Proc. Indian natn Sci. Acad.(PINSA) **B66** Nos 2 & 3 pp 97-120 (2000)

Molecular Basis of P53 Gene Deregulation: An Overview

B R DAS* and B K NAYAK
Molecular Oncology and Medical Biotechnology Division
Institute of Life Sciences, Nalco Square, Chandrasekharpur, Bhubaneswar-751 023

(Receivepd on 11 October 1999; after revision 22 March 2000; Accepted on 13 April 2000)

Initially described as an oncogene, at the end of 1980s, the wild-type p53 gene was later shown to be capable of suppressing the proliferation of transformed cells. In the following years, an increasing number of studies demonstrated its role in a variety of cellular outcomes, most notably cell cycle control and apoptosis. These cellular effects of wild-type p53 can reduce cancer incidence through elimination of cancer-prone cells from the replicative pool. Thus, p53 function is essential for the maintenance of the non-tumorigenic phenotype of cells. Indeed, functional inactivation of the p53 protein is one of the most common alterations observed in human cancers and plays a major role in malignant transformation. P53 is known to activate and repress a number of genes and in the process it controls the different cellular processes. Thus, it is well established that p53 acts as a transcriptional regulator. However, the mechanisms leading to inactivation/deregulation of p53 gene itself is not clearly defined. In the present review an attempt has been made to look into the different aspects like gene mutation, p53 protein accumulation, rearrangement, methylation, deregulation of NF1 transcription factors and their role in p53 function/ dysfunction.

Key Words: p53, mutation, rearrangement, p53 promoter, methylation, NF1, Phosphorylation

Introduction

In the past decade, cellular oncogenes have attracted the attention of biologists intent on understanding the molecular origin of cancers. As the present decade unfolds, oncogenes are yielding their place at center stage to a second group of actors, the tumour suppressor genes, which promise to teach us equally important lessons about the molecular mechanisms of cancer pathogenesis. The early evidence for the existence of tumour suppressor genes came from somatic cell hybridization, which showed that fusion of tumour cells with normal cells almost invariably results in the outgrowth of nontumorigenic hybrids (Harris et al. 1969). During the past few years interest in tumour suppressor genes has been building exponentially, and with good reason. Cancer researchers have accumulated convincing evidences that loss of growth inhibition exerted by these genes plays a major role in cancer. But while the spotlight has been on all known tumour suppressor genes, one of them, p53, has drawn most of the attention, because mutations in that gene contribute to the development of upto 50% of all human cancers.

*Corresponding author: e-mail : brdils@hotmail.com

Abbrevations *UTR*, Untranslated Region; *PCNA*, Proliferating Cell Nuclear Antigen; *CDK*, Cyclin Dependent Kinase; *IGFBP*, Insulin -like Growth Factor Binding Protein; *GADD45*-Growth Arrest and DNA Damage gene 45; *DP1*, Dimerization Partner 1; *USF*-Upstream Stimulatory Factor; *PCAF*-p300/CBP Associated Factor (CBP-CREB-binding protein); *MDM2*, Murine Double Minute; *CBP-CREB* Binding Protein (CREB-Cyclic AMP Responsive Element Binding Protein); *JMY*-Junction Mediating and Regulatory Protein

