Alterations in tumour suppressor gene p53 in human gliomas from Indian patients

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Alterations in the tumour suppressor *p53* gene are among the most common defects seen in a variety of human cancers. In order to study the significance of the *p53* gene in the genesis and development of human glioma from Indian patients, we checked 44 untreated primary gliomas for mutations in exons 5–9 of the *p53* gene by PCR-SSCP and DNA sequencing. Sequencing analysis revealed six missense mutations. The incidence of p53 mutations was 13·6% (6 of 44). All the six mutations were found to be located in the central core domain of p53, which carries the sequence-specific DNA-binding domain. These results suggest a rather low incidence but a definite involvement of p53 mutations in the gliomas of Indian patients.

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1. Introduction

Glioma, a neoplasm of neuroglial cells, is the most common type of brain tumour, constituting more than 50% of all brain tumours. Gliomas account for about 2% of the malignant tumours in adults. Malignant gliomas are the leading cause of central nervous system (CNS) tumour-related death. Patients with glioblastoma have a life expectancy of less than one year even after surgery, chemo- and radiotherapy (Burger and Scheithauer 1993). The prognosis is even worse in children with brain stem malignant gliomas, the death occurring within 6–12 months after diagnosis (Epstein and McCleary 1986). A better understanding of the biological pathway leading to glial tumourigenesis is essential to arrive at new therapeutic modalities.

The *p53* tumour suppressor gene is the most frequently altered gene in human cancer, including brain tumours. The p53 protein is a transcription factor involved in maintaining genomic integrity by controlling cell cycle progression and cell survival (Levine 1997; Somasundaram and El-Deiry 2000). Consistent with this view, about 50% of primary human tumours carry mutations in the *p53* gene (Hollstein *et al* 1991; Levine *et al* 1991). The function of p53 is critical to the efficiency of many cancer treatment procedures, because radiotherapy and chemotherapy act in part by triggering programmed cell death in response to DNA damage. Consequently, tumours which carry mutations in p53 are often difficult to treat, and their prognosis is poor. The characterization of the biochemical pathways by which p53 alteration triggers tumourigenesis is the foundation for the design of novel therapeutic approaches.

Karyotype analysis and restriction fragment length polymorphism (RFLP) analysis of glioma have shown several numerical and structural alterations of chromosomes (Bigner *et al* 1988; Libermann *et al* 1985; James *et al* 1988; Fujimoto *et al* 1989). One of the alterations frequently seen in glioma is LOH on chromosome 17p, which leads to deletion of the region that includes p53 (Chung *et al* 1991; Frankel *et al* 1992; Fults *et al* 1992; von Deimling *et al* 1992; Ghosh *et al* 1994; Rasheed *et al*

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1994; Jain *et al* 1999). Studies at many laboratories have shown that approximately 25% of the glioma carries mutations in *p53* gene (Nigro *et al* 1989; Chung *et al* 1991; Mashiyama *et al* 1991; Ohgaki *et al* 1991; Frankel *et al* 1992; Fults *et al* 1992; Saxena *et al* 1992; von Deimling *et al* 1992; Louis *et al* 1993).

In the study reported here, we tested 44 glioma samples from Indian patients for mutations in the p53 tumour suppressor gene by PCR-SSCP method. We found the *p53* gene mutated in 13·6% (6/44) of the gliomas.

2. Materials and methods

2.1 *Tumour samples*

Glioma tissue samples were obtained from patients who underwent surgery at Manipal Hospital, Bangalore, India. Forty-four patients with different grades of glioma were chosen for the study. Tissue samples were kept frozen at – 70°C. Total DNA was isolated from a part of the frozen samples. Tissue sections were used to identify the histopathological grade and the type of glioma.

2.2 *Isolation of DNA*

A commercial DNA isolation kit (Qiagen, USA) was used to isolate genomic DNA from 25 mg of glioma tissue.

