Brain Pathology 8: 599-613(1998)

REVIEW ARTICLE

p53 and Brain Tumors: From Gene Mutations to Gene Therapy

Giulia Fulci[1,2], Nobuaki Ishii[1], Erwin G. Van Meir[1,2]

[1] Laboratory of Tumor Biology and Genetics, Neurosurgery Dept., University Hospital (CHUV), 1011 Lausanne, Switzerland
[2] Laboratory of Molecular Neuro-Oncology, Department of Neurological Surgery and Winship Cancer Center, Emory University, Atlanta, Georgia 30322, USA.

The p53 tumor suppressor gene (*TP53*) is the most frequently altered gene in human cancer and is also found mutated in several types of brain tumors. Loss of p53 function plays a central role in the development of cancer. The characterization of the biochemical pathways by which p53 alteration triggers tumorigenesis is the foundation for the design of novel therapeutic approaches.

Investigations of the intracellular mechanisms at the origin of p53 tumor suppressive functions have shown that p53 is a transcription factor able to sense a variety of cellular insults and induce a dual response: cell growth arrest/senescence or apoptosis. Less well studied are p53's influences on extracellular events such as tumor angiogenesis, immunology and invasion. Here, we review these findings and specifically discuss their implications for brain tumor genesis, molecular diagnosis and prognosis. Of clinical importance are the findings that brain tumors with wild type (wt) or mutant p53 status may respond differently to radiation therapy and that novel therapeutic strategies using *TP53* gene transfer or specifically targeting tumor cells with mutated p53 are being evaluated in clinical trials.

Corresponding Author:
Erwin G. Van Meir, PhD, Associate Professor, Laboratory of Molecular Neurooncology, Winship Cancer Center, Emory University, 1365-B Clifton Road, NE., Room B5103, Atlanta, Georgia 30322, USA; Tel.: 404-778-5227; Fax: 404-778-4472; E-mail: evanmei@emory.edu

Introduction

p53 is a 53kDa phosphoprotein that has an important function in preventing tumor formation by influencing both the intra- and extra-cellular environment. p53 exerts its tumor suppressive function by sensing cellular damage and integrating this signal into a response where cell cycle arrest and repair mechanisms take place, or where cell elimination by apoptosis or senescence occurs. More recent data show an important role for p53 in angiogenesis and tumor invasion, two fundamental processes in malignancy (reviewed in 56,60).

Biochemically, p53 forms tetramers and is a transcriptional activator of a specific subset of target genes containing a p53 binding domain (Table 1). p53 can also influence cellular functions by interacting with a variety of cellular proteins (Table 2); notably, its interaction with CBF/CBP and TBP inhibits transactivation of genes containing CCAAT and TATA regulatory elements (Table 3).

The variability of pathways in which p53 is involved could constitute an explanation for the high frequency of mutations found at the *TP53* locus in human tumors. Here, we review the molecular and physiological functions of p53 and examine how their loss favors brain tumor development. These findings show the importance of the *TP53* gene in astrocytic tumor formation and allowed the development of therapies for tumor cells with disrupted p53 tumor suppressor pathways.

p53 Function: intracellular effects

The major role postulated for p53 in cells of most human tissues is maintenance of genomic stability; hence its appelation as the "guardian" of the genome. This is accomplished in two ways: either directly by participating in the mechanisms maintaining DNA integrity or, indirectly, by inducing cell cycle arrest, senescence or apoptosis in damaged cells.

p53 and the mechanisms maintaining DNA integrity. p53 may influence genome integrity/stability through

Genes	Function	P53 binding sequences	References**
A28-RGS14	regulator of G protein signaling		Buckbinder L., 1997, P.N.A.S.,* 4:7868
BAI1	inhibitor of angiogenesis		(80)
Bax	apoptosis promoter	tcACAAGTTa AGACAAGCCT	(78)
CDKN1 (p21)	inhibitor of cyclin dependent kinases	GAACATGTCC cAACATGTTg	(30)
Cyclin G (murine)	cell cycle regulator	AGACcTGCCC GGGCAAGCCT	Okamoto K., 1994, EMBO J., 13:4816
Cytokeratine 8	major component of intermediate type of filament	ctGCcTcCaC	Mukhopadhyay T., 1996, Anticanc. Res., 16:105
DDR protein tyrosine kinase	receptor protein tyrosine kinase		Sakuma S., 1996, FEBS Lett., 398:165
EGF receptor	epithelial growth factor	AGACTAGgCC GAGCTAGCCC GAGCgAGCTC	Deb SP., 1994, Oncogene, 9:1341
EI24 (murine)	unknown function		Lehar SM., 1996, Oncogene, 12:1181
Fas/APO1	inducer of apoptosis		Owen-Schaub LB., 1995, Mol. Cell Biol, 15:3032
GADD45	inhibitor of cell entry in S phase by binding to PCNA	GAACATGTCT AAGCATGCTg	Kastan MB.,1992, Cell, 71:587
GML	GPI-anchored gene	AtGCTTGCCC AGGCATGTCC	Furuhata T., 1996, Oncogene, 13:1965
GD-AIF	inhibitor of angiogenesis		(111)
HIC-1	tumor suppressor		Makos-Wales M., 1995, Nat. Med., 1:570
IGF binding protein 3	inhibitor of IGF mitogenic signaling	AAACAAGCCac cAACATGCTT	Buckbinder L., 1995, Nature, 377:646
MDM2	p53 inhibitor	GGtCAAGTTg GGACAcGTCC GAGCTaAGTCc tGACATGTCT	Oliner JD., 1992, Nature, 358:80
MSH2	DNA-mismatch repair	AGGCTAGTTT AAGtTtcCTT	Sherer S.J., (1996), Biochem. Bioph. Res. Comm., 221:722
Muscle MCK (murine)	ATP generator	tGGCAAGCCT tGACATGgCC	Weintraub H., 1991, P.N.A.S.,* 88:4570 Zhao J., 1994, Mol. Cell. Biol., 14:8483
P2XM	ATP-gated ion channel	GAACAAGggC GAGCTTGTCT	Urano T., 1997, Canc. Res., 57:3281
PCNA	proliferating cell nuclear antigen promoter	GAACAAGTCC GGGCATaTgT	Morris GF., 1996, P.N.A.S.,* 93:895
PIG1	galectin-7	tAACcTGgTTc	(86)
PIG2	guanidinoacetate N-methyl transferarse	ccctcctCCgt	(86)
PIG3	quinone oxidoreductase homologue	GAGgccaaCa	(86)
PIG4	serum amyloid A	GTGCggaGgac	(86)
PIG5	normal keratinocyte mRNA	tGGggccGCag	(86)
PIG6	proline oxidase homologue	tcctTggaCCt	(86)
PIG7	TNF-a-induced mRNA	ctGggccTgaa	(86)
PIG8	etoposide-induced mRNA	AGctggtTTCc	(86)
PIG9	tax1-binding protein	cGtCccGgagc	(86)
PIG10	actin-binding protein	AGctcacTCCa	(86)
PIG11	unknown	AGGCTgtCCag	(86)
PIG12	microsomal glutathione transferase homologue	tGAgTccCTgt	(86)
PIG13	unknown	AGAtgctgdag	(86)
TGFa	growth regulator	GGGCAgGCCC tGcCTAGTCT AGcCAAGTCT tGGCAAGCgg	Shin TH, 1995, Mol. Cell Biol. 15: 4694
TGFb	growth regulator		(31)
TSP-1	angiogenesis inhibitor		(24)
WIG 1	unknown		Varmehziaie S., 1997, Oncogene, 15:2699
WIP-1	nuclear phosphatase		Fiscella M., 1997, P.N.A.S.,* 94:6048

*P.N.A.S.: Proc. Natl. Acad. Sci. USA.
**Due to space limitations references in tables are not cited in bibliography unless they appear in article text

Table 1. Genes transactivated by wild type p53.

several functions: i) cells expressing mutant p53 protein show an increase of spontaneous **intra-chromosomal recombination**, a hallmark of cancer (13, 74). This might be due to loss of wt p53's ability to bind and inactivate Rad 51, a protein involved in homologous recombination (104) (Table 2). ii) p53 interacts through its C-terminal domain with transcription-repair TFIIH-associated factors such as ERCC 2 and ERCC 3 (Table 2) involved in **strand-specific DNA repair** (120). iii) p53 prevents **multiple DNA replication** before mitosis (27), a process that may lead to polyploidy, a regular feature of cancer. The relationship between *TP53* status and genomic ploidy has been examined in a series of 15 low grade astrocytomas that progressed to higher grade gliomas. All the anaplastic recurrences of gliomas harboring *TP53* mutations were non diploid, whereas two out of seven gliomas with intact *TP53* remained diploid (113). Similarly, *TP53* loss preceded genome duplication in a p53-null glioblastoma (3). iv) Lack of p53 results in high frequency of **gene amplification** in normal human fibroblasts when treated with PALA, a drug able to induce cell growth arrest by inhibition of uridine biosynthesis (64). *TP53* mutation, however, is not a prerequisite for DNA amplification. Most glioblastoma which show amplification and rearrangement of the *EGFR* gene express wt p53 and those with mutated forms of p53 rarely amplify *EGFR* (118). Other examples are *MDM2* and *N-MYC* gene amplifications which occur in wt p53 containing astrocytoma (88) and neuroblastoma (49), respectively. v) Correct spindle bipolarity and spindle microtubule assembly are essential to ensure balanced **chromosome segregation** during cell

