoclonal antibodies to the growth fraction-associated Ki-67 antigen (Gerdes et al., 1984) have been used to assess the proliferative activity of tumour cells as the Ki-67 labelling index (LI) has been shown to correlate with the degree of

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malignancy in different types of tumours, including gliomas (Zuber et al., 1988; Raghavan et al., 1990). Preliminary results of this study have been presented in part to the British Neurooncology Society (Jaros et al., 1991a) and to the British Neuropathological Society (Jaros et al., 1991b).

Materials and methods

Fresh specimens of 78 CNS tumours were obtained during neurosurgery in Newcastle General Hospital between April 1988 and May 1990. They were divided into several portions: (i) used for karyotyping employing methods developed for solid tumours (Adam et al., in preparation); (ii) fixed in formalin, embedded in paraffin, and sections stained with haematoxylin and eosin for histopathological assessment (iii) snap-frozen in arctic pre-cooled with liquid nitrogen and stored at −70°C for immunohistochemistry. Histopathological assessment of the tumours was made according to the WHO classification system (Rorke et al., 1985), and the degree of malignancy was graded according to Kernohan’s system (Kernohan et al., 1949).

Frozen sections of the tumours were cut at 6 μm, mounted on silanized glass slides and allowed to dry overnight. The sections were fixed in 1:1 mixture of chloroform:acetone for 10 min at room temperature, dried for 10 min, and incubated with mouse monoclonal antibodies to human p53 (PAb 1801 from Cambridge Research Biochemicals) at a 1:1600 dilution (titled to detect p53 protein expressed in control human lung carcinoma material); or a mouse monoclonal Ki-67 antibodies (Dako) at a 1:25 dilution; or with mouse monoclonal antibodies to EGFR (EGFR1 from Amersham) at a 1:50 dilution (titled on normal human skin), followed by biotinylated anti-mouse antibodies (Vectastain) at a 1:200 dilution, streptavidin-biotin-HRP (Amersham) at a 1:100 dilution, DAB at 0.5 mg ml⁻¹ and counterstained with haematoxylin. All the dilutions of antibodies were prepared in Tris-buffered saline, pH 7.6, containing 1.5% normal preimmune horse serum. As a control for endogenous peroxidase the primary antibodies were omitted on serial sections from each block. Another serial section was stained with haematoxylin and eosin for histopathology.

Immunohistochemically processed sections from all the tumours were examined and those containing nuclei labelled with the p53 or the Ki-67 antibody were classified as p53 + ve (p53 positive) or Ki-67 + ve tumours, and were quantified on a Nikon microscope at x 400 magnification using a square graticule. When regional heterogeneity of labelling was detected in the tumour, counting areas were chosen to include areas with high and low density of p53 positive cells and also areas in which serial sections showed variation in the Ki-67 or the EGFR labelling. In each area between 901 and 1566 tumour cells were counted from systematically randomised fields. Endothelial cells were not included in the counts, even when in some of the tumours they were labelled with Ki-67 (though never with the p53) antibody. The p53 or Ki-67 LI was calculated as a percentage of labelled tumour cells out of the total number of tumour cells counted (LI = 100 × number of labelled nuclei + total number of nuclei). The highest Ki-67 LI detected in individual tumours was considered to represent the proliferative potential within the tumour (Raghavan et al., 1990), and was therefore used in quantitative analysis. Because the EGFR labelling of tumours varied both in terms of cellular intensity and proportion of tumour labelled, calculating a simple EGFR LI may have been inadequate. Instead, for each tumour the labelling intensity was scored on a scale from -ve to ++ + + +, and the percentage of EGFR reactive tumour cells was calculated using an eyepiece with square graticule. EGFR labelling factor was then calculated for each tumour by multiplying the percentage of labelled area by the labelling intensity score was scored as (−), and by 2.34, or 5, respectively, if the labelling intensity was scored as +, + +, ++ + or +++ ++. Student t-test was used to analyse p53, EGFR or Ki-67 labelling as continuous variables in relation to tumour grade, and to analyse Ki-67 labelling in relation to p53 or EGFR labelling as categorised variables. Pearson’s correlation coefficient (r) was used to determine the strength of association between the continuous variables Ki-67 LI, p53 LI, EGFR labelling factor, and patient’s age.

The prognostic importance of each of the variables with natural categorisation, i.e. sex, histological grade (1, 2 vs 3, 4), p53 (+ ve vs − ve), EGFR (+ ve vs − ve), extent of surgery (total, subtotal, partial, biopsy), radiotherapy (Y/N), and chemotherapy (Y/N) was assessed using Log-Rank test (Petos et al., 1977). The continuous variables, i.e. age and Ki-67 LI were separately entered into the Cox regression model (Cox, 1972) to yield relative risks and P-values. This avoids the need of possibly ‘data-driven’ categorisation of the variables although Ki-67 was also considered in the form of <5% vs >5% and analysed using the Log-Rank test. All variables apart from sex, radiotherapy, and chemotherapy were entered into the multivariate analysis. The multivariate analysis was performed by using a forward stepwise application of Cox’s Regression model via the BMDP statistical package (Program 2). Variables selected as statistically significant by this procedure had P < 0.10. 95% confidence intervals for the relative risks in the multivariate procedure are given by $e^{\text{coef} ± 1.960\text{SE(coef)}}$.

Results

Clinical histopathological data

These data are summarised in columns 1–5, and 10–13 of Table I. Only the astrocytoma group was sufficiently large to make the Log-Rank test of survival possible. The range of follow-up time was 0–180 weeks (median = 39 weeks). All except one of the patients who are still alive (patient number 276) have reached the 100 weeks follow-up (Table I). Out of the total of 43 astrocytoma patients, three patients who died of other causes were not included in the Log-Rank analysis. The length of patient’s survival was significantly related to their age at diagnosis (younger patients showing longer survival: $P = 0.0002$; Table II), and to the histopathological grade of their tumour ($P < 0.0001$; Table III and Figure 1). In Figure 1 note that patients with malignant grades had a significantly reduced survival time – only 6% of patients with grades 3 and 4 were alive at 100 weeks following diagnosis compared to 89% of patients with grade 1 and 2 tumours. Sex of the astrocytoma patients, radiotherapy, chemotherapy or surgery did not significantly affect their survival (Table III), though surgery was weakly significant ($P = 0.06$).

Cytogenetic data

These data are shown in column 9 of Table I. Cytogenetic analysis was performed on short-term cultures of 74% of the tumours; 50% of the tumours were successfully karyotyped. Chromosomal abnormalities were found in 21% of all the tumours: in 13 astrocytomas grade 3 and 4, one angioglioma grade 4, one malignant choroid plexus papilloma, and one metastatic melanoma. In the astrocytomas and the angio- glioma the most common abnormality was aneuploidy of sex chromosomes, in particular loss of chromosome Y. Trisomy of chromosome 7, where EGFR gene is known to be localised, was found in one astrocytoma only (patient No. 113). None of the patients were found to have gross rearrangements or deletions of chromosome 17p, where p53 gene is localised.