The product of the p53 tumour suppressor gene was first discovered in 1979 complexed to the Simian Virus 40 (SV40) large T antigen (in SV40-transformed rodent cells) and adenovirus E1B oncoproteins (Lane & Crawford 1979, Linzer & Levine 1979). Initially p53 was classified as a tumour antigen. Transfection of the p53 gene into rodent embryo fibroblasts suggested that p53 was an oncogene as it was capable of immortalizing these cells by itself or transforming them in conjunction with the ras oncogene (Eliyahu et al. 1984, Rovinski & Benchimol 1988). Only in the last few years it has become clear that wild-type p53 behaves as a negative growth regulator or tumour suppressor gene. The earlier transfection studies demonstrating oncogenic properties of p53 were misleading because mutant forms of p53 cDNA were used (Hinds et al. 1989). Many mutant forms of p53 are indeed capable of behaving in an oncogenic manner (Levine et al. 1991). The evidence for the tumour suppressor activity of wild-type p53 is now conclusive. Transfection of wild-type p53 into tumour cell lines reduces or terminates cell growth and division (Baker et al. 1990; Mercer et al. 1990). Loss of wild-type p53 alleles is exceedingly common in human tumours; the first p53 allele may incur a point mutation, and the remaining wild type allele is often lost in the progression of the tumour (Nigro et al. 1989; Hollstein et al. 1991; Caron de Fromentel & Soussi 1992). While co-transfection of mutated p53 and ras causes transformation of rodent embryo fibroblasts in culture (Eliyahu et al. 1984; Parada et al. 1984), addition of wild type p53 DNA to mutant p53 and ras results in a marked decrease in transformed colonies (Eliyahu et al. 1989; Finlay et al. 1989). Human families with Li-Fraumeni syndrome, an inherited predisposition to cancer, have a mutated germ line p53 gene (Malkin et al. 1990; Srivastava et al. 1990). Some mice with Friend virus-induced erythroleukemia have rearranged or deleted p53 alleles in their tumour cells (Ben David & Bernstein 1991). Finally, it has been shown that p53-deficient mice generated by gene targeting methods, with two p53 null alleles, develop normally, but are susceptible to tumours at an young age (Donehower et al. 1992).

Structure of p53 Gene

The p53 gene has been found to be highly conserved in evolution. It has been isolated from many different species, including Xenopus levis (Soussi et al. 1987), rainbow trout (Caron de Fromentel et al. 1992), chicken (Louis et al. 1988), monkey (Rigaudy & Eckhart 1989), rat (Soussi et al. 1988), mouse (Oren & Levine 1983) and human (Matlashewski et al. 1984; Lamb & Crawford 1986).

P53 gene (coding region): In humans, p53 spans a region of 20 Kb and is located in the short arm of chromosome 17, 17p 13.1 (Benchimol et al. 1985). The mouse homologue is relatively smaller (12 Kb) and resides on chromosome 11 (Czosnek et al. 1984). The relatively complex structural organization of the p53 gene is quite similar among different species. A considerable similarity exists between mouse and human p53. In both cases, the gene is split into 11 exons separated by 10 introns. The first untranslated exon in both mouse and human is followed by an unusually large intron sequence. The much greater size of the human p53 gene is partly due to the longer intron sequences, particularly the first intron, which is 10 Kb in human and 6 Kb in mouse (Beinz-Tadmor et al. 1985; Lamb & Crawford 1986). The 2.5 Kb intron 9 of the human is about three times longer than the 0.83 Kb mouse intron. The human exons vary in length from 22 to 1,268 bp; exons 2, 4, 5, 7 and 8 code for five clusters of amino acid residues which are highly conserved during evolution.

P53 promoter: The human p53 gene appears to be controlled by two promoter elements separately regulated during cellular differentiation (Reisman et al. 1988; Tuck & Crawford 1989). Primer extension experiments indicated that one promoter lies in intron 1, about 1000 bp downstream of the first p53 exon, while the other promoter is located 100 to 250 bp upstream of the noncoding first exon (Reisman et al. 1988). Unlike typical genes transcribed by RNA polymerase II, p53 gene does not contain TATA or CAAT boxes; nor they are like the housekeeping gene promoters, highly GC rich (Beinz - Tadmor et al. 1985; Lamb & Crawford, 1986). Perhaps because of this and a potential stem loop structure in the 5' part of the gene (Beinz et al. 1984), attempts to map the transcriptional start site have yielded quite different results. In the human gene, the major transcription start site is at position -114 from the 3' end of exon 1 (Tuck & Crawford 1989). Data for the mouse gene

indicate a major start site at -216 with at least two other alternative start sites (Beinz et al. 1984, Beinz - Tadmor et al. 1985).