Proteins	References**
Viral factors	
Adenovirus 2, 5 & 12 E1B	Sarnow P., 1982, Cell, 28:387 Yew PR, 1992, Nature, 357:82
Epstein Barr Virus (EBV) EBNA-5	Szekely L., 1993, P.N.A.S.,* 90:5455
Epstein Barr Virus (EBV) BZLF1	Zhang Q., 1994, Mol. Cell Biol., 14:1929
Hepatitis B Virus X protein	Wang XW., 1994, P.N.A.S.,* 91:2230
Human Cytomegalovirus (HCMV) IE84	Speir E., 1994, Science, 265:391
Human Herpesvirus 6 (HHV6) orf1	Kashanchi F., 1997, Oncogene, 14:359
Human Papilloma Virus (HPV) 16 & 18 E6	Scheffner M., 1993, Cell, 75:495
Simian Virus 40 (SV40) large T antigen	Lane DP., 1979, Nature, 278:261
General transcription factors	
1) TFIID components	
TAFII31	Lu H., 1995, P.N.A.S.,* 92:5154
TAFII40 (drosophila)	Thut CJ., 1995, Science, 267:100
TAFII60 (drosophila)	Thut CJ., 1995, Science, 267:100
TATA box binding protein (TBP)	Horikoshi N., 1995, Mol. Cell Biol., 15:227
2) TFIIH components	
TFIIH	Xiao H., 1994, Mol. Cell. Biol., 14:7013
CSB	(120)
ERCC2 (XPD)	(120)
ERCC3 (XPB)	(120)
p62	Leveillard T., 1996, EMBO J., 15:1615
3) TFIIIB components	
TFIIIB90	Chesnokov I., 1997, Mol. Cell. Biol., 16:7084
Transcriptional activators	
CCAAT binding factor (CBF)	Agoff SN., 1993, Science, 259:84
CBP	Gu W., 1997, Cell, 90:595
p300	Avantaggiati ML., 1997, Cell, 89:1175
Sp1	Borellini F., 1993, J. Biol. Chem., 268:7923
WT1	Maheswaran S., 1995, Genes Develop., 9:2143
p53 posttranscriptional modulators	
c-Abl	Goga A., 1995, Oncogene, 11:791
Casein kinase II	Filhol O., 1992, J. Biol. Chem., 267:20577
Redox repair protein Ref1	Jayaraman L., 1997, Genes Develop., 11:558
p53 turnover components	
MDM2	Momand J., 1992, Cell, 69. 1237; Oliner JD., 1992, Nature, 358:80
MDM-X	Shvarts A., 1996, EMBO J., 15:5349
Ubiquitin and conjugating enzymes	Scheffner M., 1993, Cell, 75:495
Others	
70kD heat shock protein	Graeber TG., 1994, Mol. Cell Biol., 14:6264
Hypoxia inducible factor 1a	An W.G., 1998, Nature, 392:405
p53-BP1 and p53-BP2	Iwabuchi K., 1994, P.N.A.S.,* 91:6098
p73b	Kaghad M., 1997, Cell, 90:809
Rad 51	(104)
S100b	Baudier J., 1992, P.N.A.S.,* 89:11627
Topoisomerase 1	Gobert C., 1996, Biochem., 35:5778

*P.N.A.S.: Proc. Natl. Acad. Sci. USA.
**Due to space limitations references in tables are not cited in bibliography unless they appear in article text.

Table 2. Proteins which interact with p53.

division. p53 interacts with the centrosome during interphase and controls centrosome duplication (17). When spindle assembly is blocked by anti-microtubule agents p53 prevents cell cycle progression (23). In mouse embryonic fibroblasts lacking p53, abnormal centrosome amplification and imbalanced chromosome segregation are observed (32).

Cell cycle arrest/senescence and apoptosis. The second set of mechanisms by which p53 accomplishes maintenance of genomic stability is arrest of the cell cycle and/or apoptosis. Both pathways are induced by DNA damage and have as their ultimate result the repair or elimination of DNA damaged cells. Therefore, these two mechanisms are very important to avoid tumor initiation and/or progression both in the brain and in other human tissues. The decision for p53 whether to act as an apoptosis or cell growth arrest inducer depends on the cell type, its molecular background and the retinoblastoma pathway (39, 40, 85).

a) p53 and cell cycle arrest/senescence. p53 is able to suppress the cell cycle at the G1 and G2/M transitions (2, 63, 103) . In leukemia cells a reversible G1 cell cycle arrest might give time to damaged DNA to be repaired, but in human fibroblasts p53 cell cycle arrest is an irreversible senescent-like process (28, 97). Some studies suggest that p53-induced growth arrest is reversible in human glioma cells (75), but others show that *TP53* transfer induces particular morphological changes reminiscent of a quiescent/senescent cellular state (112).

The main **molecular mechanism** by which p53 induces cell growth arrest is acting as a transcription factor. Many genes involved in cell cycle regulation are induced by p53 (Table 1), but the main effector of the p53-mediated cell cycle arrest is p21, a 21 kDa protein encoded by the *CDKN*1 gene (30) (Table 1). p21 interacts with cyclin-dependent kinase complexes and inhibits their activity, required for cell cycle progression (37). p21 induction through *CDKN*1 gene transfer, or, indirectly, after *TP53* transfer arrests glioma cell growth (51, 112). This was accompanied by loss of tumorigenic capacity in vivo both in peripheral and intracerebral xenograft models (19). However, the absence of *CDKN*1 gene mutations in human glioma (108) suggests that p21 is a growth suppressor rather than a genuine tumor suppressor. Other genes involved in cell cycle control that were found regulated by p53 in human glioma cell lines are PCNA and TGFβ (31, 76) (Tables 1 and 3).

b) p53 and apoptosis. Transfer of *TP53* can induce apoptosis in different cell types, including human glioma cells (61). Evasion of the pathways leading to apoptosis is critical for the development of tumors and their restoration holds promise for clinical treatment (reviewed in 56, 60). For example, p21 is an important downstream mediator of p53-dependent cisplatin-induced apoptosis (58).

Although different stimuli were shown to induce p53-dependent apoptosis, such as DNA damage, expression of the myc adenovirus E1A proteins and withdrawal of growth factors (reviewed in 56), the **molecular mechanisms** of p53 induced apoptosis are still unclear. In some cases it seems that p53 transactivating function is important to determine cell susceptibility to apoptosis, while in other cases apoptosis is mediated by p53 tran-

Genes	References**
Viral factors	
Hepatitis B Virus (HBV) X	Takada S., 1996, Virology, 216:80
Herpes Simplex Virus (HSV) thymidine kinase	Yuan JN., 1993, Biochem. Biophys. Res. Comm., 191:662
Herpes Simplex Virus (HSV) UL9	Subler MA., 1992, J. Virol., 66:4757
Human Cytomegalovirus (CMV) early promoter	Subler MA., 1992, J. Virol., 66:4757
Human Immunodeficiency Virus (HIV) Type I LTR	Subler MA., 1992, J. Virol., 66:4757 Subler MA, 1994, J. Virol., 68:103
Human Papilloma Virus (HPV) Types 6, 16 & 18 Long Control Regions	Desaintes C., 1995, Oncogene, 10:2155
Human T cell Lymphotropic Virus (HTLV) Type I LTR	Subler MA., 1992, J. Virol., 66:4757
Rous Sarcoma Virus LTR	Subler MA., 1992, J. Virol., 66:4757
Simian Virus 40 (SV40) immediate early promoter and enhancer	Subler MA., 1992, J. Virol., 66:4757 Perrem K, 1995, Oncogene, 11:1299
Oncogenes	
c-Fos	Ginsberg D., 1991, P.N.A.S.,* 88:9979
c-Jun	Ginsberg D., 1991, P.N.A.S.,* 88:9979
b-Myb	Lin D., 1992, P.N.A.S.,* 89:9210
DNA replication associated factors	
DNA polymerase a	Lin D., 1992, P.N.A.S.,* 89:9210
Polymerase III transcribed templates	Chesnokov., 1996, Mol. Cell Biol., 16:7084
Topoisomerase IIa	Wang Q., 1997, Mol. Cell Biol., 17:389
Cell cycle associated factors	
Basic FGF	(109)
Bcl-2	(77)
Cyclin A	Yamamoto M., 1994, Exp. Cell Res., 210:94
IGF-I receptor	Werner H., 1996, P.N.A.S.,* 93:8318
IGF-II	Zhang L., 1996, Canc. Res., 56:1367
PCNA	(76)
Others	
b-Actin	Ginsberg D., 1991, P.N.A.S.,* 88:9979
Fibronectin	Iotsova V., 1996, Cell Growth Diff., 7:629
Human O6-methylguanine-DNA methyltransferase	Harris LC, 1996, Canc. Res., 56:2029
Hsc70 heat shock protein	Ginsberg D., 1991, P.N.A.S.,* 88:9979
Hsp70 heat shock protein	Agoff SN., 1993, Science, 259:84
Interleukine-6	Santhanam U., (1991), P.N.A.S.,* 88:7605 Margulies L., (1993), J. Biol. Chem., 268:15096
Multiple Drug Resistance gene I	Chin KV., 1992, Science, 255:459
Nitric Oxide Synthase 2	Forrester K., 1996, P.N.A.S.,* 93:2442
p53	Ginsberg D., 1991, P.N.A.S.,* 88:9979
Rat brain creatine kinase	Zhao J., 1994, Mol. Cell Biol., 14:8483
Retinoblastoma	Shiio Y., 1992, P.N.A.S.,* 89:5206
Serum glucocorticoid-inducible protein kinase	Maiyar AC., 1997, Mol. Endocrinol., 11:312
VEGF	(115)

**P.N.A.S.: Proc. Natl. Acad. Sci. USA.
**Due to space limitations references in tables are not cited in bibliography unless they appear in article text.

Table 3. Genes repressed by p53

scriptional repression activity. Both cases are in agreement with p53's ability to induce the expression of the BAX gene and to repress the Bcl-2 gene (Tables 1 and 3) (77, 78). Recently, a new model for p53 induced apoptosis was proposed, where p53 transcriptionally induces redox-controlling genes that promote an increase in reactive oxygen species (ROS) (Table 1) which consequently leads to oxidative damage and apoptosis (86).