Immunohistochemistry of p53

No labelling was detected in normal neocortical (12 cases) and normal cerebellar (four cases) tissue adjacent to the tumours. The p53 labelling of tumours was restricted to the tumour cell nuclei. No cytoplasmic labelling was observed, and no endothelial cells were labelled, even in areas showing...
Table 1  Summary of clinical, histopathological, immunohistochemical, and cytogenetic data

| Patient number | Sex | Age yrs | Site of tumour | Histological diagnosis and grade (1-4) | p53 LI % | EGFR labelling intensity - to ++++ | Ki67 LI % | Cytogenetic analysis | Treatment | Therapy | Chemo | Survival time PostOp Weeks |
|----------------|-----|---------|----------------|--------------------------------------|----------|----------------------------------|----------|----------------------|-----------|---------|-------|--------------------------|
| 183            | M   | 18      | Cerebellar Mid | Astro 1 cystic                       | 0.0      | -                                | 1.0      | Normal               | Total exc | +       | -     | 129 alive                |
| 188            | M   | 15      | Cerebellar     | Astro 1 cystic                       | 0.0      | -                                | 1.2      | Normal               | Total exc | -       | -     | 2 other causes            |
| 217            | F   | 7       | Cerebellar Mid | Astro 1 cystic                       | 0.0      | -                                | 0.5      | Normal               | Total exc | -       | +     | 115 alive                |
| 73             | M   | 63      | Left Front     | Astro 2                              | 0.0      | -                                | 0.1      | Not Done             | Subtot exc | +       | -     | 178 alive                |
| 82             | F   | 19/12   | Cerebellar     | Astro 2                              | 0.0      | +                                | 2.3      | Normal               | Subtot exc | -       | -     | 175 alive                |
| 104            | M   | 16      | Right Front    | Astro 2                              | 0.0      | +                                | 0.0      | Failed               | Total exc | +       | -     | 162 alive                |
| 138            | F   | 4       | Cerebellar     | Astro 2                              | 0.0      | -                                | 0.4      | Normal               | Total exc | -       | -     | 152 alive                |
| 236            | M   | 24      | Left Temp Lobe | Astro 2                              | 2.8      | -                                | 7.5      | Failed               | Biopsy    | -       | +     | 92 alive                 |
| 267            | M   | 4       | Hypothalamic   | Astro 2                              | 0.0      | -                                | 2.3      | Not Done             | Subtot exc | -       | -     | 65                       |
| 276            | M   | 5/52    | Posterior      | Astro 2                              | 0.0      | -                                | 3.1      | Failed               | Biopsy    | -       | +     | 11                       |
| 60             | M   | 68      | Left Front     | Astro 3                              | 0.0      | -                                | 7.5      | Not Done             | Part exc   | -       | -     | 11                       |
| 67             | M   | 47      | Right Front    | Astro 3                              | 0.0      | +                                | 0.1      | Not Done             | Part exc   | +       | -     | 180 alive                |
| 126            | M   | 30      | Right Temp Lobe| Astro 3                              | 29.4     | -                                | 3.4      | 46XY/46XY t(1;14)    | Subtot exc | +       | +     | 113                      |
| 147            | F   | 37      | Left Temp      | Astro 3                              | 22.0     | +/+++                            | 1.7      | Normal               | Part exc   | -       | -     | 1 other cause            |
| 154            | F   | 63      | Left Temp      | Astro 3                              | 1.2      | +/+++                            | 1.4      | Normal               | Part exc   | -       | -     | 9                        |
| 167            | F   | 44      | Left Temp      | Astro 3                              | 0.0      | -                                | 7.7      | Not Done             | Part exc   | -       | -     | 2                        |
| 194            | M   | 61      | Left Temp      | Astro 3                              | 15.2     | +/+++                            | 9.4      | Not Done             | Biopsy    | -       | -     | 2                        |
| 203            | M   | 42      | Left Post      | Astro 3                              | 2.2      | +/+++                            | 5.7      | Normal               | Subtot exc | +       | -     | 65                       |
| 207            | M   | 52      | Left Temp      | Astro 3                              | 0.0      | +/+++                            | 11.5     | Failed               | Part exc   | +       | -     | 39                       |
| 245            | M   | 52      | Left Temp      | Astro 3                              | 0.0      | +/+++                            | 12.9     | 45Y/46XY             | Biopsy    | +       | +?  | 26                       |
| 237            | F   | 44      | Left Temp      | Astro 3                              | 0.0      | +/+++                            | 2.1      | Failed               | Part exc   | +       | -     | 53                       |
| 273            | M   | 33      | Cerebellar     | Astro 3                              | 1.6      | -                                | 4.5      | 45Y/46XY             | Part exc   | -       | -     | 9                        |
| 285            | M   | 12      | Left Parietal  | Astro 3                              | 0.0      | +                                | 7.5      | 46XY/46XY (16) = ? del 16p | Subtot exc | +       | +     | 77                       |