2.3 *Primers for PCR and DNA sequencing*

The primers used were oligonucleotides complementary to the sequence flanking the exon/intron junctions of exons 5–9 (Kovach *et al* 1991). The sequence of the primers is as follows: exon 5, 5′-CTGACTTTCAACTCTG-3′ (forward) and 5′-AGCCCTGTCGTCTCT-3′ (reverse); exon 6, 5′-CTCTGATTCCTCACTG-3′ (forward) and 5′-ACCCCA GTTGCAAACC-3′ (reverse); exon 7, 5′-TGCTTGCCACA GGTCT-3′ (forward) and 5′-ACAGCAGGCCAGTGT-3′ (reverse); exon 8, 5′-AGGACCTGATTTCCTTAC-3′ (forward) and 5′-TCTGAGGCATAACTGC-3′ (reverse); exon 9, 5′-TATGCCTCAGATTCACT-3′ (forward) and 5′-ACTTGATAAGAGGTCC-3′ (reverse). The same sets of primers were used for DNA sequencing.

2.4 *Polymerase chain reaction*

Genomic DNA (100 ng) was used for amplification in 25 µl of reaction mixture. The composition of the 10X buffer used for all exons (except exon 7) was: 100 mM Tris-HCl (pH 9·0), 15 mM $MgCl_2$, 500 mM KCl, 0·1% gelatin. For exon 7, the composition was: 100 mM Tris-HCl (pH 8·3), 35 mM $MgCl_2$, 250 mM KCl. Each PCR reaction had a 1X PCR buffer, 100 µM dNTPs (Gibco BRL), 5 µCi $a[^{32}P]$dATP, 5 pmol of each primer (Microsynth) and 0·5 U of Taq DNA polymerase (Bangalore Genei). PCR conditions for exon 5, 6 and 8 were 94°C for 5 min, 30 cycles of 94°C for 1 min, 57°C for 1 min and 72°C for 30 s and a final cycle of 72°C for 10 min. The condition for exon 7 was 94°C for 5 min, 30 cycles of 94°C for 1 min, 65°C for 1 min and 72°C for 10 s, and a final cycle of 72°C for 10 min. The condition for exon 9 was 94°C for 5 min, 30 cycles of 94°C for 1 min, 56°C for 1 min and 72°C for 15 s, and a final cycle of 72°C for 10 min.

2.5 *Single strand conformation polymorphism analysis*

Labelled PCR products were checked for amplification by 2% agarose gel electrophoresis, diluted to 10 to 100-fold in a loading and denaturing buffer (95% formamide, 0·25% bromophenol blue, 0·25 xylene cyanol, 10 mM EDTA, pH 8·0 and 0·1 N NaOH), heated at 85°C for 5 min and then subjected to single strand conformation polymorphism (SSCP) analysis using 6% non-denaturing polyacrylamide gel with 5% glycerol and 0·5X TBE as running buffer. Electrophoresis was carried out at 500 V for 3·5 h at 22°C for exons 5, 6, 7 and 9. For exon 8, the conditions remained the same, except that the gel was run at 15°C. Samples that showed band shifts were chosen for further study.

2.6 *Direct DNA sequencing*

Bands showing abnormal shifts in the SSCP analysis were purified from acrylamide gel using Microcon spin columns (Millipore) and used as templates to reamplify the fragment with the same set of primers originally used for amplification. The amplified DNA fragments were purified with a QIAquick PCR purification kit (Qiagen) and subjected to cycle sequencing (fmol cycle sequencing kit; Promega) in both directions. Sequencing reactions were carried out in a thermal cycler under the following cycling conditions: 95°C for 2 min, 30 cycles of 95°C for 30 s, 42°C for 30 s, and 70°C for 1 min. The samples were electrophoresed on a 6% polyacrylamide denaturing gel. Gels were dried under vacuum at 80°C, and exposed for Phosphor Imaging.

3. Results

3.1 *PCR-SSCP analysis for detection of mutations*

PCR fragments generated from exons 5 to 9 of the *p53* gene were subjected to SSCP analysis. All 44 glioma

samples were checked for mutations. DNA samples showing mobility shifts or abnormal bands were scored as positive for mutation. Of the 44 samples analysed, six tumour DNAs showed an altered pattern in PCR-SSCP analysis (figure 1; table 1). Exon 5 analysis revealed the presence of mutation in samples G2 (figure 1a), G10 (data not shown) and G20 (figure 1b). Sample 34 showed a differentially migrating band in exon 7 (figure 1c). Samples G23 and G33 (figure 1d) showed additional bands in exon 8 moving differently from normal bands.