P53 mRNA: The mature and spliced p53 mRNA is 2.2-2.5 Kb in size and is expressed in a cell type-dependent manner (Oren et al. 1983, Soussi et al. 1990). The p53 mRNA has the capacity to form a stable stem-loop structure that involves 5'-untranslated region (5'-UTR) spanning nucleotides -216 to +284 (Beinz-Tadmor et al. 1985, Mosner et al. 1995). Human p53 mRNA also contains a long 3' UTR of 1176 nucleotides with an Alu-like repetitive sequence element of ~ 470 bp located immediately upstream of the poly (A) tail (Matlashewski et al. 1984). The Alu-like sequence is in the reverse transcriptional orientation with respect to the p53 gene. The Alu-like element in the 3' UTR of human p53 mRNA is predicted to form an independent secondary structure that does not have long-range interactions with other regions of p53 mRNA. In the presence of a poly (A) tail, the secondary structure formed by the Alu-like element is predicted to remain essentially intact except that a 50 nucleotide U-rich sequence at the 5' boundary of the Alu-like sequence will interact with the poly (A) tail. The extended base pairing between U and A residues will further stabilize the secondary structure formed by the Alu-like element. It has been demonstrated that the 5' UTR and 3' UTR regions in the p53 mRNA suppress translation of p53 mRNA (Mosner et al. 1995, Fu et al. 1996).

P53 protein: The cloning and sequencing of p53 cDNAs from a variety of species has enabled detailed analysis of structural and evolutionary features of the p53 protein. The human p53 protein contains 393 amino acids and has been divided structurally and functionally into three domains (figure 1) i.e., (i) transcriptional activation domain, (ii) sequence specific DNA binding domain, oligomerization domain and nonspecific DNA binding domain and (iii) multi-functional carboxy-terminal domain (Cho et al. 1994). The different p53-interacting factors are summarized in table-1.

Functions of p53: P53 controls a number of key cellular events and among these, the best documented are cell cycle control (Kastan et al. 1992, Agarwal et al. 1998, Gire & Wynford-Thomas 1998), apoptosis (Polyak et al. 1997, Smith & Fornace

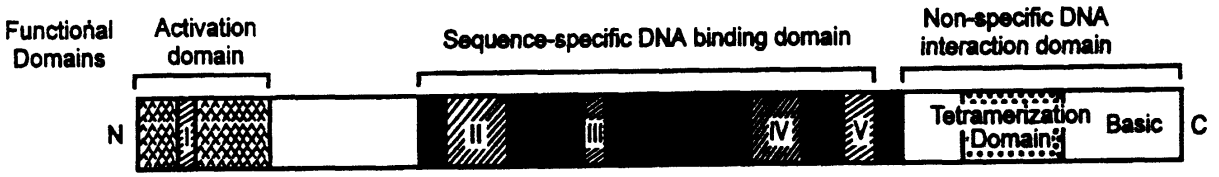

Figure 1 Map showing the functional domains of human p53 protein

Table 1 *Different cellular and viral proteins interacting with p53*

A. Oncogene products

MDM2- blocks p53 transcriptional activation domain (Wu et al. 1993)

c-Abl - brings about *p53* mediated cell-cycle arrest (Goga et al. 1995)

B. Transcription factors

TATA binding protein- binds amino and carboxyl terminal domain of *p53* (Horikoshi et al. 1995)

C. Coactivators

P300- acts as a cofactor for *p53* in transcriptional regulation (Lill et al. 1997)

CBP- acts as a cofactor for *p53* in transcriptional regulation (Gu et al. 1997)

JMY- acts as a cofactor for *p53* in transcriptional regulation (Shikama et al. 1999)

D. Other tumour suppressors

WT1- alters *p53* activities (Maheswaran et al. 1995)

E. Viral proteins

SV40 T antigen- blocks *p53* binding domain (Levine 1997)

Human papilloma virus E6- promotes the degradation of *p53* (Levine 1997)