(continued overleaf)
| Patient number | Sex | Age yrs | Site of tumour | Histological diagnosis and grade (1-4) | p53 LI % | labelling intensity (0-4) | Ki67 LI % | Cytogenetic analysis | Treatment | Surgery | Therapy | Chemo | Survival time PostOp Weeks |
|----------------|-----|---------|----------------|--------------------------------------|----------|-------------------------|----------|----------------------|-----------|---------|---------|------|--------------------------|
| 69             | M   | 35      | Right Temp Lobe | Astro 4 Recur                        | 19.7     | ++/+/++++              | 100      | 13.8                 | Not Done  | Part exc | –       | +    | 47                       |
| 75             | M   | 54      | Right Pariet Lobe| Astro 4                              | 0.0      | +/+++++                | 100      | 21.0                 | Not Done  | Biopsy   | +       | –    | 48                       |
| 112            | M   | 33      | Left Front Lobe | Gliomulti 4                          | 14.3     | –                      | 99       | 1.5                  | 45X-Y/46XY/  | Subtot exc | –       | –    | 25                       |
| 113            | M   | 65      | Right Occip Lobe| Astro 4                              | 0.0      | +/+++++                | 100      | 15.3                 | 45X-Y/47XXY| Subtot exc | –       | –    | 3 other causes           |
| 116            | F   | 58      | Right Temp Lobe | Gliomulti 4                          | 0.0      | +/+/++++               | 100      | 11.0                 | Normal    | Part exc  | +       | –    | 65                       |
| 134            | F   | 59      | Right Temp Lobe | Astro 4                              | 3.5      | ++/+/++++              | 100      | 14.4                 | 47XX9p+ +10q| Subtot exc | –       | –    | 37                       |
| 139            | M   | 44      | Left Frong Lobe | Gliomulti 4                          | 0.0      | –/+                   | 80-85    | 8.1                  | Not Done  | Total exc | –       | –    | 24                       |
| 142            | F   | 41      | Right Temp Lobe | Gliomulti 4                          | 3.2      | ++/+/++++              | 100      | 5.1                  | Not Done  | Part exc  | +       | –    | 50                       |
| 150            | M   | 37      | Right Pariet Temp Lobe | Gliomulti 4 | 15.5      | –                      | 50       | 7.8                  | 45X-Y/46XY/  | Subtot exc | –       | –    | 19                       |
| 158            | M   | 49      | Left Post Pariet Lobe | Astro 4 | 0.0      | ++/+/++++              | 100      | 13.4                 | 45X-Y/46XY| Subtot exc | –       | –    | 51                       |
| 165            | F   | 50      | Right Front Lobe | Gliomulti 4                          | 61.9     | ++/+/++++              | 100      | 16.7                 | 46XX/46XX  | Subtot exc | –       | –    | 37                       |
| 169            | M   | 67      | Left Temp Pariet Lobe | Astro 4 | 0.0      | ++/+/++++              | 100      | 0.6                  | 45X-Y/46XY| Subtot exc | –       | +    | 24                       |
| 170            | F   | 55      | Right Temp Lobe | Gliomulti 4                          | 5.6      | +/+++++                | 100      | 13.3                 | Normal    | Part exc  | +       | –    | 86                       |
| 212            | F   | 55      | Right Pariet Lobe | Astro 4                              | 4.7      | +/–                    | 100      | 8.9                  | Not Done  | Subtot exc | –       | –    | 5                        |
| 238            | M   | 67      | Right Occip Pariet Lobe | Astro 4 | 0.3      | ++/++                  | 99       | 5.1                  | Failed    | Part exc  | +       | –    | 40                       |
| 252            | M   | 60      | Right Front Lobe | Astro 4                              | 0.0      | ++/+/++++              | 100      | 20.3                 | Failed    | Subtot exc | +       | –    | 35                       |
| 258            | M   | 68      | Right Pariet Occip Lobe | Gliomulti 4 | 10.5     | +/+/++++               | 100      | 6.7                  | 45X-Y/46XY| Subtot exc | –       | –    | 17                       |
| 263            | F   | 45      | Left Amyg Thal Hypothal Pedun Pit | Astro 4 | 0.1      | +/+/++++               | 100      | 3.8                  | Not Done  | No Surgery | –       | –    | 0                        |
| 270            | M   | 59      | Right Temp Lobe | Gliomulti 4                          | 15.7     | –                      | 100      | 21.6                 | 45X-Y/46XY| Part exc  | +       | –    | 24                       |
| No | M/F | Tumour Location | Tumour Type | Tumour Grade | Outcome | Status | Comments |
|----|-----|----------------|-------------|--------------|---------|--------|----------|
| 278 | M 37 | Right Temp Lobe | Astro 4 | 0.8 | Failed | Part exc | + | – | 39 |
| 108 | F 7 | Left Front Pariet Lobe | GigGlio | 0.8 | Normal | Total exc | + | + | 95 alive |
| 18 | M 14 | Left Front Lobe | Astrobl 3 | 0.0 | Failed | Part exc | + | – | 143 alive |
| 210 | M 23 | Right Front Left Parasag Lobe | Astrobl 3 | 2.6 | Normal | Total exc | + | + | 51 alive |
| 239 | F 62 | Right Front Lobe | Oligo 2 | 0.0 | Failed | Subtot exc | + | – | 52 alive |
| 202 | M 20 | Floor 4th Ventr | Epend 2 | 0.0 | Failed | Total exc | + | – | 52 alive |
| 144 | F 21/12 | Post Fos | Epend 3 | 0.0 | Normal | Total exc | + | + | 97 alive |
| 168 | M 52 | Fil Term | EpendMyx | 0.0 | Failed | Total exc | – | – | 65 alive |
| 229 | F 59 | Fil Term | EpendMyx | 0.0 | Not Done | Subtot exc | + | – | 54 alive |
| 71 | F 30 | Left Occip Lobe | PNET | 26.5 | Failed | Total exc | – | – | 113 alive |
| 98 | M 14 | Cерв Mid | PNET | 26.5 | Normal | Total exc | + | + | 93 alive |
| 231 | F 5 | Right Front Pariet Lobe | PNET cystic | 0.0 | Normal | Total exc | – | – | 105 alive |
| 288 | F 5 | Theca L4/5 | PNET Metas | 0.0 | Normal | Biopsy | + | + | 35 alive |
| 289 | F 3 | Left Front Temp Lobe | PNET Metas | 0.0 | Normal | Biopsy | + | + | 35 alive |
| 237 | M 55 | Left Lat Ventr | Chor Plex Papill early malignant | 0.0 | Failed | Total exc | – | – | 31 |
| 286 | M 8/52 | Left Chor | Chor Plex Papill early malignant | 0.0 | Failed | Total exc | – | – | 45X-21 or -22 |
| 291 | M 55 | 4th Ventr | Chor Plex Papill non-malignant | 0.0 | Failed | Total exc | – | – | 24 alive |
| 200 | M 25 | Left Temp Pariet Lobe | Angioglioma 3 | 0.0 | Failed | Part exc | + | – | 67 alive |
| 175 | M 62 | Right Temp Lobe | Angioglioma 4 | 0.0 | 45X-Y/46X-Y + 20 | Not Done | ? | – | 1/7 other causes |
| 221 | F 31 | Cерв | Haemangioblast | 0.0 | 45X-Y/46X-Y + 20 | Not Done | ? | – | 2 other causes |
| 146 | F 38 | Medulla | Haemangioblast | 0.0 | Normal | Total exc | – | – | 1 |
| 227 | F 10 | Lat Hypo Thal | Dysgerminoma | 3.9 | Failed | Biopsy | + | – | 54 alive |
| 283 | M 55 | Right BasSk | ChondroSarc | 0.0 | Failed | Total exc | – | – | 35 alive |
| 52 | M 5 | Right CPA | Angioma | 0.0 | Normal | Total exc | – | – | 134 alive |
| 68 | M 45 | Pit | Pit Adenoma | 0.0 | Failed | Subtot exc | + | – | 118 alive |
| 164 | M 52 | Pit | Pit Adenoma | 0.0 | Failed | Part exc | + | – | 75 alive |
| 233 | M 54 | Pit | Pit Adenoma | 0.0 | Failed | Part exc | + | – | 50 alive |

(continued overleaf)
endothelial proliferation. The nuclear labelling did not correlate with any particular tumour cell type. This was most obvious in such morphologically heterogeneous tumours such as glioblastoma multiforme or giant cell tumours where both small and large nuclei were either labelled or unlabelled (Figures 2 and 3). The intensity of the nuclear labelling within the tumour also varied: lesser or more intensely labelled nuclei were intermingled in an irregular fashion (Figures 2 and 3).