Other studies using somatotropic progenitors, immortalized by expression of the SV40 T-antigen, show p53 induced apoptosis independent from new RNA and protein synthesis (18). This was confirmed by deletion studies showing that p53-induced apoptosis could be prevented by removal of the N-terminal proline-rich domain of p53, although this did not affect p53's transactivation capacity. This domain likely plays an important role in triggering apoptosis by interacting with other proteins through its Pro-X-X-Pro motifs (93,119). Taken together, all these data indicate that p53 may have separate transcription-dependent and -independent pathways to induce cell death and suggest that the chosen apoptotic mechanism is cell type specific.

Contradictory data have been obtained in the study of p53 involvement in radiation-induced apoptosis in glioblastoma cells. In one study, inactivation of endogenous wt p53 by expression of a dominant negative p53 mutant in U87MG cells prevented apoptosis and made the cells resistant to irradiation (127). In another study, radiation response was shown to depend on p53-induced G1 arrest and p53-independent apoptosis in the same cells (35 .

p53 Function: effects on the microenvironment

p53 and angiogenesis. Angiogenesis is a complex process through which new blood vessels are formed from preexisting ones. Without neovascularization tumors remain small and dormant, a condition where cell renewal is balanced with cell death through apoptosis. Tumor establishment, growth and metastasis occur only with a switch to the angiogenesis phenotype, a state where unbalanced production of activators and inhibitors induces active growth of blood vessels. Both the physiological state of the tumor cells and alterations in specific genes trigger this switch. p53 can affect this process by different mechanisms in different tissues. First, in BT549 human breast carcinoma cells, the reintroduction of a wt *TP53* gene augments the secretion of angiogenesis inhibitor thrombospondin-1 (TSP-1)(24, 116). Second, in cultured fibroblasts from Li-Fraumeni patients the angiogenic switch coincides with loss of *TP53* (24) , which is consistent with the capacity of p53 to induce TSP-1 and repress VEGF expression in these cells (Tables 1 and 3)(115). In contrast, in glioblastoma cell line LN-Z308, p53 is not involved in the regulation of TSP-1 or VEGF (83), but induces the release of an as yet unidentified inhibitor of angiogenesis (111). Recently, a candidate for this activity has been identified (80) (Table 1).

Further data show that mutant p53 can confer an angiogenic gain of function. Experiments made in human glioblastoma cell line U87MG suggest that mutant p53 induces the release of bFGF, whereas wt p53 represses the same factor (Table 3) (109). Finally, in NIH-3T3 cells mutant p53 synergizes with protein

kinase C (PKC) and 12-0-tetradecanoylphorbol-13-acetate (TPA) to enhance VEGF production (52).

p53 and invasion. Astrocytoma of all grades invade surrounding normal brain areas, resulting in difficult clinical management of the patients and high recurrence rates. It has recently been shown that induction of wt p53 in osteosarcoma cells partially reduced their invasive capacity (62). The high frequency of p53 mutations in glioblastoma and the invasive nature of this tumor, certainly warrants examining a potential role for p53 in this process.

p53 and the immune system. The accumulation of either wt or mutated forms of p53 in most human cancers suggests that p53 might elicit immune responses that could be exploited for immunotherapy. The finding of p53 antibodies in sera of tumor patients has demonstrated the presence of a B-cell response to p53 accumulation (67, 96). Such a response constitutes an early way to detect *TP53* mutations in the progression of lung cancer (68). These antibodies were mostly IgG, consistent with a T cell dependent response. A T cell response was also evidenced when p53-derived peptides were used, suggesting that injections of p53 peptides could be a way to enhance immunological recognition and killing of tumor cells by cytotoxic T lymphocytes (CTL)(126). Recent experiments have shown that tumor development could be inhibited by MHC class I restricted CTL responses specific for mutated or wt p53 peptides (73, 92, 114).

These preliminary results should encourage the evaluation of this kind of anticancer immunotherapy for brain tumors.

Another area of interest which has not been explored to date is to evaluate whether cells which have inactivated p53 may have a different susceptibility to immune system recognition than those expressing wt p53.

TP53 mutation spectrum in brain tumors

The specific nucleotides found mutated in genes altered in tumors are the relics of a combined chemical and biological process. They reflect the exposure of some tissues to specific carcinogens and the chemical interactions of these with cellular DNA and repair systems. The mutations that will ensue undergo an indirect biological selection process since only cells in which they confer a growth advantage leading to cancer will survive. Therefore, analysis of the mutation spectrum of such genes can give us some clues to the etiology and molecular pathogenesis of neoplasia (36, 45).

cell lines	origin	induction mode §	TP53 mutations	references
EN-1	type-1 astrocyte	ENU	249: ATC (I) ➔ AAC (N)	(43)
EN-2	type-1 astrocyte	ENU	111: TTC (F) ➔ GTC (V)	(43)
EN-3	type-1 astrocyte	ENU	229: ATC (I) ➔ AGC (S)	(43)
EN-5	type-1 astrocyte	ENU	278: AGT (S) ➔ ATT (I)	(43)
EN-6	type-1 astrocyte	ENU	111: TTC (F) ➔ TCC (S)	(43)
9L*	unknown	NMU	277: GGG (G) ➔ GAG (E)	(5, 10)
T9*	unknown	NMU	wild-type	(22)
C6	unknown	NMU	wild-type	(5, 10)
RT-2	unknown	ASV	wild-type	(9, 47)
RG-2 (D74)	unknown	ENU	unknown	(1, 8, 89)

§ ENU: N-ethyl-N-nitrosourea,
NMU: N-nitroso-methylurea
ASV: avian sarcoma virus
* 9L and T9 are subcultures of the same initial cell line propagated independantly (Barth RF (1998) Journal of Neuro-Oncology 36: 91-102).

Table 4. TP53 mutations in transformed rat glioma cell lines.

TP53 mutation sites. All classes of mutations (deletions, insertions, transitions and transversions) occur in the *TP53* gene. Point mutations which alter p53 function cluster in the hydrophobic central part of the protein (87% in exons 5-8), where many base substitutions alter the protein's conformation and/or its function. This is partially biased due to the fact that most investigators have limited their analyses to exons 5-8. Frame shift and nonsense mutations that truncate the molecule are also situated outside of this region (reviewed in 34). Although more than 250 codons in *TP53* are potential human mutation sites, 25% of all mutations found in human tumors comprise only five of these codons (175, 245, 248, 249 and 273).

Crystallographic analysis allowed to realize that the frequency of *TP53* point mutations decreases at increased distances from the regions involved in p53 DNA binding and that two classes of mutations can be distinguished: mutations of codons directly involved in DNA binding and mutations of residues important for the stable folding of the protein. Two of the most frequently mutated codons (248 and 273) are situated at the large loop 3 and at the β strand loop helix motif, respectively, and are therefore crucial points of p53/DNA contacts, whereas, the hot spot mutation 175 is part of the large loop 2 and has an important role in stabilizing the p53/DNA complex (21) (Figure 1).

TP53 mutations in tumors of the CNS. Analysis of the *TP53* status and overexpression of p53 have been well documented in primary central nervous system (CNS) tumors (Figure 1). Mutations in *TP53* are almost restricted to tumors of astrocytic origin. Currently, the most sensitive protocol for *TP53* mutation detection shows frequencies of 67% in anaplastic astrocytoma,

Figure 1. Representation of all p53 amino acid mutations found in brain tumors as reported by the IARC database: http://www.iarc.fr. The colors of the boxes indicate the tumor type (right hand legend), the letters and symbols in the boxes indicate the type of mutation (left hand legend), black squared boxes indicate the starting site of a frameshift (left hand legend). Mutations at the three hotspot sites (175, 248, 273) are indicated with black columns with a distinctive color and the total number of mutations on the top.On the top left hand legend the particular mutations for each of these are listed. Regions and residues implicated in DNA interaction and protein stability as well as the conserved domains of the protein and the intron/exon boundary sites are indicated (left hand legend).

and 41% in glioblastoma multiforme (106). Mutation frequencies are much lower in oligodendroglioma (13%), medulloblastoma (11%), pilocytic astrocytoma (<5%) and virtually absent in other CNS tumors (reviewed in 54).

The finding of a lower frequency of mutations in glioblastoma (41%) versus anaplastic astrocytoma (67%) lead to hypothesize that glioblastoma was a pathological entity that might be genetically diverse. This idea was reinforced when much lower frequencies of *TP53* mutations (11%) were found in glioblastoma that occured without clinical evidence of a prior less malignant lesion (called *de novo* or primary glioblastoma) as compared to those resulting from the recurrence of a grade II/III astrocytoma (called secondary glioblastoma) (67%) (121). Analysis of other genetic defects in glioblastoma also suggested at least two subtypes (see below). These data also suggested that a large fraction (59%) of glioblastoma occured without the apparent need for a mutation in the *TP53* gene. This result was compatible with several scenarios: i) these glioblastoma develop independently of the p53 pathway, ii) the p53 pathway is altered by alternative mechanisms, iii) wt p53 produced in these cells is functionally inactive and, iv) these tumors have homozygous *TP53* deletions and the wt *TP53* alleles detected derive from non-tumoral cells. The last two issues were addressed by examining *TP53* status and p53 function in glioblastoma cell lines. About 25% have wt *TP53* alleles and express p53 that is transcriptionally competent *in vivo* (110, 112). Cell lines null for *TP53* do occur but are uncommon (3). In conclusion, this suggests that some

glioblastoma can occur through a p53-independent pathway or that the need for p53 mutation is bypassed by alterations in other genes of the same pathway. It was demonstrated that amplification of mdm2, a negative regulator of p53 occurs in about 10% of tumors (88). Mutations in *p19*^{ARF}, a gene encoding a protein able to downregulate mdm2 may provide a similar effect (128), but this remains to be examined in brain tumors.