3.2 *Identification of mutations by sequence analysis*

The bands that showed abnormal shifts in all the samples mentioned above were purified from gel and subjected to DNA sequencing. The sequencing revealed the presence of mutation in all the six samples. Figure 2 shows the results of sequencing of some of the samples. Exon 5 of samples G2 and G10 had the same missense mutation (G–T), resulting in the conversion of valine to phenylalanine at codon 172 (figure 2a). Exon 5 of sample G20 had the missense mutation (T–G), resulting in the conversion of valine to glycine at codon 173 (data not shown). Exon 7 of sample G34 showed the missense mutation (T–G), which converted leucine to arginine at codon 257 (data not shown). Exon 8 of G23 had the missense mutation (C–T), which caused the conversion of arginine to cysteine at codon 273 (figure 2b). G33 had the missense mutation (T–G) in exon 8, which resulted in the conversion of phenylalanine to cysteine at codon 270 (figure 2c).

4. Discussion

The most frequent chromosomal abnormalities associated with glioma are gains of chromosome 7, losses of 10, 9p and 17p and *EGFR* gene amplification (Libermann *et al* 1985; Bigner *et al* 1988; James *et al* 1988; Fujimoto *et al* 1989). The loss of 17p is usually associated with mutations in the *p53* gene (Chung *et al* 1991; Frankel *et al* 1992; Fults *et al* 1992; von Deimling *et al* 1992; Ghosh *et al* 1994; Jain *et al* 1999). The involvement of p53 in the development of glioma from Indian patients has been shown by loss of heterozygosity (LOH) analysis (Chattopadhyay *et al* 1997; Gosh *et al* 1994). The present study was carried out to determine the frequency of p53 mutation in glioma derived from the Indian population. We analysed 44 glioma samples to check for mutations in

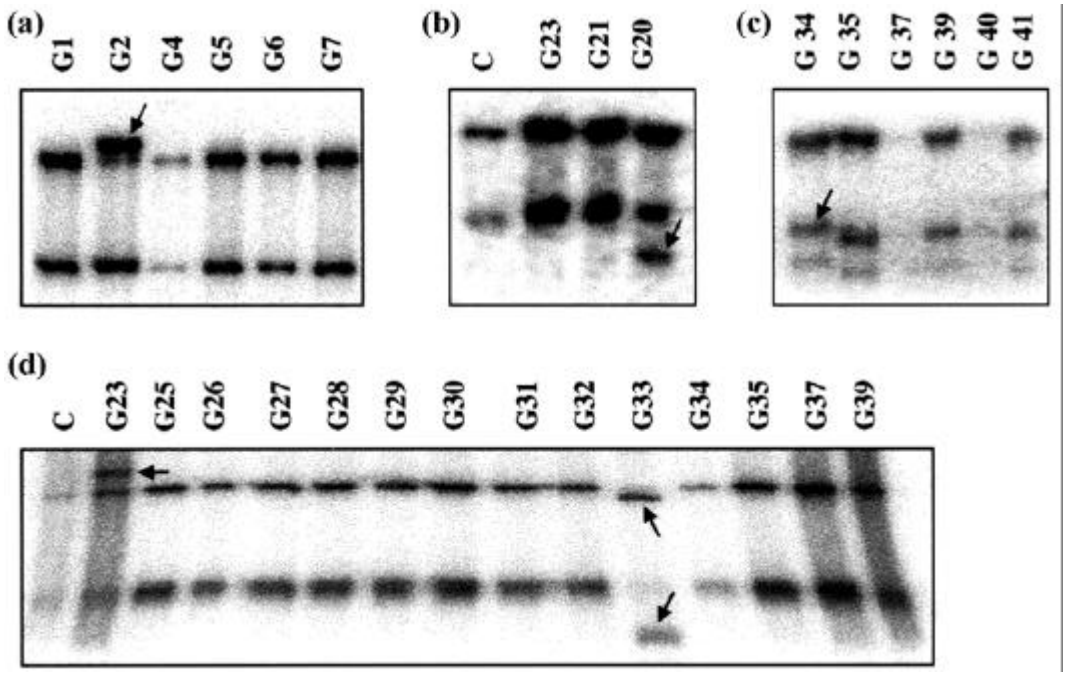

Figure 1. PCR-SSCP analysis of glioma samples. The exons 5, 6, 7, 8 and 9 of *p53* gene were amplified in the presence of $\alpha[^{32}p]$dATP using the total genomic DNA as template. The radiolabelled PCR products of exon 5 (**a** and **b**), exon 7 (**c**) and exon 8 (**d**) were run on a SSCP gel and autoradiographed. Only representative gels showing abnormal bands in comparison to bands in the control lanes are shown. Arrows show the abnormal bands in exon 5 of sample G2 (**a**), G10 (not shown), G20 (**b**); exon 7 of sample G34 (**c**); exon 8 of sample G23 (**d**) and G33 (**d**).