Table II
Univariate analysis (Cox's regression) for age and Ki-67 LI data

| Variable | Coefficient | SE | Relative risk | P-value |
|----------|-------------|----|---------------|---------|
| Age      | 0.0378      | 0.0106 | 1.038          | 0.0002  |
| Ki-67 LI | 0.077       | 0.021  | 1.08          | 0.0001  |

Table III
Univariate analysis (Log-rank test)

| Variable | X² | P-Value |
|----------|----|---------|
| Sex (F vs M) | 0.07 | 0.79  |
| p53 (+ vs VE vs -VE) | 4.44 | 0.035  |
| EGFR (+ vs VE vs -VE) | 7.34 | 0.007  |
| Ki-67 LI (<5% < 18.3% >5%) | 20.50 | <0.0001 |
| Surgery (total vs subtotal vs partial vs biopsy) | 7.37 = X²; 0.06 |
| Histopathology grade (1,2 vs 3,4) | 18.30 | <0.0001 |
| Radiotherapy (+ vs -) | 0.62 | 0.43  |
| Chemotherapy (+ vs -) | 0.01 | 0.94  |

*Note, 3 degrees of freedom.

Figure 1 Survival curves for patients with astrocytoma grade I and 2 (n = 9) and patients with astrocytoma grade 3 and 4 (n = 31). Log-Rank statistic = 18.30; P < 0.0001; d.f. = 1.
Figure 2 Positive p53 mutant protein nuclear labelling in astrocytoma grade 4 (glioblastoma multiforme) with PAB 1801; both small and large nuclei were either labelled (brown) or unlabelled (blue). Scale bar = 30 μm.

Figure 3 Positive p53 mutant protein nuclear labelling in giantocellular glioma with PAB 1801; both small and large nuclei were either labelled (brown) or unlabelled (blue). Scale bar = 30 μm.

Table IV Expression of p53 and EGF receptor proteins in CNS tumours

| Tumour type | 1 | 2 | 3 | 4 | 5 |
|-------------|---|---|---|---|---|
|             | 2 p53 + ve | EGF + ve | Ratio | % | Ratio | % |
| Astrocytoma Grade 1 | 0/4 | 0% | 0/4 | 0% |
| Grade 2 | 1/6 | 17% | 2/6 | 33% |
| Grade 3 | 5/13 | 38% | 11/13 | 85% |
| Grade 4* | 13/20 | 65% | 9/20 | 95% |
| Total | 19/43 | 44% | 32/43 | 74% |
| Primitive neuro-ectodermal tumours | 1/6 | 17% | 4/6 | 67% |
| Astroblastoma | 1/2 | 2/2 |
| Giantocellular glioma | 1/1 | 1/1 |
| Oligodendroglioma | 0/1 | 0/1 |
| Ependymoma Grade 2/3 | 0/2 | 0/2 |
| Myxopapillary | 0/2 | 0/2 |
| Angioblastoma | 0/3 | 1/2 |
| Choroid plexus papilloma | 0/3 | 3/3 |
| CNS dyserminoma | 2/2 | 1/2 |
| Pituitary tumour | 0/3 | 0/3 |
| Haemangioblastoma | 0/3 | 2/3 |
| Chordoma | 0/1 | 0/1 |
| Tumours in Von Recklinghausen’s neurofibroma* | 0/1 | 0/1 |
| neurofibrosarcoma* | 1/1 | 1/1 |
| Angioma (developmental abnormality) | 0/1 | 0/1 |
| Metastatic tumours in CNS | 0/1 | 0/1 | 0/1 |
| carcinoma | 0/1 | 0/1 |
| melanoma | 1/1 | 0/1 |

*Includes glioblastoma multiforme. *Associated with spinal roots.

*Intrinsic to cerebrum.

Table V p53, EGFR, and Ki-67 labelling in relation to histopathological tumour grade

| Grade | p53 LI | EGFR labelling factor | Ki-67 LI |
|-------|--------|-----------------------|---------|
|       | Mean (s.d.,n) | vs 3 & 4; P<0.0001 | Mean (s.d.,n) | d.f. = 41 |
| 1 & 2 | 110.1(31.6;10) | vs 3; P = 0.01 | 1.1(1.0;10) | d.f. = 21 |
| 3 | 14.1(12.2;13) | 248.6(113.1;13) | 8.9(9.1;13) |
| 4 | 12.4(16.3;20) | 323.0(136.1;20) | 13.4(7.3;20) |

Patients with p53 + ve astrocytomas had a reduced survival (P = 0.035; Table III) – only 11% of these patients were alive at 100 weeks following operation and diagnosis compared to 36% of patients with p53-ve tumours (Figure 5). The number of cases with tumours in the other categories were too small to attempt survival analyses.

Immunohistochemistry of EGFR

No labelling was seen in normal neocortical (12 ×) and normal cerebellar (4 ×) nervous tissue adjacent to tumours or in tumour endothelial cells. The EGFR labelling of tumours was restricted to cytoplasmic regions and, in some instances, to cell membranes of tumour cells in the EGFR + ve tumours (Figures 6 and 7). This is in contrast to normal human epidermis, which was used to determine the optimal dilution of the EGFR antibody, where the labelling was associated exclusively with cell membranes.

Eleven out of 16 types of tumours examined had EGFR positive cells (Table IV, column 1 and 4). It should be noted that a higher proportion of all astrocytomas was labelled with EGFR (74%) than p53 antibody but that, similar to p53 labelling, none of the grade 1 tumours were labelled, and the proportion of positive tumours increased with tumour grade (Table IV, column 5). In some tumours the EGFR labelling was intense and uniform both in terms of distribution and intensity (Figure 6) but in other tumours it was fainter (Figure 7) or patchy and of variable intensity (see also Table 1, column 7). The variability of these two parameters was taken into account by calculating EGFR labelling factor for each tumour (see Material and methods). Astrocytomas grades 3 & 4 had a significantly higher mean labelling factor than grades 1 and 2 (P<0.0001; Table V) indicating that the intensity/area of EGFR labelling increased with malignancy.
grade. Other tumours with high degree of EGFR labelling included PNETs, astroblastomas, oligodendrogliomas, choroid plexus papillomas, and angiogliomas (Table I, column 7).