The most frequent *TP53* alterations are GC-AT transversions that occur at CpG sites by deamination of 5' methylcytosine and are considered to be spontaneous. There are no brain tumor-specific mutations, the three most commonly mutated codons are in order of frequency: 273, 248 and 175. In other human tumors the most frequently mutated codons are 248, 249 and 175 (reviewed in 15). Whether this has any meaning is uncertain since there is no data indicating a defined role of specific mutants in brain tumorigenesis.

Clearly, a very interesting question is to determine whether loss of wt p53 biological function is essential for progression from low grade astrocytomas to secondary glioblastoma and if this genetic event is an initial, early or late step during glioblastoma development. In contrast to carcinoma where *TP53* gene mutations occur late during tumor progression, *TP53* mutations occur at similar frequencies in astrocytoma grade II, III and in secondary glioblastoma suggesting that they are an early event in the progression of this tumor type (81, 117). Further analysis in tumor pairs of patients showing malignant recurrence from low grade or anaplastic astrocytoma to secondary glioblastoma showed that most cells of the recurrent tumors harbored mutated *TP53*. These mutations were already present in cells of the primary tumors suggesting that recurrence had occured by clonal expansion of cells with mutated *TP53* (41, 100, 105). Since only a fraction of the cells in the original low grade tumors harbored *TP53* mutations one might conclude that *TP53* is an early progression event rather than "the" initiation event. Such interpretation is complicated, however, with the difficulty of excluding the substantial presence of normal brain in these "diffuse" tumors. Thinking of *TP53* mutation as an early progression event is in accordance with wt p53 function in the cell. In fact, since cells lacking wt p53 should be genetically unstable, they might accumulate additional alterations at an increased rate, facilitating progression to a more malignant stage.

There is also evidence that *TP53* mutation may be "the" initiation event in astrocytoma. Brain tumors occur in patients with germline *TP53* mutations; families with Li-Fraumeni syndrome present an incidence of CNS tumors of 13%, most of which (73%) are astrocytomas (55). Also, a significant fraction of patients with multifocal glioma showed constitutively mutated *TP53* alleles (59). This theory is reinforced by the observation that the pattern of mutations in sporadic and inherited brain tumors is similar. Further evidence derives from spontaneous immortalization of astrocytes derived from *TP53* knockout mice and their subsequent transformation *in vitro* (14, 125). All this information is consistent with *TP53* mutations accompanying astrocytoma initiation/early progression, but does not prove causality. Causal involvement of *TP53* alteration in the disease was provided by functional studies restoring p53 function in cell lines, demonstrating growth arrest or apoptosis as described above. These studies were biased by the use of vectors leading to overexpression of *TP53* and the use of cell lines with mutated p53 derived from de novo glioblastoma (Ishii et al, manuscript in preparation). Stronger evidence for p53 involvement in the transformation of the astrocytic lineage will have to await specific generation of astrocytoma in animals engineered to lack *TP53* alleles in these cells, either through transplantation studies or generation of tissue-specific knockouts.

Carcinogenesis of CNS tumors. Many epidemiology studies have been designed to identify risk factors for brain tumor development. However, the data on non-inherited factors are controversial and a specific exposure or causative environmental agent has not been identified yet, with the exception of therapeutic irradiation to the brain (discussed in detail in (25). Only experimental studies in rodents have shown that several classes of chemical carcinogens selectively induce tumors in the CNS. Nitrosourea-derivatives and other alkylating agents are considered as powerful compounds which cause CNS neoplasms in rats after systemic administration. These agents induce the formation of oligodendrogliomas, astrocytomas, ependymomas, primitive neuroectodermal tumors (PNETs) with neural differentiation, and malignant schwannomas (53). This wide variation of histology may indicate that most cell types which construct central and peripheral nervous tissues are susceptible to such carcinogens. The ultimate carcinogen of these alkylating agents is considered to be the methyl or ethyl cation. Interaction of these cations with cellular DNA causes gene mutations leading to malignant transformation of cells. For example, N-ethyl-N-nitrosourea (ENU) induces adduct formation on the O^6 position of guanine. This is misrepaired into adenine and causes G:C→A:T transitions.

TP53 gene alterations were found in rat glioma cell lines which are transformed by N-ethyl-N-nitrosourea (ENU) (43, 44) (Table 4). All the mutations were located in conserved domains II-V which show 92% homology between human and rat and harbor approximately 95% of *TP53* gene mutations in human tumors. In contrast, spontaneously immortalized and transformed rat type 1 astroglial cell lines showed no alterations in the *TP53* gene (43). This might suggest that cell immortalization may be regulated by gene alterations other than *TP53*, and that *TP53* gene mutation may correlate more with later stages of carcinogenesis. However, as the DNA mutations found in the *TP53* gene were not always compatible with the mechanism of carcinogenesis associated with ENU, other mutagenic factors are not excluded. Furthermore, these results in type 1 rat astrocytes contrast with those obtained with cortical astrocytes of mice where *TP53* nullizygosity was shown to confer immortality (14, 125).

Biological role of mutant p53. Mutant p53 proteins may have different biological activities compared to wt p53. Usually, they have longer half life and may have cytoplasmic as well as nuclear localization (4). Experimental evidence has shown that in general mutant p53 proteins are not able of specific DNA binding and transactivation and have lost the capacity to arrest cell growth. Nevertheless, it is important not to consider all p53 mutants as similar because individual hot spot mutants differ in their properties and may have different behaviors in specific cell types, which might explain why p53 mutation profile differences exist between histological subtypes of cancer. Although there are examples which indicate that these differences are due to exposure to a specific carcinogenic risk (26), at this point we cannot exclude that some p53 point mutations can give tissue specific growth advantages.

It has been shown that heterooligomers between wt and mutant p53 peptidic chains may have an altered conformation which results in a protein tetrameric complex unable to bind DNA and activate transcription. Thus, the presence of a mutant *TP53* allele can have a **dominant negative** effect on the wt allele (102). Nevertheless, it is not possible to predict p53's biological function based only on its conformation. For example, the two "hot spot" DNA contact mutants (248W and 273H) have completely lost p53 biological activity, but maintain wt conformation (82), and heterooligomers between these mutants and wt p53 are, in some cases, able to specifically bind DNA (91). Moreover, the frequent conformational mutant 175H does not seem to be always fully dominant over endogenous wt p53. By induction of mdm2 protein and retention of G1 arrest after irradiation, it was shown that colorectal adenoma cells maintain wt p53 activity when transfected with 175H-p53, but lose it when transfected with 273H-p53 (124). These contradictory data indicate that the dominant effect of mutant over wt p53 could be dependent on the type of p53 mutation as well as on the genetic background of the cell. An issue that has not yet been explored is that cells expressing both mutated and wt *TP53* alleles may contain heterooligomers that lose wt p53-mediated cell cycle control but maintain other biochemical functions mediated by p53.

Some p53 mutants seem to be capable of conferring increased tumorigenicity and metastatic potential on a p53-null background; as an example, it was shown that mutated *TP53* alleles may attenuate p21-mediated growth arrest in glioblastoma cells (51). This **gain of function** of p53 mutants can be achieved by different mechanisms: (i) the ability to stimulate the transcription of several cellular and viral promoters (6, 29, 31, 69, 70, 109, 123); (ii) interaction with cellular proteins p38 and p42 (20); (iii) synergism with protein kinase C in order to induce the expression of the VEGF gene (52); (iv) antagonism of deregulated c-myc-mediated enhancement of apoptosis and decrease in leukemogenicity (65).

Still, there are data that deny the capacity of p53 mutants to confer tumorigenic and metastatic potential. Harvey et al. (38) have shown that a specific mutant *TP53* transgene accelerates tumor development in heterozygous but not nullizygous p53-deficient mice, which indicates the possibility of a dominant-negative effect, but not of a gain of function by mutant p53.

p53 as a prognostic factor for astrocytic gliomas

The limitation of classification based on morphological criteria for glioma has stimulated research for diagnostic and prognostic factors based on molecular analysis. This approach now suggests that two types of glioblastomas exist: type 1 would be characterized by *TP53* gene mutations, would predominantly occur in younger patients and have better prognosis. In contrast, type 2 tumors would show amplification of the *EGFR* gene, loss of heterozygosity on chromosome 10, be commonly found in older age and have poor outcome (118). In a number of cancers, including breast (12,101), lung (46), gastric (72) and colorectal (33), mutation of the *TP53* gene is correlated with more aggressive tumors, a higher rate of metastasis, and worse prognosis. Similar studies were performed on astrocytic tumors,

but the correlation between *TP53* gene status and their prognosis is not clarified yet. Here, we will discuss similar studies on astrocytic tumors by focusing on those where *TP53* status was determined by sequencing, since in our opinion p53 immunohistochemistry does not allow reliable *TP53* status determination. Some researchers suggest that *TP53* mutations have no effect on survival of patients with astrocytic tumors (87). Others propose that *TP53* mutation could be an unfavorable factor in childhood malignant gliomas (84), but this is biased by the fact that *TP53* mutations occur almost exclusively in pediatric brain stem gliomas, which are known to have a very poor prognosis. Another group thinks that relative poorer prognosis of gemistocytic astrocytomas as compared to other types of grade II tumors may be linked to their higher frequency of *TP53* mutation (122). In contrast, the existence of *TP53* mutations in recurrent high-grade tumors may indicate better prognosis (113).