1997), differentiation and development (Rotter et al. 1994, Hall & Lane 1997). Each of these biological roles of p53 contributes to the ability of p53 to limit the tumorigenicity of cells. Thus, p53 decreases the chance of mutant cell populations arising and acts as 'guardian of the genome' (Lane 1992). Although we do not yet fully understand how p53 elicits its effects upon cells, it is clear that the transcriptional activating function of p53 is a major component of its biological effects (Ko & Prives 1996, Levine 1997). Identification of transcriptional targets of p53 has been critical in discerning pathways by which p53 affects global cellular outcomes such as growth, arrest and death (figure 2). A substantial number of genes have been claimed to contain p53-binding sites and/ or response elements and thus to have the potential to be target genes. P53 activates the expression of several genes like p21 (El-Deiry et al. 1993), mdm2 (Wu et al. 1993), GADD45 (Kastan et al.1992, Zhang et al. 1998), cyclin G (Zauberman et al. 1995), Bax (Miyashita & Reed, 1995) and IGF-BP3 (Buckbinder et al. 1995) and also known to repress genes like Insulin like growth factor 1 receptor gene (Werner et al.1996), H19 gene (Dugimont et al. 1998), mouse DP1 promoter (Gopalakrishnan et al. 1998), HVB enhancer (Ori et al. 1998), Hepatitis C virus (Ray et al.1998) and TF III B (Cairns & White 1998). Thus, it is well established that p53 acts as a general transcription factor and regulates the expression of several genes. But the precise mechanisms leading to p53 gene inactivation/deregulation are not well defined. Moreover, most of the available informations are in in vitro systems. However, several studies have been conducted in solid tumours only on mutational aspects of p53. Whereas there is a scarcity of information in several other aspects of p53 gene in solid tumours. With this background, in this review an attempt has been made to assess the different mechanisms responsible for p53 gene inactivation during the process of tumorigenesis.

Mutation in p53 Gene

The p53 gene and its protein product have become the center of intensive study ever since it became clear that more than 50% of human cancers contain mutations in this gene (Hollstein et al. 1991, Greenblatt et al. 1994, Hollstein et al. 1996, Levine

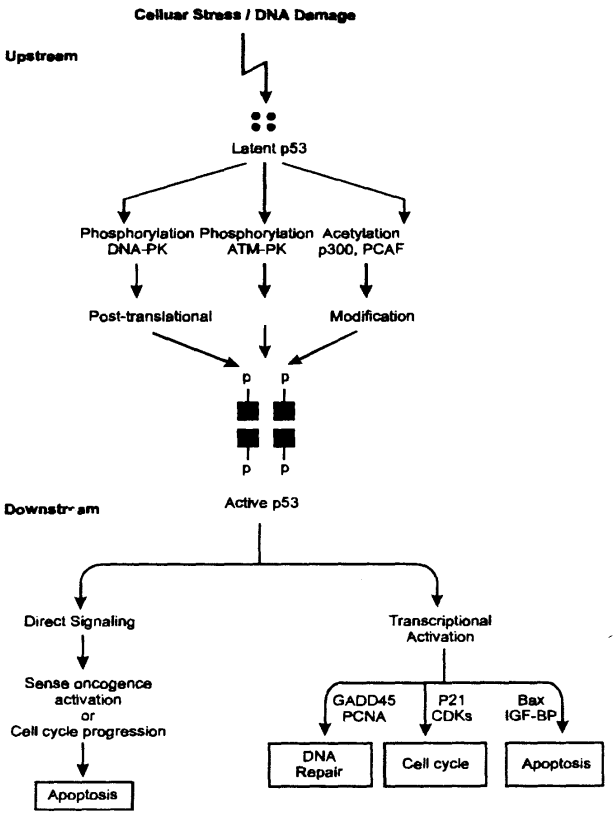

Figure 2 The events in p53 activation (upstream events) and the biological functions of p53 (downstream events).

1997). Most p53 mutations found in human tumours are confined to exons 4-8, which correspond to the highly conserved sequences of the protein. In addition, mutations at specific codons ("hot spots": codons 175, 245, 248, 249, 273, 282) occur very frequently (Greenblatt et al. 1994). The incidence, distribution and nature of p53 mutations show tumour type, tissue and exogenous carcinogen specificity.