Patients with EGFR +ve astrocytomas appeared to have reduced survival ($P = 0.007$; Table III) – only 13% of these patients were alive at 100 weeks following diagnosis compared to 60% of EGFR-ve patients (Figure 8). The number of cases with tumours in the other categories were too small to attempt survival analysis.

![Figure 5](image)

**Figure 5** Survival curves for patients with p53 negative astrocytomas (p53neg--; $n = 22$) and patients with p53 positive astrocytomas (p53pos--; $n = 18$). Log-Rank statistic = 4.44; $P = 0.035$; d.f. = 1.

![Figure 6](image)

**Figure 6** Intense positive EGFR labelling (brown) in astrocytoma grade 4 (glioblastoma multiforme) with EGFR1 antibody; the blood vessel ($V$) is unlabelled. Scale bar = 30 μm.

![Figure 7](image)

**Figure 7** Faint EGFR labelling (pale brown) in gigantocellular glioma with EGFR1 antibody. Scale bar = 30 μm.

**Immunohistochemistry of Ki-67**

At the cellular level the Ki-67 antibody reactivity had an exclusively nuclear distribution, and was either uniform or granular (Figure 9). Most tumours had at least some Ki-67 labelled nuclei with the exception of one grade 2 astrocytoma in tuberous sclerosis, one angioglioma, one chordoma and one angioma (Table I, column 8). Heterogeneity in Ki-67 nuclear labelling was found in around of 20% astrocytomas (Table I, column 8). However, the mean values of the Ki-67, LI, representing the proliferative potential of the tumour (see Material and methods), showed a statistically significant increase with increasing grade of astrocytoma malignancy (1 and 2 vs 3; $P = 0.01$; 3 vs 4; $P = 0.006$; Table V).

In the Univariate analysis the astrocytoma patients' Ki-67 LI, when analysed as a continuous variable by Cox regression analysis, showed a strong relationship to the length of survival ($P > 0.0001$; Table II). When analysed by the Log-Rank test patients with Ki-67 LI > 5% (5.1 to 30.9%) had a reduced survival ($P < 0.0001$; Table III) – none of these patients survived beyond 86 weeks following diagnosis compared with 63% of patients with Ki-67 LI of < 5% (0.1 to 3.9%) who were still alive at 100 weeks (Figure 10). The number of cases with tumours in the other categories were too small to attempt survival analysis.

![Figure 8](image)

**Figure 8** Survival curves for patients with EGFR receptor negative astrocytomas (EGFRneg--; $n = 10$) and patients with EGFR receptor positive astrocytomas (EGFRpos--; $n = 30$). Log-Rank statistic = 7.34; $P = 0.007$; d.f. = 1.

![Figure 9](image)

**Figure 9** Positive Ki-67 nuclear labelling (brown) in astrocytoma grade 4 (glioblastoma multiforme); a blood vessel ($V$) displays one labelled endothelial cell nucleus (arrow). Scale bar = 30 μm.
Relationship between p53, EGFR and Ki-67 labelling

In about 25% of the astrocytomas regional heterogeneity was detected either in p53, EGFR, or Ki-67 labelling, and therefore more than one area was counted in those tumours (Table I, columns 6, 7, 8). Note that in most cases only one of the three antibodies showed heterogeneity, in a few cases two antibodies but never all three simultaneously. The relationship between p53, EGFR and Ki-67 labelling was determined in serial sections from corresponding areas but not between different areas. The p53 + ve astrocytomas had a significantly higher mean Ki-67 LI than p53-ve astrocytoma (Table VI), although the values of the continuous variables, p53 LI and Ki-67 LI, did not show any correlation within the individual tumours (r = 0.19; P = 0.17). A pictorial example of absence of correlation should be noted in Figure 4 where regional heterogeneity in the p53 labelling cannot be explained by differences in proliferative activity between the two areas since they both had an almost identical Ki-67 LI of 9%.

The continuous variables, EGFR labelling factor and Ki-67 LI, were significantly correlated within the individual tumours (r = 0.32; P = 0.018). The EGFR + ve astrocytomas had a significantly higher mean Ki-67 LI than EGFR-ve astrocytomas (P = 0.028; Table VI). Astrocytomas which were both EGFR + ve and p53 + ve had a somewhat higher Ki-67 LI than astrocytomas which were EGFR + ve but p53-ve, though the difference was not significant (P = 0.49; Table VI). Astrocytomas not expressing either EGFR or p53 proteins had the lowest mean Ki-67 LI (P < 0.0001; Table VI), although the continuous variables, p53 LI and EGFR labelling factor, showed no correlation within individual tumours (r = 0.04; P = 0.86). The absence of correlation is probably due to variability in p53 LI between tumours (see Table I, column 6), and also due to non-overlapping regional heterogeneity in EGFR and p53 labelling within individual tumours.

The effect of EGFR and p53 expression on patient's survival was not cumulative (P = 0.66; Figure 11) – similar proportions of patients with EGFR + ve & p53 + ve tumours (12%) were alive at 100 weeks following diagnosis compared to patients with EGFR + ve & p53-ve tumours (15%). In contrast, patients with EGFR-ve & p53-ve tumours had a significantly better survival rate than both of the previous groups (67%; P = 0.016; Figure 11).

Multivariate analysis

The list of all variables which were analysed for prognostic importance by univariate analysis are shown in Table II (Cox’s Regression for continuous variables) and in Table III (Log-Rank test for categorised variables). Note that the univariate prognostic importance of Ki-67 LI is of the same order of magnitude (P < 0.0001) whether it is considered as a

![Graph](image)

**Figure 10** Survival curves for patients with Ki-67 LI below 5% (<5% - - - ; n = 16) and patients with Ki-67 LI above 5% (>5% - - - ; n = 24). Log-Rank statistic = 20.50; P < 0.0001; d.f. = 1.