From a clinical perspective, postsurgical determination of *TP53* status would be of great importance and could help to establish new treatment strategies for the astrocytic tumors. There is still much controversy as to the therapeutic protocols to be used in these patients. The roles of chemotherapy and radiotherapy remain unclear, both in low- and high-grade astrocytomas (94). Recent studies in human carcinomas, such as colorectal (11), ovarian (90, 98), and acute lymphoblastic leukemia (71), as well as laboratory tests in glioma (50) and other tumor cell lines (66), have shown that mutation of p53 inactivates the p53-dependent apoptotic pathway and induces chemo- and radio-resistance. Replacement of normal p53 function, or stimulation of the apoptotic pathway leads to re-establishment of chemosensitivity (79, 98). Therefore, it has come as a surprise that glioblastoma with wt *TP53* have much poorer prognosis than those with mutant *TP53*. This might be due to the mutation of other genes in the former group making them more radioresistant (107). Since only 35 patients were analyzed in this study it will be important to confirm this finding in larger patient populations.

Gene therapy

Since conventional treatment protocols against astrocytic gliomas have shown their limitations in improving the prognosis of the patients, new strategies are required for a therapeutic breakthrough. The number of these alterations and the quasi-impossibility to target all tumor cells do not give much sense to a pure gene replacement strategy. However, gene therapy acquires a new meaning for cancer therapy if it is used to regulate biological functions. The transferred genes become molecular tools delivering products that stably counterbalance altered physiological equilibriums characteristic to the local tumor microenvironment.

Most current approaches aim to induce selective tumor cell death by apoptosis or senescence after gene transfer (95). With this respect, the wt *TP53* gene appears a suitable candidate for the therapy against the astrocytic gliomas because of its cell autonomous roles in cell cycle arrest after DNA damage, its activation of apoptosis or senescence, and its cell extrinsic potential to induce secretion of anti-angiogenic molecules. Badie et al., used adenoviral vectors to transfer human wt *TP53* genes into established 9L glioma in rat brain. *TP53* gene transduction inhibited tumor growth by 40% and induced cell phenotypic changes *in vitro* (7). Köck et al (57) further investigated the effects of two adenoviruses containing wt *TP53* genes under the control of different promoters on six human glioblastoma cell lines. Growth suppression of human glioblastoma cells *in vitro* and subcutaneous xenografted tumors in nude mice was obtained independently of endogenous *TP53* status in these cells. Apoptosis and tumor regression were only detected when cells were infected with the virus expressing high levels of p53. Tumors regressed to undetectable sizes for 2 months upon 3 intratumoral injections, but recurred 1 month later in 3/5 animals. Li et al (61) also reported that overexpression of p53 protein produced by adenovirus-mediated wt *TP53* gene transfer induces apoptosis of human glioma cells regardless of endogenous *TP53* status. Despite of several advantages, adenoviruses express proteins that trigger immune responses, thereby repeated dosing might be limited. As gene expression may not be permanent, and as glioma cells infiltrate normal brain tissue, repeated delivery of vectors would be necessary for successful therapy.

Unlike viral vectors, DNA-liposome complexes are non-infectious, non-immunogenic, and show low toxicity in vivo. Hsiao et al. injected a liposome-wt *TP53* complex into a cavitary glioblastoma model. This model is created by injecting glioma cells into the peritoneal cavity of nude mice, supposedly mimicking the postsurgical cavity remaining in glioblastoma patients (48). Liposome-DNA complexes penetrated over 20 tumor cell layers and massive necrosis was induced. Tumor cells near the necrotic area expressed exogenous wt p53. Furthermore, mice bearing *TP53* treated tumors survived significantly longer compared to those treated with vector controls (average survival 26 days vs. 15

days). *In vitro*, their study showed that cell growth was inhibited by 54%. Since low efficacy of liposome transfection is the main disadvantage, a number of strategies for enhancing the efficacy have been investigated. (16,48).

In conclusion, reviewing published data revealed that re-introduction of wt *TP53* genes into human glioma inhibit their growth to some degree. However, from a clinical point of view, it is doubtful whether this partial growth inhibition will have a clinical effect on glioma patients by itself, but it could provide useful in combination therapy. The ongoing phase 1 clinical trials with p53 gene transfer will soon address the safety of these treatments. Development of novel gene transfer technology and introducing these new concepts in the clinic will contribute to improved efficacy of this modality in the future, hopefully leading to substantial improvement of the prognosis of patients with CNS tumors.

Viral therapy specific for tumor cells with mutant p53

When an adenovirus infects a cell, p53 gets upregulated due to expression of the viral E1A gene product. p53 increase would normally result in cell cycle arrest or apoptosis, but the virus has evolved a mechanism to circumvent this by expressing the E1B 55kDa protein whose function is to bind and inactivate p53. In tumor cells lacking p53 function, the *E1B* gene is not necessary for viral replication. This feature was exploited to design a new replication-competent adenovirus for cancer therapy. This virus carries an alteration in the *E1B* gene, restricting its replication capacity to tumor cells lacking wt p53 expression. Since virus replication in infected cells induces cell lysis and new virus release, strong antitumor effects were expected.

In vitro, the viruses were 100-fold more toxic to *TP53* mutant tumor cells of a variety of tissues than to normal cells. Anti-tumoral activity against established tumor xenografts in nude mice were also demonstrated after intratumoral or intravenous injection, and this acted in synergy with chemotherapeutic agents. No toxicity to the host animals was observed (42).

Phase I clinical trials are now being conducted to clarify whether specific and non-specific cytotoxic effects might occur. Human patients have an immune system primed with adenovirus and all *in vivo* studies so far were carried out in immuno-deficient mice. The immune system may improve tumor regression by destroying the virus-infected tumor cells, but this might simultaneously reduce virus efficacy. Also, it is not clear whether suppressing the immune system of cancer patients is safe, especially in conjunction with chemotherapeutic agents.

If this virus turns out to be effective in human cancers, it will carry some advantages to treat those malignant gliomas with *TP53* gene alterations since virus spread might reach to deeply invaded tumor cells into the normal brain tissue. This will depend on virus diffusion rate, for virus replication will not occur in normal astrocytes of the infiltrated peritumoral areas.

Acknowledgments

We are grateful to Drs. K. Roemer, K.G. Wiman and P. Walker for critically reading the manuscript, to Larry R. Paul, M.A., for his expert editorial assistance and to Dr. O. Bögler for advice concerning figure 1.

We thank Drs. H. Fillmore and W.C. Broaddus for providing p53 status for cell-line T9.

The Laboratory of Tumor Biology and Genetics is supported by grants 31-49194.96 and 4037-44729 from the swiss National Science Foundation and grant KFS172-9-1995 from the swiss Anti-Cancer Research Foundation (to EGVM). The Laboratory of Molecular Neuro-Oncology is supported by MBNA, N.A.

References

1. Aas AT, Tonnessen TI, Brun A, Salford LG (1995) Growth inhibition of rat glioma cells in vitro and in vivo by aspirin. *J. Neuro-Oncol.* 24: 171-80
2. Agarwal ML, Agarwal A, Taylor WR, Stark GR (1995) p53 controls both the G2/M and the G1 cell cycle checkpoints and mediates reversible growth arrest in human fibroblasts. *Proc. Natl. Acad. Sci. USA.* 92: 8493-7
3. Albertoni M, Daub DM, Arden KC, Viars CS, Powell C, Van Meir EG (1998) Genetic instability leads to loss of both p53 alleles in a human glioblastoma. *Oncogene.* 16: 321-326
4. Ali IU, Schweitzer JB, Ikejiri B, Saxena A, Robertson JT, Oldfield EH (1994) Heterogeneity of subcellular localization of p53 protein in human glioblastomas. *Cancer Res.* 54: 1-5
5. Asai A, Miyagi Y, Sugiyama A, Gamanuma M, Hong SH, Takamoto S, Nomura K, Matsutani M, Takakura K, Kuchino Y (1994) Negative effects of wild-type p53 and s-Myc on cellular growth and tumorigenicity of glioma cells. Implication of the tumor suppressor genes for gene therapy. *J. Neuro-Oncol.* 19: 259-68
6. Avantaggiati ML, Ogryzko V, Gardner K, Giordano A, Levine AS, Kelly K (1997) Recruitment of p300/CBP in p53-dependent signal pathways. *Cell.* 89: 1175-84
7. Badie B, Drazan KE, Kramar MH, Shaked A, Black KL (1995) Adenovirus-mediated p53 gene delivery inhibits 9L glioma growth in rats. *Neuro. Res.* 17: 209-16