Exogenous carcinogens and p53 mutation: Early analyses of the database have revealed examples of mutation patterns consistent with fingerprints of DNA damage induced by defined exogenous carcinogens. These examples include G®T transversion at codon 249 (AGG®AGT) in hepatocellular carcinomas of patients with high dietary exposure to Aflatoxin B1 (Hainut et al. 1998) and tandem CC®TT transitions, a typical signature of UV-induced mutagenesis in non-melanoma skin cancer (Nakazawa et al. 1994).. However, in most cancers, the mutation pattern is complex. Cancers

associated with tobacco smoking are a good illustration of this complexity. Smoking is a major risk factor in several cancers, including cancers of the oral cavity, esophagus, lung and bladder. In all pathologies, the prevalence of p53 mutations is generally higher in smokers than in nonsmokers (Brennan et al. 1995, Montesano et al. 1996). However, the spectrum of p53 mutations varies from one pathology to the other. In lung cancers, G®T transversions, a typical signature of mutagenesis by benzo(a)pyrene, are particularly frequent. Experimental evidence shows that benzo(a)pyrene preferentially forms adducts at codons 157, 248 and 273, which are all mutation hotspots in lung cancers (Denissenko et al. 1996). In squamous cell carcinoma of the oral cavity and of the esophagus, the predominant types of mutations are transitions or transversions at A:T base pairs and G®A transitions. In these cancers, the combined consumption of tobacco and alcohol is considered to be a cumulative risk factor, and the observed spectrum is consistent with a role of N-nitrosamines (G:C transitions) and of metabolites of ethanol, such as acetaldehyde (mutations at A:T bases) (Montesano et al. 1996). In bladder cancer, the mutation spectrum is dominated by mutations at G:C base pairs (75%, including 28% of transitions at non-CpG). The major tobacco carcinogen(s) in these tumours are thought to be aromatic amines such as 4-aminobiphenyl. Interestingly, the mutation spectrum is similar in bladder cancers of workers occupationally exposed to aromatic amines as in the general population, supporting the notion that the carcinogens involved are identical in both groups (Taylor et al. 1996). Thus, a complex carcinogenic mixture such as tobacco smoke can have a different mutagenic impact in different tissues. These examples demonstrate that more sophisticated molecular epidemiological studies with exposure cohorts matched for various parameters that could influence the mutation spectrum (such as age, sex, ethnic origin, etc.) are required to determine the role of exogenous agents in the generation of such mutation spectra.

P53 mutations in different geographic populations: P53 mutations have been shown to be common in breast (Elledge & Allred 1994, Falette et al. 1998, Iacopetta et al. 1998)), colonic (Purdie et al. 1991), lung (Iggo et al. 1990), and several other types of human cancers. P53 mutation pattern in different tumours differs in different ethnically and geographically diverse groups/population. Mutation analysis in lung cancer patients of United States, many European countries, and Japan have indicated 70% mutations among small cell lung cancer patients and 24% among adenocarcinoma patients (Greenblatt et al. 1994, Harris 1996a, Harris 1996b, Velculescu & El Deiry 1996). The predominant mutation in these patients is G:C ® T:A (Greenblatt et al. 1994, Gottlieb & Oren 1996). However, a study done by Takagi et al. (1995) on 35 Chinese female lung cancer patients in Hong Kong reported a low frequency (7 of 35, 20%) of p53 mutation. Notably, a distinct mutation spectrum of the p53 gene with a prevalence of single-base deletions in 3 of 7 (43%) mutations was observed. Recently, Wang et al. (1998) found that mutation frequency of the p53 gene was low i.e., 18% (11 of 60) in lung cancer patients from Taiwan. However, distinct patterns of gene mutation were found. A majority (9 of 11, 82%) of the mutations in lung cancer patients in Taiwan were nonmissense mutations i.e., deletions and nonsense mutations. P53 mutation profile also differs in breast cancer patients from different regions. Hartmann et al. (1995) have reported 33% mutations in exons 5-8 in primary breast cancers from different population. US Midwest Caucasian women and African-American women have different mutational patterns. Midwest caucasian women have a large proportion of deletions and insertions, whereas the African - American women have more A:T ® G:C transitions. Their patterns also differ from that reported in a Scottish population (Coles 1992). However, most of the mutations in breast cancer are restricted to exon 7 (Davidoff et al. 1991a,b, Runnebaum 1991). But our results on Indian population have shown mutations in either exon 5 or exon 6 (Nayak et al. 1996). Similarly the spectrum of p53 mutation in oral cancer from Indian population indicates mutation in exon 5-9 (32%) (Tandle et al. 1994) and 21% (Munirajan et al. 1996). Unlike earlier Indian reports we did not find any mutation in p53 exons 5-8 in 48 oral cancer patients (Patnaik et al. 1999a). Similar to our reports in eastern Indian population, reports are available on oral carcinomas from Srilankan betel/tobacco chewers who have shown overexpression of p53 protein without any mutations in exons 5-9 of the p53 gene