exons 5–9 of *p53* gene by the PCR-SSCP method. We found that 13·6% of the tumours tested positive for p53 mutations (table 1). To our knowledge, ours is the first study of p53 mutation in glioma from the Indian population.

The central core of p53, which spans from amino acids 100 to 300, contains the sequence-specific DNA-binding domain. This is the domain where majority of p53 mutations have been found to occur. In our study, all six mutations were mapped to this domain.

The occurrence of p53 mutations in glioma has generally been associated with age. While the p53 alterations are rare among pediatric age groups, their incidence is high in young adults, and low among the older age group (Louis *et al* 1993; Rasheed *et al* 1994). This study shows that the incidence of p53 mutations amongst young adults (19–50 years) is 18% (5/27) and amongst patients > 50 years old is 6·7% (1/15). No mutations of the *p53* gene among the pediatric age group could be detected (0/2).

Table 1. Details of glioma samples.

Sample	Mutated exon	Domain*	Codon	Base	Amino acid	Age/sex	Histopathological grade	Type**
G 1	–	–	–			46/M	III	Anaplastic astrocytoma
G 2	5	DBD	172	G to T	Valine to Ph. alanine	32/M	III	Anaplastic astrocytoma
G 3	–	–	–			40/F	III	Anaplastic astrocytoma
G 4	–	–	–			48/M	IV	GBM
G 5	–	–	–			53/M	II	Astrocytoma
G 6	–	–	–			38/M	II	Oligoastrocytoma
G 7	–	–	–			59/M	IV	GBM
G 8	–	–	–			70/M	IV	GBM
G 9	–	–	–			12/F	II	Pilocytic astrocytoma
G 10	5	DBD	172	G to T	Valine to Ph. alanine	68/F	IV	GBM
G 13	–	–	–			38/F	III	Anaplastic astrocytoma
G 15	–	–	–			22/M	III	Anaplastic astrocytoma
G 17	–	–	–			68/M	IV	GBM
G 18	–	–	–			21/F	IV	GBM
G 19	–	–	–			36/M	III	Anaplastic astrocytoma
G 20	5	DBD	173	T to G	Valine to glycine	43/M	III	Anaplastic oligoastrocytoma
G 21	–	–	–			46/F	II	Astrocytoma
G 23	8	DBD	273	C to T	Arginine to cysteine	30/M	II	Astrocytoma
G 24	–	–	–			27/M	II	Astrocytoma
G 25	–	–	–			58/F	III	Anaplastic astrocytoma
G 26	–	–	–			68/F	IV	GBM
G 28	–	–	–			25/F	III	Oligodendroastrocytoma
G 29	–	–	–			22/M	III	Anaplastic astrocytoma
G 30	–	–	–			49/M	IV	GBM
G 31	–	–	–			14/M	II	Astrocytoma
G 32	–	–	–			27/M	II	Astrocytoma
G 33	8	DBD	270	T to G	Ph. alanine to cysteine	25/M	II	Astrocytoma
G 34	7	DBD	257	T to G	Leucine to arginine	37/M	II	Astrocytoma
G 35	–	–	–			28/M	IV	GBM
G 37	–	–	–			64/M	IV	GBM
G 39	–	–	–			35/M	IV	Gemistocytic astrocytoma
G 40	–	–	–			52/F	IV	GBM
G 41	–	–	–			50/F	IV	GBM
G 42	–	–	–			44/M	IV	GBM
G 43	–	–	–			41/M	III	Anaplastic astrocytoma
G 44	–	–	–			52/M	IV	GBM
G 45	–	–	–			35/M	II	Astrocytoma
G 47	–	–	–			52/M	IV	GBM
G 48	–	–	–			54/M	IV	GBM
G 49	–	–	–			21/M	IV	GBM
G 50	–	–	–			63/M	II	Pilocytic astrocytoma
G 52	–	–	–			68/M	III	Anaplastic astrocytoma
G 53	–	–	–			55/M	III	Anaplastic astrocytoma
G 54	–	–	–			42/F	IV	GBM

*DBD refers to DNA binding domain of p53; **GBM refers to glioblastoma multiforme.