8. Baldwin NG, Rice CD, Tuttle TM, Bear HD, Hirsch JI, Merchant RE (1997) Ex vivo expansion of tumor-draining lymph node cells using compounds which activate intracellular signal transduction. I. Characterization and in vivo anti-tumor activity of glioma-sensitized lymphocytes. *J. Neuro-Oncol.* 32: 19-28
9. Beckman WJ, Powers SK, Brown JT, Gillespie GY, Bigner DD, Camps JJ (1987) Differential retention of rhodamine 123 by avian sarcoma virus-induced glioma and normal brain tissue of the rat in vivo. *Cancer.* 59: 266-70
10. Benda P, Someda K, Messer J, Sweet WH (1971) Morphological and immunochemical studies of rat glial tumors and clonal strains propagated in culture. *J. Neurosurg.* 34: 310-23
11. Benhattar J, Cerottini JP, Saraga E, Metthez G, Givel JC (1996) p53 mutations as a possible predictor of response to chemotherapy in metastatic colorectal carcinomas. Int. *J. Cancer.* 69: 190-2
12. Bergh J, Norberg T, Sjogren S, Lindgren A, Holmberg L (1995) Complete sequencing of the p53 gene provides prognostic information in breast cancer patients, particularly in relation to adjuvant systemic therapy and radiotherapy. *Nat. Med.* 1: 1029-34
13. Bertrand P, Rouillard D, Boulet A, Levalois C, Soussi T, Lopez BS (1997) Increase of spontaneous intrachromosomal homologous recombination in mammalian cells expressing a mutant p53 protein. *Oncogene.* 14: 1117-1122
14. Bögler O, Huang HJS, Cavenee WK (1995) Loss of wild-type p53 bestows a growth advantage on primary cortical astrocytes and facilitates their in vitro transformation. *Cancer Res.* 55: 2746- 51
15. Bögler O, Huang HJS, Kleihues P, Cavenee WK (1995) The p53 gene and its role in human brain tumors. *Glia.* 15: 308-27
16. Boulikas T (1996) Liposome DNA delivery and uptake by cells. *Oncol. Rep.* 3: 989-995
17. Brown CR, Doxsey SJ, White E, Welch WJ (1994) Both viral (adenovirus E1B) and cellular (hsp 70, p53) components interact with centrosomes. *J. Cell Physiol.* 160: 47-60
18. Caelles C, Helmberg A, Karin M (1994) p53-dependent apoptosis in the absence of transcriptional activation of p53-target genes. *Nature.* 370: 220-3
19. Chen J, Willingham T, Shuford M, Bruce D, Rushing E, Smith Y, Nisen PD (1996) Effects of ectopic overexpression of p21(WAF1/CIP1) on aneuploidy and the malignant phenotype of human brain tumor cells. *Oncogene.* 13: 1395-403
20. Chen Y, Chen PL, Lee WH (1994) Hot-spot p53 mutants interact specifically with two cellular proteins during progression of the cell cycle. *Mol. Cell. Biol.* 14: 6764-72
21. Cho Y, Gorina S, Jeffrey PD, Pavletich NP (1994) Crystal structure of a p53 tumor suppressor-DNA complex: understanding tumorigenic mutations. *Science.* 265: 346-355
22. Crafts D, Wilson CB (1977) Animal models of brain tumors. *Natl. Canc. Inst. Mon.* 46: 11-7
23. Cross SM, Sanchez CA, Morgan CA, Schimke MK, Ramel S, Idzerda RL, Raskind WH, Reid BJ (1995) A p53-dependent mouse spindle checkpoint. *Science.* 267: 1353-6
24. Dameron KM, Volpert OV, Tainsky MA, Bouck N (1994) Control of angiogenesis in fibroblasts by p53 regulation of thrombospondin-1. *Science.* 265: 1582-4
25. Davis FG, Preston-Martin S (1998) Epidemiology. In: *RUSSELL AND RUBINSTEIN'S Pathology of Tumors of the Nervous Sytem*, Bigner DD, McLendon RE, Bruner JM, 17-33, London
26. Denissenko MF, Pao A, Tang M, Pfeifer GP (1996) Preferential formation of benzo[a]pyrene adducts at lung cancer mutational hotspots in P53. *Science.* 274: 430-2
27. Di Leonardo A, Khan SH, Linke SP, Greco V, Seidita G, Wahl GM (1997) DNA rereplication in the presence of mitotic spindle inhibitors in human and mouse fibroblasts lacking either p53 or pRb function. *Cancer Res.* 57: 1013-1019
28. Di Leonardo A, Linke SP, Clarkin K, Wahl GM (1994) DNA damage triggers a prolonged p53-dependent G1 arrest and long-term induction of Cip1 in normal human fibroblasts. *Genes Develop.* 8: 2540-51
29. Dittmer D, Pati S, Zambetti G, Chu S, Teresky AK, Moore M, Finlay C, Levine AJ (1993) Gain of function mutations in p53. *Nature Genet.* 4: 42-46
30. El-Deiry WS, Tokino T, Velculescu VE, Levy DB, Parsons R, Trent JM, Lin D, Mercer WE, Kinzler KW, Vogelstein B (1993) WAF1, a potential mediator of p53 suppression. *Cell.* 75: 817-825
31. Fujiwara T, Mukhopadhyay T, Cai DW, Morris DK, Roth JA, Grimm EA (1994) Retroviral-mediated transduction of p53 gene increases TGF-beta expression in a human glioblastoma cell line. *Int. J. Cancer.* 56: 834-9
32. Fukasawa K, Choi T, Kuriyama R, Rulong S, Vande Woude G (1996) Abnormal centrosome amplification in the absence of p53. *Science.* 271: 1744-7
33. Goh HS, Yao J, Smith DR (1995) p53 point mutation and survival in colorectal cancer patients. *Cancer Res.* 55: 5217-21
34. Greenblatt MS, Bennett WP, Hollstein M, Harris CC (1994) Mutations in the p53 tumor suppressor gene: clues to cancer etiology and molecular pathogenesis. *Cancer* Res. 54: 4855-78
35. Haas-Kogan D, Yount G, Haas M, Levi D, Kogan SS, Hu L, Vidair C, Deen DF, Dewey WC, Israel MA (1996) p53-dependent G1 arrest and p53-independent apoptosis influence the radiobiologic response of glioblastoma. *Int. J. Radiat. Onco.l Biol. Phys.* 36: 95-103
36. Hainaut P, Soussi T, Shomer B, Hollstein M, Greenblatt M, Hovig E, Harris CC, Montesano R (1997) Database of p53 gene somatic mutations in human tumors and cell lines: updated compilation and future prospects. *Nucl. Ac. Res.* 25: 151-7
37. Harper JW, Adami GR, Wei N, Keyomarsi K, Elledge SJ (1993) The p21 Cdk-interacting protein is a potent inhibitor of G1 cyclin-dependent kinases. *Cell.* 75: 805-816

38. Harvey M, Vogel H, Morris D, Bradley A, Bernstein A, Donehower LA (1995) A mutant p53 transgene accelerates tumour development in heterozygous but not nullizygous p53-deficient mice. *Nature Genet.* 9: 305-11

39. Haupt Y, Barak Y, Oren M (1996) Cell type-specific inhibition of p53-mediated apoptosis by mdm2. *EMBO J.* 15: 1596-606

40. Haupt Y, Rowan S, Oren M (1995) p53-mediated apoptosis in HeLa cells can be overcome by excess pRB. *Oncogene.* 10: 1563-71

41. Hayashi Y, Yamashita J, Yamaguchi K (1991) Timing and role of p53 gene mutation in the recurrence of glioma. *Biochem. Biophys. Res. Comm.* 180: 1145-1150

42. Heise C, Sampson-Johannes A, Williams A, McCormick F, Von Hoff D, Kirn DH (1997) ONYX-015, an E1B gene-attenuated adenovirus, causes tumor-specific cytolysis and antitumoral efficacy that can be augmented by standard chemotherapeutic agents. *Nat. Med.* 3: 639-45

43. Hiraga S, Arita N, Ohnishi T, Izumoto S, Taki T, Higuchi M, Iwaisako K, Sakoda S, Yamamoto Y, Hayakawa T (1996) Establishment of spontaneously immortalized rat type 1 astroglial cell lines: the role of p53 in astroglial carcinogenesis. *Glia.* 18: 185-99

44. Hiraga S, Arita N, Ohnishi T, Izumoto S, Taki T, Yamamoto H, Higuchi M, Hayakawa T (1995) Transformation of type 1 astrocytes with N-ethyl-N-nitrosourea: establishment of an in vitro system and the role of the p53 gene. *Glia.* 13: 51-63

45. Hollstein M, Shomer B, Greenblatt M, Soussi T, Hovig E, Montesano R, Harris CC (1996) Somatic point mutations in the p53 gene of human tumors and cell lines: updated compilation. *Nucl. Ac. Res.* 24: 141-6

46. Horio Y, Takahashi T, Kuroishi T, Hibi K, Suyama M, Niimi T, Shimokata K, Yamakawa K, Nakamura Y, Ueda R, et al (1993) Prognostic significance of p53 mutations and 3p deletions in primary resected non-small cell lung cancer. *Cancer Res.* 53: 1-4

47. Hsiao M, Tse V, Carmel J, Costanzi E, Strauss B, Haas M, Silverberg GD (1997) Functional expression of human p21(WAF1/CIP1) gene in rat glioma cells suppresses tumor growth in vivo and induces radiosensitivity. *Biochem. Biophys. Res. Comm.* 233: 329-35

48. Hsiao M, Tse V, Carmel J, Tsai Y, Felgner PL, Haas M, Silverberg GD (1997) Intracavitary liposome-mediated p53 gene transfer into glioblastoma with endogenous wild-type p53 in vivo results in tumor suppression and long-term survival. *Biochem. Biophys. Res. Comm.* 233: 359-364

49. Imamura J, Bartram CR, Berthold F, Harms D, Nakamura H, Koeffler HP (1993) Mutation of the p53 gene in neuroblastoma and its relationship with N-myc amplification. *Cancer Res.* 53: 4053-8

50. Iwadate Y, Fujimoto S, Tagawa M, Namba H, Sueyoshi K, Hirose M, Sakiyama S (1996) Association of p53 gene mutation with decreased chemosensitivity in human malignant gliomas. *Int. J. Cancer.* 69: 236-40

51. Jung JM, Li H, Kobayashi T, Kyritsis AP, Langford LA, Bruner JM, Levin VA, Zhang W (1995) Inhibition of human glioblastoma cell growth by WAF1/Cip1 can be attenuated by mutant p53. *Cell Growth Differ.* 6: 909-13

52. Kieser A, Weich HA, Brandner G, Marme D, Kolch W (1994) Mutant p53 potentiates protein kinase C induction of vascular endothelial growth factor expression. *Oncogene.* 9: 963-9

53. Kleihues P, Aguzzi A, Ohgaki H (1995) Genetic and environmental factors in the etiology of human brain tumors. *Toxicol. Lett.* 3: 601-605