(Ranasinghe et al. 1993). Thus the mutation pattern differs in different geographic population. The reason for these differences is not very clear. However, this suggests that different etiological role of the p53 gene and unique environmental/ genetic factors may be involved in tumorigenesis of different cancer patients.

Mutation in p53 promoter. Mutations in p53 gene are mainly found in exons 5 - 8 (Hollstein et al. 1991, Gottlieb et al. 1994, Levine 1997). But the involvement of p53 promoter mutations in cancers is not known. However, mutation in promoter of other tumour suppressor genes like WT1 (Grubb et al. 1995) and Rb (Sakai et al. 1991) have been reported. Further, it has been demonstrated that mutations change the binding sites of transcription factors like SP1 and ATF which are involved in the regulation of Rb gene expression (Sakai et al. 1991). Earlier workers have reported deletions in Rb gene promoter in human prostate carcinoma (Bookstein et al. 1990). Recently, Nayak and Das (1999a) have indicated no deletion and no point mutation in the p53 promoter in breast tumours. Similar results have also been observed in oral cancer patients (Patnaik & Das unpublished data). So it is speculated that mutation in the p53 promoter is probably not a significant factor in tumorigenesis. However, p53 promoter mutation studies should be undertaken in different types of tumours before reaching a conclusion.

Expression of p53 protein

Detection of p53 mutations by Immunohistochemistry (IHC) is based on the accumulation of mutant p53 in cells due to conformational changes in the p53 polypeptide that result in increased stability (Finlay et al. 1988, Levine et al. 1991). On the other hand, wild-type p53 protein has a short half-life (5 to 45 min) and is not usually detected by IHC (Reich & Levine 1984). The correlation between accumulation of the p53 protein in neoplastic cells and the presence of missense mutation in the gene is not always in concordance. There are cases in which p53 gene mutations are not linked to protein accumulation, but these are generally consistent with a molecular alteration that does not lead to correct transcription or translation of p53. Indeed, tumours with nonsense or frameshift mutations that result in the production of an unstable truncated protein are negative by immunostaining as expected, as are cells with mutations in the RNA splice sequences that cannot be correctly transcribed (Anelli et al. 1995). Although p53 protein overexpression may correlate with gene mutation in some tumours such as bladder carcinomas (Esrig et al. 1993), high p53 expression may occur without apparent genetic alteration in others (Zhang et al. 1992, Kennedy et al. 1994, Xu et al. 1994). For example, in a study of 25 childhood hepatic tumours, p53 protein overexpression was found in 17 tumours, but gene mutation was not detected in any of the 17 cases (Kennedy et al. 1994). Our results have clearly demonstrated a positive correlation between p53 mutation and p53 overexpression in male breast tumours and an overexpression of p53 even without gene mutation in female breast tumours (Nayak et al. 1996). Besides, we have found 45.7% immunoreactivity in oral tumour samples of eastern Indian population (Baral et al. 1998). This percentage differs from the reported data from northern (Kour et al. 1996) and western (Tandle et al. 1994) India. The reason behind this may be the different mode of tobacco consumption. The population we had studied was particularly habituated with chewable smokeless tobacco i.e. tobacco wrapped betel quid (pan), tobacco paste (Gudakhu), whereas people of the northern and western India were used to take bidies, cigarettes (tobacco smoke) along with chewable tobacco. This may ultimately lead to a difference in 'field cancerization' in the oral cavity and thereby cause a variation in the rate of p53 expression between eastern India and northern/western India. However, the observed 45.7% protein expression was without any gene mutation. Several explanations have also been given to account for the phenomenon of high protein expression without mutation, including high expression of wild-type p53 protein (Battifora et al. 1994), mutation in areas other than the hot spots (Xu et al. 1994), sampling error in molecular studies (Wynford-Thomas 1992, Ambros et al. 1994), and conformational changes of wild-type protein (Zhang et al. 1992). Although it is possible that wild-type protein may be detected in tumours by immunohistochemical means, particularly when sensitive techniques are used, high protein expression has been frequently demonstrated in the absence of detectable mutation using antibodies with proven specificity for mutant proteins (Gannon et al. 1990). This phenomenon