About 83% of the mutations were found in young adults (5/6), while only 17% were found in the older group (1/6). Thus our results confirm the relationship between p53 mutation and the age of the patient, as shown previously by others. Mutations of p53 were detected both in low-grade (grade II) and high-grade (grade III, IV) gliomas, with frequencies varying between 25 and 35% (Ohgaki *et al* 1995). Our results show that the p53 mutation frequency is higher in low-grade gliomas than in high-grade gliomas. Grade II gliomas, which mainly comprise astrocytomas, had a frequency of 25% (3/12), while grade III gliomas, which are mainly anaplastic astrocytomas, had a frequency of 15·4% (2/13). In contrast to this, the p53 mutation occurred at a low frequency of 5·4% (1/19) among grade IV glioblastoma multiforme cases. Three of the six p53 mutations were found in grade II gliomas.

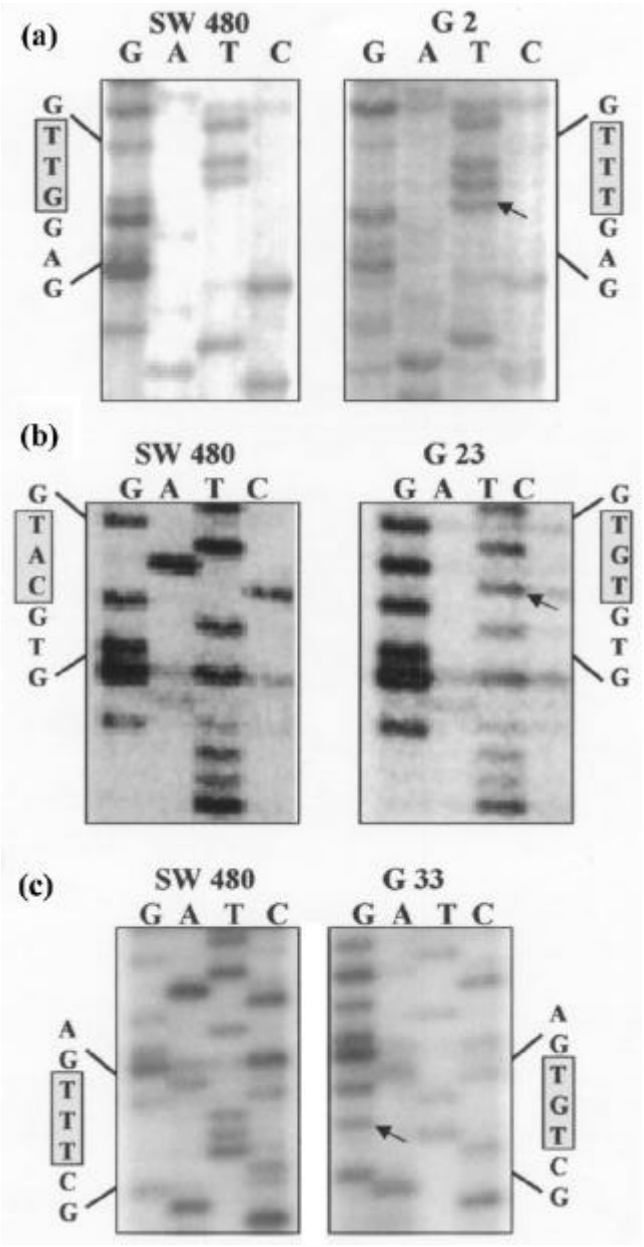

Figure 2. DNA sequence analysis of abnormal bands for selected samples. (**a**) Patient G2 has a missense mutation (G–T) in exon 5. (**b**) Patient G23 has a missense mutation (C–T). (**c**) Patient G33 has a missense mutation (T–G). The DNA sequence of control sample (left) and the patient (right) are shown. Total DNA from SW480 cells was used as control sample. Normal and mutated codons are boxed and shaded. The cell line SW480 carries a mutation in exon 8 at codon 273 due a missense mutation (G–A), which results in the conversion of normal codon CGT to CAT.