54. Kleihues P, Ohgaki H, Eibl RH, Reichel MB, Mariani L, Gehring M, Petersen I, Holl T, von Deimling A, Wiestler OD, et al. (1994) Type and frequency of p53 mutations in tumors of the nervous system and its coverings. *Recent Results Cancer Res.* 135: 25-31

55. Kleihues P, Schauble B, zur Hausen A, Esteve J, Ohgaki H (1997) Tumors associated with p53 germline mutations: a synopsis of 91 families. *Am. J. Pathol.* 150: 1-13

56. Ko LJ, Prives C (1996) P53 - Puzzle and Paradigm. *Genes Develop.* 10: 1054-1072

57. Köck H, Harris MP, Anderson SC, Machemer T, Hancock W, Sutjipto S, Wills KN, Gregory RJ, Shepard HM, Westphal M, Maneval DC (1996) Adenovirus-mediated p53 gene transfer suppresses growth of human glioblastoma cells in vitro and in vivo. I*nt. J. Cancer.* 67: 808-15

58. Kondo S, Barna BP, Kondo Y, Tanaka Y, Casey G, Liu J, Morimura T, Kaakaji R, Peterson JW, Werbel B, Barnett GH (1996) WAF1/CIP1 increases the susceptibility of p53 non-functional malignant glioma cells to cisplatin-induced apoptosis. *Oncogene.* 13: 1279-85

59. Kyritsis AP, Yung WK, Leeds NE, Bruner J, Gleason MJ, Levin VA (1992) Multifocal cerebral gliomas associated with secondary malignancies. *Lancet.* 339: 1229-30

60. Levine AJ (1997) p53, the cellular gatekeeper for growth and division. *Cell.* 88: 323-31

61. Li HW, Lochmuller H, Yong VW, Karpati G, Nalbantoglu J (1997) Adenovirus-mediated wild-type p53 gene transfer and overexpression induces apoptosis of human glioma cells independent of endogenous p53 status. J. Neuropath. *Exp. Neur.* 56: 872-878

62. Li J, Hu SX, Perng GS, Zhou Y, Xu K, Zhang C, Seigne J, Benedict WF, Xu HJ (1996) Expression of the retinoblastoma (RB) tumor suppressor gene inhibits tumor cell invasion in vitro. *Oncogene.* 13: 2379-86

63. Lin D, Shields MT, Ullrich SJ, Appella E, Mercer WE (1992) Growth arrest induced by wild-type p53 protein blocks cells prior to or near the restriction point in late G1 phase. *Proc. Natl. Acad. Sci. USA.* 89: 9210-4

64. Livingstone LR, White A, Sprouse J, Livanos E, Jacks T, Tlsty TD (1992) Altered cell cycle arrest and gene amplification potential accompany loss of wild-type p53. *Cell.* 70: 923-35

65. Lotem J, Sachs L (1995) A mutant p53 antagonizes the deregulated c-myc-mediated enhancement of apoptosis and decrease in leukemogenicity. *Proc. Natl. Acad. Sci. USA.* 92: 9672-6

66. Lowe SW, Bodis S, McClatchey A, Remington L, Ruley HE, Fisher DE, Housman DE, Jacks T (1994) p53 status and the efficacy of cancer therapy in vivo. *Science.* 266: 807-10

67. Lubin R, Schlichtholz B, Bengoufa D, Zalcman G, Tredaniel J, Hirsch A, de Fromentel CC, Preudhomme C, Fenaux P, Fournier G, et al (1993) Analysis of p53 antibodies in patients with various cancers define B-cell epitopes of human p53: distribution on primary structure and exposure on protein surface. *Cancer Res.* 53: 5872-6

68. Lubin R, Zalcman G, Bouchet L, Tredanel J, Legros Y, Cazals D, Hirsch A, Soussi T (1995) Serum p53 antibodies as early markers of lung cancer. *Nat. Med.* 1: 701-2

69. Ludes MJ, Subler MA, Shivakumar CV, Munoz RM, Jiang P, Bigger JE, Brown DR, Deb SP, Deb S (1996) Transcriptional activation of the human epidermal growth factor receptor promoter by human p53. *Mol. Cell. Biol.* 16: 6009-19

70. Margulies L, Sehgal PB (1993) Modulation of the human interleukin-6 promoter (IL-6) and transcription factor C/EBP beta (NF-IL6) activity by p53 species. *J. Biol. Chem.* 268: 15096-100

71. Marks DI, Kurz BW, Link MP, Ng E, Shuster JJ, Lauer SJ, Carroll D, Brodsky I, Haines DS (1997) Altered expression of p53 and mdm-2 proteins at diagnosis is associated with early treatment failure in childhood acute lymphoblastic leukemia. *J. Clin. Oncol.* 15: 1158-62

72. Martin HM, Filipe MI, Morris RW, Lane DP, Silvestre F (1992) p53 expression and prognosis in gastric carcinoma. *Int. J. Cancer.* 50: 859-62

73. Mayordomo JI, Loftus DJ, Sakamoto H, De Cesare CM, Appasamy PM, Lotze MT, Storkus WJ, Appella E, DeLeo AB (1996) Therapy of murine tumors with p53 wild-type and mutant sequence peptide-based vaccines. *J. Exp. Med.* 183: 1357-65

74. Mekeel KL, Tang W, Kachnic LA, Luo CM, Defrank JS, Powell SN (1997) Inactivation of p53 results in high rates of homologous recombination. *Oncogene.* 14: 1847-1857

75. Mercer WE, Shields MT, Amin M, Sauve GJ, Appella E, Romano JW, Ullrich SJ (1990) Negative growth regulation in a glioblastoma tumor cell line that conditionally expresses human wild-type p53. *Proc. Natl. Acad. Sci. USA.* 87: 6166-6170

76. Mercer WE, Shields MT, Lin D, Appella E, Ullrich SJ (1991) Growth suppression induced by wild-type p53 protein is accompanied by selective down-regulation of proliferating-cell nuclear antigen expression. *Proc. Natl. Acad. Sci. USA.* 88: 1958-1962

77. Miyashita T, Krajewski S, Krajewska M, Wang HG, Lin HK, Liebermann DA, Hoffman B, Reed JC (1994) Tumor suppressor p53 is a regulator of bcl-2 and bax gene expression in vitro and in vivo. *Oncogene.* 9: 1799-805

78. Miyashita T, Reed JC (1995) Tumor suppressor p53 is a direct transcriptional activator of the human bax gene. *Cell.* 80: 293-9

79. Nguyen DM, Spitz FR, Yen N, Cristiano RJ, Roth JA (1996) Gene therapy for lung cancer: enhancement of tumor suppression by a combination of sequential systemic cisplatin and adenovirus-mediated p53 gene transfer. *J. Thorac. Cardiov. Sur.* 112: 1372-6

80. Nishimori H, Shiratsuchi T, Urano T, Kimura Y, Kiyono K, Tatsumi K, Yoshida S, Ono M, Kuwano M, Nakamura Y, Tokino T (1997) A novel brain-specific p53-target gene, BAI1, containing thrombospondin type 1 repeats inhibits experimental angiogenesis. *Oncogene.* 15: 2145-2150

81. Ohgaki H, Eibl RH, Schwab M, Reichel MB, Mariani L, Gehring M, Petersen I, Holl T, Wiestler OD, Kleihues P (1993) Mutations of the p53 tumor suppressor gene in neoplasms of the human nervous system. *Mol Carcinog.* 8: 74-80

82. Ory K, Legros Y, Auguin C, Soussi T (1994) Analysis of the most representative tumour-derived p53 mutants reveals that changes in protein conformation are not correlated with loss of transactivation or inhibition of cell proliferation. *EMBO J.* 13: 3496-504

83. Plate KH, Breier G, Weich HA, Mennel HD, Risau W (1994) Vascular endothelial growth factor and glioma angiogenesis: coordinate induction of VEGF receptors, distribution of VEGF protein and possible in vivo regulatory mechanisms. *Int J Cancer.* 59: 520-9

84. Pollack IF, Hamilton RL, Finkelstein SD, Campbell JW, Martinez AJ, Sherwin RN, Bozik ME, Gollin SM (1997) The relationship between TP53 mutations and overexpression of p53 and prognosis in malignant gliomas of childhood. *Cancer Res.* 57: 304-9

85. Polyak K, Waldman T, He TC, Kinzler KW, Vogelstein B (1996) Genetic determinants of p53-induced apoptosis and growth arrest. *Genes Develop.* 10: 1945-52

86. Polyak K, Xia Y, Zweier JL, Kinzler KW, Vogelstein B (1997) A model for p53-induced apoptosis. *Nature.* 389: 300-305

87. Rainov NG, Dobberstein KU, Bahn H, Holzhausen HJ, Lautenschlager C, Heidecke V, Burkert W (1997) Prognostic factors in malignant glioma : Influence of the overexpression of oncogene and tumor-suppressor gene products on survival. *J. Neuro-Oncol.* 35: 13-28

88. Reifenberger G, Liu L, Ichimura K, Schmidt EE, Collins VP (1993) Amplification and overexpression of the MDM2 gene in a subset of human malignant gliomas without p53 mutations. *Cancer Res.* 53: 2736-9

89. Rice CD, Baldwin NG, Biron RT, Bear HD, Merchant RE (1997) Ex vivo expansion of tumor-draining lymph node cells using compounds which activate intracellular signal transduction. II. Cytokine production and in vivo efficacy of glioma-sensitized lymphocytes. *J. Neuro-Oncol.* 32: 29-38

90. Righetti SC, Della TG, Pilotti S, Menard S, Ottone F, Colnaghi MI, Pierotti MA, Lavarino C, Cornarotti M, Oriana S, Bohm S, Bresciani GL, Spatti G, Zunino F (1996) A comparative study of p53 gene mutations, protein accumulation, and response to cisplatin-based chemotherapy in advanced ovarian carcinoma. *Cancer Res.* 56: 689-93