**Table VI** Ki-67 labelling in relation to p53 and EGFR labelling

|       | Ki-67 LI | P-value | Degrees of freedom |
|-------|----------|---------|--------------------|
|       | Mean (SD,n) |         |                    |
| p53-ve | 6.2(6.6;24) | 0.036   | 41                |
| p53 + ve | 11.8(10.2;19) |         |                    |
| EGFR-ve | 4.1(6.3;11) | 0.028   | 41                |
| EGFR + ve | 10.7(8.3;32) |         |                    |
| p53-ve & EGFR + ve | 9.0(7.3;14) | 0.49     | 30                |
| p53 + ve & EGFR + ve | 11.2(10.2;18) |         |                    |
| p53-ve & EGFR-ve | 2.3(2.7;10) | <0.0001 | 40                |
| EGFR + ve (= p53-ve & EGFR + ve plus p53 + ve & EGFR + ve) | 10.7(8.3;32) |         |                    |
| p53 + ve & EGFR-ve | 21.6 (1)   |         |                    |
continuous or categorical (<5% vs >5%) variable. Sex, radiotherapy and chemotherapy had non-significant univariate P-values, while surgery was of weak statistical importance (P = 0.06; Table III). All variables apart from sex, radiotherapy and chemotherapy were entered into a multivariate analysis to determine whether they influence the patient’s prognosis independently or are associated with each other. The multivariate analysis was performed by using a forward stepwise application of Cox’s Regression model. Tables VII and VIII shows that the only variables selected as statistically significant (P < 0.10) by this procedure were histopathological grade and Ki-67 LI. Although several of the variables had a significant prognostic importance following univariate analysis (Tables II and III), the multivariate analysis reveals histopathological grade as the overwhelming dominating factor with Ki-67 LI being the only other variable with prognostic information once histopathological grade has entered the model. By employing the regression procedure with only Ki-67, EGFR and p53 labelling as independent variables, only Ki-67 labelling was significant (P = 0.0002), and therefore the controlling variable. This is because many of the variables do not influence the survival of astrocytoma patients independently, and are interrelated with each other as follows, histopathological grade and age: 1 and 2 vs 3 and 4; P < 0.0001; histopathological grade and Ki-67 LI; 1 and 2 vs 3: P = 0.01; 3 vs 4; P = 0.006; histopathological grade and EGFR labelling factor: 1 and 2 vs 3 and 4; P < 0.0001; age and Ki-67 LI; r = 0.37, P = 0.014; age and EGFR labelling factor: r = 0.57, P < 0.0001; Ki-67 LI and EGFR labelling factor: r = 0.32, P = 0.0018; except for histopathological grade and p53 LI: P = 0.83; age and p53 LI: r = 0.06, P = 0.77; Ki-67 LI and p53 LI: r = 0.19, P = 0.17; and EGFR labelling factor and p53 LI: r = 0.04, P = 0.86.

Discussion

This study demonstrates immunohistochemically detectable levels of p53 protein in tumour cell nuclei of many CNS and non-CNS tumours. The monoclonal antibody used in this study can recognize both normal and mutant forms of human p53 proteins (Banks et al., 1986; Rodrigues et al., 1990) but the labelling almost certainly represents accumulation of nonfunctional p53 mutants only. The mutants are detectable by immunohistochemistry (Cattoretti et al., 1988; Iggo et al., 1990; Rodrigues et al., 1990) because of their metabolic stability, and their cellular levels are elevated 10–100 fold above normal values (Finlay et al., 1989). In the present series none of the normal CNS tissue adjacent to the tumour was labelled with PAb 1801 antibody. Also, the labelling of tumour cells is unlikely to represent the somewhat elevated levels of normal p53 seen in actively proliferating cell populations (Dippold et al., 1981; Levin & Momand, 1990) because some tumours with large growth fractions were not labelled with the PAb 1801 antibody, whilst in p53 +ve tumours the p53 LI did not correlate with the growth fraction size. In addition, no endothelial cells were labelled in any of the tumours, even in areas where endothelial proliferation, and Ki-67 labelling were present.

Our finding that p53 mutants are expressed in astrocytomas, primitive neuroectodermal tumour, astroblastomas, gigantocellular glioma, neurofibrosarcoma, CNS dysgerminomas, and melanoma extends the number of human tumours with identified mutations in p53 gene. So far, the list included carcinomas of the breast, lung, colorectum, and liver, and also neurofibrosarcoma, osteosarcoma and glioblastomas multiforme (Masuda et al., 1987; Cattoretti et al., 1988; Nigro et al., 1989; Iggo et al., 1990; Rodrigues et al., 1990; Menon et al., 1990; Bressac et al., 1991; Husu et al., 1991). Using karyotyping we have not detected abnormalities of chromosome 17p (where gene p53 is localised) in any of the p53 +ve tumours in this study. This may not be surprising since accumulation of p53 protein is almost certainly an outcome of point mutations in the p53 gene (Nigro et al., 1989) which is beyond resolution of karyotyping.

In the present series the p53 labelling was exclusively localised in tumour cell nuclei, whilst in other tumour types either nuclear or a combined nuclear and cytoplasmic p53 labelling has been found (Iggo et al., 1990; Rodrigues et al., 1990). The nuclear localisation of p53 indicates presence of transforming p53 mutants. Normal p53 protein is thought to have a role in regulating gene expression or DNA replication (Michaelovitz et al., 1991), and it appears that nuclear localisation of p53 mutants is essential for their transforming activity (Shaullsky et al., 1990). The reason for some p53 mutants accumulating in the nuclei is unclear. Cytoplasmic accumulation of some p53 mutants has been reported to occur because a conformational change in their molecule leads them to form complexes with cytoplasmic heat-shock cognate protein 70 (hsc70; Sturtzbecher et al., 1988). It would be of interest to see whether the p53 mutants that accumulate in nuclei of different tumour types in this and other studies share a particular conformational change, and bind to an as yet unidentified nuclear protein.

In astrocytomas this study has found that the nuclear

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**Table VII** Variables that achieved P < 0.10 following forward stepwise Cox regression on all variables, Ki-67 LI analysed as continuous variable

| Variable      | Coefficient | SE   | Relative risk (95% C.I.)* | P-value | \(\chi^2\) to enter model |
|---------------|-------------|------|--------------------------|---------|--------------------------|
| Histological grade | 2.920       | 1.050| 18.50 (2.37,145.2)        | 0.005   | 26.23                    |
| Ki-67 LI      | 0.044       | 0.025| 1.045 (0.99,1.10)         | 0.08    | 2.99                     |

*95% C.I. = 95% Confidence Interval for Relative Risk is given by \(\text{coefficient} \pm 1.645\text{coefficient}\).

**Table VIII** Variables that achieved P < 0.10 following forward stepwise Cox regression on all variables, Ki-67 LI categorical <5% vs >5%

| Variable      | Coefficient | SE   | Relative risk (95% C.I.)* | P-value | \(\chi^2\) to enter model |
|---------------|-------------|------|--------------------------|---------|--------------------------|
| Histological grade | 2.694       | 1.082| 14.80 (1.78,123.2)        | 0.012   | 26.23                    |
| Ki-67 LI      | 0.975       | 0.508| 1.921 (0.98,7.17)         | 0.055   | 4.31                     |