Other mechanisms implicated in the abnormal functioning of p53 are the amplification of *MDM2* gene and the inactivation of $p14^{ARF}$ gene product. The binding of MDM2 protein to p53 results not only in the inhibition of p53-mediated transcription, but also in the rapid degradation of p53 (Oliner *et al* 1993; Haupt *et al* 1997; Kubbutat *et al* 1997). The binding of $p14^{ARF}$ to the MDM2 protein inhibits the MDM2-mediated degradation of p53 (Kamijo *et al* 1998; Stott *et al* 1998; Zhang *et al* 1998; Orlow *et al* 1999). Studies of the status of *MDM2* and $p14^{ARF}$ genes in these glioma samples are currently in progress.

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References

Bigner S H, Mark J, Burger P C, Mahaley M S, Bullard D E, Muhlbaier L H and Bigner D 1988 Specific chromosomal abnormalities in malignant human gliomas; *Cancer Res*. **88** 405–411

Burger P C and Scheithauer B W 1993 *Tumors of the central nervous system* (Washington DC: Armed Forces Institute of Pathology)

Chattopadhyay P, Rathore A, Mathur M, Sarkar C, Mahapatra A K and Sinha S 1997 Loss of heterozygosity of a locus on 17p13· 3, independent of p53, is associated with higher grades of astrocytic tumours; *Oncogene* **15** 871–874

Chung R, Whaley J, Kley N, Anderson K, Louis D, Meneon A, Hettlich C, Freiman R, Hedley-White E T, Marutza R, Jenkins R, Yandell D and Seizinger B 1991 TP53 gene mutations and 17p deletion in human astrocytomas; *Genes Chromosomes Cancer* **3** 323–331

Epstein F and McCleary E L 1986 Intrinsic brain-stem tumors of childhood: surgical indications; *J. Neurosurg*. **64** 11–15

Frankel R H, Bayona W, Koslow M and Newcomb E W 1992 p53 mutations in human malignant gliomas: comparison of loss of heterozygosity with mutation frequency; *Cancer Res*. **52** 1427–1433

Fujimoto M, Fluts D W, Thomas G A, Nakamura Y, Heilbrun M P, White R, Story J L, Naylor S L, Kagan-Hallet K S and

Sheridan P J 1989 Loss of heterozygosity on chromosome 10 in human glioblastoma multiforme; *Genomics* **4** 210–214

Fults D, Brockmeyer D, Tullous M W, Pedone C A and Cawthon R M 1992 p53 mutations and loss of heterozygosity on chromosomes 17 and 10 during human astrocytoma progression; *Cancer Res*. **52** 674–679

Ghosh M, Dinda A, Chattopadhyay P, Sarkar C, Bhatia S and Sinha S 1994 Rearrangement of p53 gene with loss of normal allele in a low grade non recurrent mixed glioma; *Cancer Genet*. *Cytogenet*. **78** 68–71

Haupt Y R, Maya R, Kaza A and Oren M 1997 Mdm2 promotes the rapid degradation of p53; *Nature* (*London*) **387** 296–297

Hollstein M, Sidransky D, Vogelstein B and Harris C C 1991 p53 Mutations in human cancers; *Science* **253** 49–53

Jain K C, Chattopadhyay P, Sarkar C, Sinha S and Mahapatra A K 1999 A pilot study of recurrence of human glial tumours in light of p53 heterozygosity status; *J. Biosci*. **24** 477–481

James C D, Carlbom E, Dumanski J P, Hansen M, Nordenskjold M, Collins V P and Cavenee W K 1988 Clonal genomic alteration in glioma malignancy stages; *Cancer Res*. **48** 5546–5551

Kamijo T, Weber J D, Zambetti G, Zindy F, Roussel M F and Sherr C J 1998 Functional and physical interactions of the ARF tumor suppressor with p53 and Mdm2; *Proc*. *Natl*. *Acad*. *Sci*. *USA* **95** 8292–8297

Kovach J S, McGovern R M, Cassady J D, Swanson S K, Wold L S, Vogelstein B and Sommer S S 1991 Direct sequencing from touch preparations of Human carcinomas: Analysis of p53 mutations in Breast carcinoma; *J. Natl*. *Cancer Inst*. **83** 1004–1009