91. Rolley N, Butcher S, Milner J (1995) Specific DNA binding by different classes of human p53 mutants. *Oncogene.* 11: 763-70

92. Ropke M, Hald J, Guldberg P, Zeuthen J, Norgaard L, Fugger L, Svejgaard A, Van Der Burg, S, Nijman HW, Melief CJ, Claesson MH (1996) Spontaneous human squamous cell carcinomas are killed by a human cytotoxic T lymphocyte clone recognizing a wild-type p53-derived peptide. *Proc. Natl. Acad. Sci. USA.* 93: 14704-7

93. Sakamuro D, Sabbatini P, White E, Prendergast GC (1997) The polyproline region of p53 is required to activate apoptosis but not growth arrest. *Oncogene.* 15: 887-98

94. Salcman M (1995) The natural history of low-grade gliomas. In: *Benign Cerebral Glioma*, M.L.J. A, 213-229, Illinois

95. Sandig V, Brand K, Herwig S, Lukas J, Bartek J, Strauss M (1997) Adenovirally transferred p16INK4/CDKN2 and p53 genes cooperate to induce apoptotic tumor cell death. *Nat. Med.* 3: 313-9

96. Schlichtholz B, Legros Y, Gillet D, Gaillard C, Marty M, Lane D, Calvo F, Soussi T (1992) The immune response to p53 in breast cancer patients is directed against immunodominant epitopes unrelated to the mutational hot spot. *Cancer Res.* 52: 6380-4

97. Serrano M, Lin AW, McCurrach ME, Beach D, Lowe SW (1997) Oncogenic ras provokes premature cell senescence associated with accumulation of p53 and p16INK4a. *Cell.* 88: 593-602

98. Shelling AN (1997) Role of p53 in drug resistance in ovarian cancer. *Lancet.* 349: 744-5

99. Shin TH, Paterson AJ, Kudlow JE (1995) p53 stimulates transcription from the human transforming growth factor alpha promoter: a potential growth-stimulatory role for p53. *Mol. Cell. Biol.* 15: 4694-701

100. Sidransky D, Mikkelsen T, Schwechheimer K, Rosenblum ML, Cavenee W, Vogelstein B (1992) Clonal expansion of p53 mutant cells is associated with brain tumour progression. *Nature.* 355: 846-7

101. Sjögren S, Inganas M, Norberg T, Lindgren A, Nordgren H, Holmberg L, Bergh J (1996) The p53 gene in breast cancer: prognostic value of complementary DNA sequencing versus immunohistochemistry. *J. Natl. Canc. Inst.* 88: 173-82

102. Srivastava S, Wang S, Tong YA, Hao ZM, Chang EH (1993) Dominant negative effect of a germ-line mutant p53: a step fostering tumorigenesis. *Cancer Res.* 53: 4452-5

103. Stewart N, Hicks GG, Paraskevas F, Mowat M (1995) Evidence for a second cell cycle block at G2/M by p53. *Oncogene.* 10: 109-15

104. Sturzbecher HW, Donzelmann B, Henning W, Knippschild U, Buchhop S (1996) p53 is linked directly to homologous recombination processes via RAD51/RecA protein interaction. *EMBO J.* 15: 1992-2002

105. Tada M, Iggo RD, Ishii N, Shinohe Y, Sakuma S, Estreicher A, Sawamura Y, Abe H (1996) Clonality and stability of the p53 gene in human astrocytic tumor cells: quantitative analysis of p53 gene mutations by yeast functional assay. *Int. J. Cancer.* 67: 447-50

106. Tada M, Iggo RD, Waridel F, Nozaki M, Matsumoto R, Sawamura Y, Shinohe Y, Ikeda J, Abe H (1997) Reappraisal of p53 mutations in human malignant astrocytic neoplasms by p53 functional assay: comparison with conventional structural analyses. *Mol. Carcin.* 18: 171-6

107. Tada M, Matsumoto R, Iggo RD, Onimaru R, Shirato H, Sawamura Y, Shinohe Y (1998) Selective sensitivity to radiation of cerebral glioblastomas harboring p53 mutations. *Cancer Res.* 58: 1793-1797

108. Tenan M, Carrara F, Di Donato S, Finocchiaro G (1995) Absence of mutations and identification of two polymorphisms in the SSCP and sequence analysis of p21CKI gene in malignant gliomas. *Int J Cancer.* 62: 115-7

109. Ueba T, Nosaka T, Takahashi JA, Shibata F, Florkiewicz RZ, Vogelstein B, Oda Y, Kikuchi H, Hatanaka M (1994) Transcriptional regulation of basic fibroblast growth factor gene by p53 in human glioblastoma and hepatocellular carcinoma cells. *Proc. Natl. Acad. Sci. USA.* 91: 9009-13

110. Van Meir EG, Kikuchi T, Tada M, Li H, Diserens AC, Wojcik BE, Huang H-JS, Friedmann T, de Tribolet N, Cavenee WK (1994) Analysis of the p53 gene and its expression in human glioblastoma cells. *Cancer Res.* 54: 649-52

111. Van Meir EG, Polverini PJ, Chazin VR, Huang H-JS, de Tribolet N, Cavenee WK (1994) Release of an inhibitor of angiogenesis upon induction of wild type p53 expression in glioblastoma cells. *Nature Genet.* 8: 171-6

112. Van Meir EG, Roemer K, Diserens AC, Kikuchi T, Rempel SA, Haas M, Huang H-JS, Friedmann T, de Tribolet N, Cavenee WK (1995) Single cell monitoring of growth arrest and morphological changes induced by transfer of wild-type p53 alleles to glioblastoma cells. *Proc Natl Acad Sci U S A.* 92: 1008-12

113. Van Meyel DJ, Ramsay DA, Casson AG, Keeney M, Chambers AF, Cairncross JG (1994) p53 mutation, expression, and DNA ploidy in evolving gliomas: evidence for two pathways of progression. *J. Natl. Canc. Inst.* 86: 1011-7

114. Vierboom MP, Nijman HW, Offringa R, van Der Voort EI, van Hall T, van Den Broek L, Fleuren GJ, Kenemans P, Kast WM, Melief CJ (1997) Tumor eradication by wild-type p53-specific cytotoxic T lymphocytes. *J. Exp. Med.* 186: 695-704

115. Volpert OV, Dameron KM, Bouck N (1997) Sequential development of an angiogenic phenotype by human fibroblasts progressing to tumorigenicity. *Oncogene.* 14: 1495-502

116. Volpert OV, Tolsma SS, Pellerin S, Feige JJ, Chen H, Mosher DF, Bouck N (1995) Inhibition of angiogenesis by thrombospondin-2. Biochem. *Biophys. Res. Comm.* 217: 326-32

117. von Deimling A, Eibl RH, Ohgaki H, Louis DN, von Ammon K, Petersen I, Kleihues P, Chung RY, Wiestler OD, Seizinger BR (1992) p53 mutations are associated with 17p allelic loss in grade II and grade III astrocytoma. *Cancer Res.* 52: 2987-90

118. von Deimling A, von Ammon K, Schoenfeld D, Wiestler OD, Seizinger BR, Louis DN (1993) Subsets of glioblastoma multiforme defined by molecular genetic analysis. *Brain Pathol.* 3: 19-26

119. Walker KK, Levine AJ (1996) Identification of a novel p53 functional domain that is necessary for efficient growth suppression. *Proc. Natl. Acad. Sci. USA.* 93: 15335-40

120. Wang XW, Yeh H, Schaeffer L, Roy R, Moncollin V, Egly JM, Wang Z, Freidberg EC, Evans MK, Taffe BG, et al (1995) p53 modulation of TFIIH-associated nucleotide excision repair activity. *Nature Genet.* 10: 188-95

121. Watanabe K, Tachibana O, Sata K, Yonekawa Y, Kleihues P, Ohgaki H (1996) Overexpression of the EGF receptor and p53 mutations are mutually exclusive in the evolution of primary and secondary glioblastomas. *Brain Pathol.* 6: 217-23

122. Watanabe K, Tachibana O, Yonekawa Y, Kleihues P, Ohgaki H (1997) Role of gemistocytes in astrocytoma progression. *Lab. Invest.* 76: 277-84

123. Werner H, Karnieli E, Rauscher FJ, LeRoith D (1996) Wild-type and mutant p53 differentially regulate transcription of the insulin-like growth factor I receptor gene. *Proc. Natl. Acad. Sci. USA.* 93: 8318-23

124. Williams AC, Miller JC, Collard TJ, Bracey TS, Cosulich S, Paraskeva C (1995) Mutant p53 is not fully dominant over endogenous wild type p53 in a colorectal adenoma cell line as demonstrated by induction of MDM2 protein and retention of a p53 dependent G1 arrest after gamma irradiation. *Oncogene.* 11: 141-9

125. Yahanda AM, Bruner JM, Donehower LA, Morrison RS (1995) Astrocytes derived from p53-deficient mice provide a multistep in vitro model for development of malignant gliomas. *Mol. Cell. Biol.* 15: 4249-59

126. Yanuck M, Carbone DP, Pendleton CD, Tsukui T, Winter SF, Minna JD, Berzofsky JA (1993) A mutant p53 tumor suppressor protein is a target for peptide-induced CD8+ cytotoxic T-cells. *Cancer Res.* 53: 3257-61

127. Yount GL, Haas KD, Vidair CA, Haas M, Dewey WC, Israel MA (1996) Cell cycle synchrony unmasks the influence of p53 function on radiosensitivity of human glioblastoma cells. *Cancer Res.* 56: 500-6

128. Zhang YP, Xiong Y, Yarbrough WG (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