*95% C.I. = 95% Confidence Interval for Relative Risk is given by \(\text{coefficient} \pm 1.645\text{coefficient}\)
expression of p53 mutants was associated with increase in tumour malignancy and poor prognosis. Using immunohistochemistry, similar observations, though without survival data, have been made in neurofibrosarcoma, and carcinomas of the breast, lung, and colorectum (Baker et al., 1989; Vogelstein et al., 1989; Iggo et al., 1990; Harris et al., 1990; Menon et al., 1990). In astrocytomas p53 expression has not yet been examined by immunohistochemistry but allele loss of chromosome 17p, and presumed mutations in p53 gene, have been reported to be associated with tumour initiation (James et al., 1989; El-Azouzi et al., 1989) which contrasts with the absence of changes in the present study. The reasons for the apparent difference between conclusions of the present and previous studies may be due to the difference in sensitivities of the different techniques employed, and may not emerge until the mechanism of tumour evolution in astrocytomas is more precisely understood at molecular level. However, several possible explanations might be considered. Firstly, low and high grade astrocytomas may originate from different tumour precursor cells (James et al., 1988). The precursor cells giving rise to low grade astrocytomas may be affected by loss-of-function p53 mutations leading to a failure to express any p53 RNA and protein, similar to that reported in a proportion of rhabdomyosarcomas, osteosarcomas, and Li-Fraumeni lesions (Masuda et al., 1987; Mulligan et al., 1990; Malkin et al., 1990). Only precursors giving rise to high grade astrocytomas may be affected by p53 mutations leading to overexpression of the p53 mutants, similar to that reported in carcinoma of the breast and lung (Cattoretti et al., 1988; Iggo et al., 1990). This scheme, however, implies that benign astrocytomas cannot progress to a malignant stage, which is contrary to clinical and histopathological observations (Russell & Rubinstein, 1989). Alternatively, if astrocytomas progress from low to higher grades (Russell et al., 1988), then changes in the p53 gene, analogous to those proposed for colorectal carcinomas: the first or initiating step involving mutation in one allele only and a synthesis of inactive mutant/normal oligomers; further loss of control is believed to result from deletion of the normal allele, leaving the cell with only a mutant allele (Nigro et al., 1989). The present immunohistochemical assay has detected p53 mutant molecules, but may not have been sufficiently sensitive to detect cells expressing oligomeric p53 mutant/normal molecules, similar to observation made by Rodrigues et al. (1990) on cell lines derived from acute lymphoblastic leukaemia. The finding that p53 LI was variably expressed between tumours, and regionally heterogeneous within tumours, indicates that p53 mutant molecules were expressed in subclones of astrocytomas which were not part of the major line of tumour development. If a precursor cell expressed p53 mutants, all the daughter tumour cells in high grade astrocytomas would also express the mutant molecules.

In this series EGFR was expressed in astrocytomas and ten other tumour types, mostly with glial and/or neuroepithelial differentiation, but not in normal brain tissue adjacent to the tumours. In astrocytomas EGFR expression was associated with increase in tumour malignancy and poor prognosis. Survival data confirm findings of previous biochemical (Liberman et al., 1984; 1985) and immunohistochemical studies on astrocytomas (Reifenberger et al., 1989), and extend them by survival data. But, unlike human breast cancer (Harris et al., 1990), this series does not support a direct association between extent of EGFR and p53 mutants: there was no correlation between p53 LI and categories of EGFR staining, possibly due to nonoverlapping regional heterogeneity in each parameter. This suggests that in astrocytomas p53 mutants and EGFR are expressed in different subclones of tumour cells, and that the associations between increase in tumour malignancy and the expression of EGFR protein or p53 mutations were independent of each other.

Overexpression of EGFR, previously found to occur in a high proportion of malignant gliomas, has been related either to an amplification of the EGFR gene, often in the form of double minutes (Liberman et al., 1984; 1985; Wong et al., 1987), or to an extra copy of chromosome 7 (Liberman et al., 1984) on which the EGFR gene is located (Shimizu et al., 1985), or to loss of control of transcriptional activity of the gene (Gerosa et al., 1989). In this study trisomy of chromosome 7 was found only in one patient, and double minutes in none. It therefore seems that in the present series the possible mechanisms responsible for EGFR overexpression are either amplification at the EGFR gene locus which is not easily detectable by karyotyping, or loss of control of transcriptional activity. Our observation that in the majority of the brain tumours EGFR labelling had a predominantly cytoplasmic expression which was explained by rapid internalisation of the EGF after ligand binding (Stoscheck & King, 1986; Humphrey et al., 1990). Alternatively, similar to human glioma cell lines which show co-expression of high levels of EGFR and one of its ligands TGF-α (Nister et al., 1988), an autocrine growth stimulation loop may operate in astrocytomas in vivo, and the cytoplasmic labelling may represent cytoplasmic binding of EGF to its ligand.

In this series high Ki-67 LI was associated with reduced survival. This is contrary to the only previous study which included survival data (Zuber et al., 1988), possibly due to a small sample size in the previous study. It is likely that the poor prognosis found in patients with Ki-67 LI higher than 5% reflects a significant correlation between mean size of the growth fraction, as determined with the Ki-67 index, and histopathological grade of malignancy. The findings of others have been summarised in a recent review (Liberman et al., 1990). The latter observation is in broad agreement with previous studies (Raghavan et al., 1990; Brown & Gatter, 1990) but the conclusions of the present study go further – the Multivariate analysis has demonstrated that the histopathological grade is the most important variable to influence the patient’s survival, when all variables are considered together. The analysis has also demonstrated that histopathological grade, age, Ki-67 LI and EGFR do not influence survival of astrocytoma patients independently but, except for the p53 labelling, are interrelated with each other. The proliferative ability of astrocytoma cells, as determined by Ki-67 LI, appeared to be positively influenced by expression of both p53 mutants and EGFR protein, since p53 +ve or EGFR +ve astrocytomas had significantly higher Ki-67 indices than p53-ve or EGFR-ve astrocytomas but the Ki-67 LI is the most important variable to influence the patient’s survival when considered together with the EGFR and p53 labelling. This finding and two other observations indicate that alternative or additive mechanisms to expression of p53 mutants and EGFR are likely to be involved in controlling tumour cell proliferation and tumour progression in astrocytomas. Firstly, in p53-ve and/or EGFR-ve astrocytomas cells may be proliferating in a nonreplicative manner, even though it was lower than in p53 +ve and/or EGFR +ve tumours. Secondly, though EGFR labelling correlated positively with Ki-67 LI, analysis of p53 LI and Ki-67 LI did not show a significant correlation. These conclusions are in keeping with earlier cytogenetic findings which have implicated multiple chromosomal abnormalities in malignant progression of gliomas in addition to 17p and 7, where p53 and EGFR genes respectively, are involved (Bigner & Wolfe, 1986; James et al., 1988). In model for progression of gliomas Bigner and Vogelstein (1990) proposed that abnormality on chromosome 17p occurs at early stages of tumorigenesis, whilst EGFR is thought to stimulate further progression of malignant gliomas. This study has demonstrated that in astrocytomas expression of both p53 mutants and EGFR can occur at early stage of EGFR expression, since their expression also represents mechanisms associated with malignant progression and poor prognosis but that expression of neither protein may be essential for this process.