Kubbutat M H G, Jones S N and Vousden K 1997 Regulation of p53 stability by MDM2; *Nature* (*London*) **387** 299–303

Levine A J 1997 p53, The cellular gatekeeper for growth and division; *Cell* **88** 323–331

Levine A J, Momand J and Finlay C A 1991 The p53 tumor suppressor gene; *Nature* (*London*) **351** 453–456

Libermann T A, Nusbaum H R, Razon N, Kris R, Lax I, Soreq H, Whittle N, Waterfield M D, Ullrich A and Schlessinger J 1985 Amplification, enhanced expression and possible rearrangement of the EGF receptor gene in primary human brain tumors of glial origin; *Nature* (*London*) **313** 144–147

Louis D N, von Deimling A, Chung R Y, Rubio M-P, Whaley J M, Eibl R H, Ohgaki H, Weistler O D, Thor A D and Seizinger B R 1993 Comparative study of p53 gene and protein alterations in human astrocytic tumors; *J. Neuropathol*. *Exp*. *Neuro*. **52** 31–38

Mashiyama S, Murakami Y, Yoshimoto T, Sekiya T and Hayashi K 1991 Detection of p53 gene mutations in human brain tumors by single strand conformation polymorphism analysis of polymerase chain reaction product; *Oncogene* **6** 1313–1318

Nigro J, Baker S J, Presisinger A C, Jessup J M, Hostetter R, Cleary K, Bigner S H, Davidson N, Baylin S, Devilee P, Glover T, Collins F S, Weston A, Modali R, Harris C C and Vogelstein B 1989 Mutations in the p53 gene occur in diverse human tumor types; *Nature* (*London*) **342** 705–708

Ohgaki H, Eibl R H, Wiestler O D, Yasargil M G, Newcomb E W and Kleihues P 1991 p53 Mutations in nonastrocytic human brain tumors; *Cancer Res*. **51** 6202–6205

Ohgaki H, Schauble B, zur Hausen A, von Ammom K and Klehues P 1995 Genetic alterations associated with the evolution and progression of astrocytic brain tumors; *Virchows Arch*. **427** 113–118

Oliner J D, Pientenpol J A, Thiagalingam S, Gyuris J, Kinzler K W and Vogelstein B 1993 Oncoprotein MDM2 conceals the activation domain of tumor suppressor p53; *Nature* (*London*) **362** 857–860

Orlow I, LaRue H, Osman I, Lacombe L, Moore L, Rabbani F, Meyer F, Fradet Y and Cordon-Cardo C 1999 Deletions of the INK4A gene in superficial bladder tumors. Association with recurrence; *Am. J. Pathol*. **155** 105–113

Rasheed B K A, McLendon R E, Herndon J E, Friedman H S, Friedman A H, Bigner D D and Bigner S H 1994 Alterations of the TP53 gene in human gliomas; *Cancer Res*. **54** 1324–1330

Saxena A, Clark W C, Robertson J T, Ikejiri B, Oldfield E H and Ali I U 1992 Evidence for the involvement of a potential second tumor suppressor gene on 17 distinct from p53 in malignant astrocytomas; *Cancer Res*. **52** 6716–6721

Somasundaram K and El-Deiry W S 2000 Tumor suppressor p53: regulation and function; *Frontiers Biosci*. **5** d424–437

Stott F J, Bates S, James M C, McConnell B B, Starborg M, Brookes S, Palmero I, Ryan K, Hara E, Vousden K H and Peter G 1998 The alternative product from the human CDKN2A locus, $p14^{ARF}$, participates in a regulatory feedback loop with p53 and MDM2; *EMBO J*. **17** 5001–5014

von Deimling A, Eibl R H, Ohgaki H, Louis D N, von Ammon K, Petersen I, Kleihues P, Chung R Y, Wiestler O and Seizinger B R 1992 p53 mutations are associated with 17p allelic loss in grade II and grade III astrocytomas; *Cancer Res*. **52** 2987–2990

Zhang Y, Xiong Y and Yarbrough W G 1998 ARF promotes MDM2 degradation and stabilizes p53: ARF-INK4a locus deletion impairs both the Rb and p53 tumor suppression pathways; *Cell* **92** 725–734

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