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References

ARITA, N., HAYAKAWA, T., IZUMOTO, S. & S. others (1989). Epidermal growth factor receptor in human glioma. J. Neurosurg., 70, 916.

BAKER, S.J., FEARON, E.R., NIGRO, J.M. & others (1989). Chromosome 17 deletions and p53 gene mutations in colorectal carcinomas. Nature, 342, 217.

BANKS, L., MATLASHEWSKI, G. & CRAWFORD, L. (1986). Isolation of human-p53 specific monoclonal antibodies and their use in the studies of human p53 expression. Eur. J. Biochem., 159, 529.

BIGLER, S.H., MARK, J., FRIEDMAN, H.S., BIEGEL, J.A. & BIGNER, D.D. (1988). Structural abnormalities in human medulloblastoma. Cancer Genet. Cytoenet., 30, 91.

BIGNER, S.H., MARK, J., MAHALEY, Jr. M.S. & BIGNER, D.D. (1984). Patterns of the early gross chromosomal changes in malignant human gliomas. Cytogenetics, 17, 103.

BIGNER, S.H. & VOGELSTEIN, B. (1990). Cytogenetics and molecular genetics of malignant gliomas and medulloblastomas. Brain Pathol., 1, 12.

BRESSAC, B., KEW, M., WANDS, J. & OTZURK, M. (1991). Selective G to T mutations of p53 gene in hepatocellular carcinoma from southern Africa. Nature, 350, 429.

BROWN, D.C. & GATTER, K.C. (1990). Monoclonal antibody Ki-67: its use in histopathology. Histopathology, 17, 489.

CAPPEN, R.L. (1987). Receptors for epidermal growth factor and other polypeptide milogons. Ann. Rev. Biochem., 56, 881.

CATTORETTI, G., RILKE, F., ANDREOLA, S., D'AMATO, L. & DELLA, D. (1988). P53 expression in breast cancer. Int. J. Cancer, 41, 178.

COX, D.R. (1972). Regression models and lifetables. J. R. Stat. Soc., 34, 187.

DIPPOLD, W.G., JAY, G., DELEO, A.B., KOURY, G. & OLD, L.J. (1981). P53 transformation-related protein: detection by monoclonal antibody in mouse and human cells. Proc. Natl Acad. Sci. USA, 84, 7186.

EL-AZOUI, M., CHUNG, R.Y., FARMER, G.E. & others (1989). Loss of distinct regions on the short arm of chromosome 17 associated with tumorigenesis of human astrocytomas. Proc. Natl Acad. Sci. USA, 86, 244.

FINLAY, C.A., HUBERD, P.W. & LEVINE, A.J. (1989). The p53 proto-oncogene can act as a suppressor of transformation. Cell, 57, 1083.

GROSA, M.A., TALARICO, D., FOGNANI, C. & others (1989). Overexpression of N-ras oncogene and epidermal growth factor receptor gene in human glioblastomas. J. Natl Cancer Inst., 61, 63.

GERDES, J., LEMKE, H., BAISH, H., WACKER, H.H., SCHWAB, U. & STEIN, H. (1984). Cell cycle analysis of a proliferation associated human nuclear antigen defined by the monoclonal antibody Ki-67. J. ImmunoL, 133, 1710.

GRIFFIN, C.A., HAWKINS, A.L., PACKER, R.J., RORKE, L.B. & EMANUEL, B.S. (1988). Chromosome abnormalities in paediatric tumours. Cancer Res., 48, 175.

HARRIS, A.L., HORAK, E., SMITH, K. & others (1990). Mutant p53 is a common genetic abnormality in human breast cancer and associated with EGFR and neaur expression. BACR 31st Meeting. Brit. J. Cancer, 62, 503 (Abstr.).

HEU, I.C., METCALF, R.A., SUN, T., WELSH, J.A., WANG, N.J. & HARRIS, C.C. (1991). Mutational hotspot in the p53 gene in human hepatocellular carcinomas. Nature, 350, 427.

HUMPHREY, P.A., WONG, A.J., VOGELSTEIN, B. & others (1990). Anti-synthetic peptide antibody reacting at the fusion junction of deletion-mutant epidermal growth factor receptors in human glioblastoma. Proc. Natl Acad. Sci. USA, 87, 4207.

HUNTER, T. (1984). The epidermal growth factor receptor gene and its product. Nature, 311, 414.

IGGO, R., GATTER, K., BARTIE, L., LANE, D. & HARRIS, A.L. (1990). Increased expression of mutant forms of p53 oncogene in primary lung cancer. Lancet, 335, 675.

JAMES, C.D., CARLIBM, E., DUMANSKI, J.P. & others (1988). Clonal genomic alterations in glioma malignancy stages. Cancer Res., 48, 5546.

JAMES, C.D., CARLIBM, E., NORDENSKJOLD, M., COLLINS, V.P. & CAVENEVE, W.K. (1989). Mitotic recombination of chromosome 17 in glioblastomas. Proc. Natl Acad. Sci. USA, 86, 2858.

JAROS, E., PEARSON, A.D.J. & PERRY, R.H. (1991b). Immunohistochemical study of p53 protein, epidermal growth factor receptor (EGFR) and Ki-67 labelling in brain tumours. 82nd meeting of British Neuropath Society. Neuopath. Appl. Neurobiol., 17, 52 (Abstr.).

JAROS, E., PERRY, R.H., PEARSON, A.D.J. & others (1991a). p53 expression in brain tumours. 10th meeting of the British Neuro- Oncology Group. Brit. J. Neurosurg., 5, 211 (Abstract).
THOMAS, G.A. & RAFFEL, C. (1991). Loss of heterozygosity on 6q, 16q, and 17p in human central nervous system primitive neuro-ectodermal tumours. Cancer Res., 51, 639.

VOGELSTEIN, B., FEARON, E.R., BAKER, S.J. & 7 others (1989). Genetic alterations accumulate during colorectal tumorigenesis. In Recessive Oncogenes and Tumour Suppression. Cavenee, W., Hastie, N. & Stanbridge (eds) p. 73. Cold Spring Harbor Laboratory Press.

WONG, A.J., BIGNER, S.H., BIGNER, D.D., KINZLER, K.W., HAMILTON, S.R. & VOGELSTEIN, B.E. (1987). Increased expression of the epidermal growth factor receptor gene in malignant gliomas is invariably associated with gene amplification. Proc. Natl Acad. Sci. USA, 84, 6899.

ZUBER, P., HAMOU, M.-F. & DE TRIBOLET, N. (1988). Identification of proliferating cells in human gliomas using the monoclonal antibody Ki-67. Neurosurgery, 22, 364.