might be due to either sampling error in molecular techniques or post-translational conformational change of the p53 protein molecule. Potential deficiencies of molecular analysis include mutation outside the exons tested and failure to replicate mutant p53 genome exponentially. Mutations may occasionally occur outside of hotspot regions, but more than 85% of mutations occur in exons 5 to 9, which are highly conserved and thought to have functional importance among different species (Levine et al. 1991). In some reports, the frequency of p53 overexpression without gene mutation is much higher than would occur by chance alone if mutations were present outside of exons 5 to 9 (Hurlimann et al. 1994, Kennedy et al. 1994, Xu et al. 1994). The other potential shortcoming of molecular analysis, i.e., failure to replicate the mutant p53 genome, is plausible, as PCR is highly exponential in nature. Focal or regional staining for p53 protein is a common immunohistochemical finding in many tumours; if the mutant p53 gene is not "selected" early in the PCR, exponential replication of the gene might not occur, and gene mutation might not be detected. Apart from these, a post-translational conformational change of the wild-type p53 protein, which would lead to increased stability of the protein, is a possible contributor to the high expression-without-mutation phenomenon. Conformational change of the protein might occur if the molecule undergoes complex formation with other nuclear proteins. For example, the cellular protein MDM-2 can bind to p53 and inactivate its function (Barak et al. 1992, Landers et al. 1994). Stabilization of p53 is also possible through binding to other proteins such as hsp 70 (Lehman et al. 1991), or viral proteins (Sarnow et al. 1982).

Besides p53 immunoreactivity in nucleus, cytoplasmic localization of p53 has also been observed by different workers in salivary gland lesions (Li et al. 1995), breast cancer (Moll et al. 1992, Bartek et al. 1993, Midgely et al. 1993, Nayak et al. 1996), small cell lung carcinomas (Iggo et al. 1990) and squamous cell carcinoma of oral cavity (Field et al. 1991, Langdon & Partridge 1992). However, studies have indicated binding of mutant p53 to heat shock proteins and as a result p53 remains back in cytoplasm (Pinhasi - Kimhi et al. 1986). Many tumours and cell lines have been identified which use cytoplasmic retention of wild-type p53 as a way to inactivate the ability of p53 to suppress growth (Moll et al. 1995, Moll et al. 1996). Recently cytoplasmic localization of p53 has been observed in REF 52/N-myc cells (Chernova et al. 1998). All these observations suggest cytoplasmic retention of p53 as a p53-inactivating mechanism. The exact reason of cytoplasmic localization of p53 is not clear. Future studies are needed to elucidate the mechanisms of cytoplasmic accumulation of p53.

Transcriptional Status of p53

In normal cells, p53 is believed to be biochemically latent (Hupp et al. 1995). In addition, p53 is a very labile protein (Oren et al. 1981, Rogel et al. 1985) and consequently, its steady-state level is usually extremely low. However, in response to appropriate signals, p53 can become both stabilized and biochemically activated, resulting in a prominent increase in overall cellular level of active p53. In situ hybridization analysis has revealed p53 mRNA expression in all cells of the mouse embryo upto embryonic day 10.5 (E10.5) (Schmid et al. 1991). Later in development, expression of p53 mRNA becomes more heterogeneous, but high levels are still seen in many tissues. The highest level of p53 mRNA is found in spleen and thymus (Rogel et al. 1985, Lozano & Levine 1991).

Transcriptional deregulation of p53 may play a role in the genesis of some tumours (Rogel et al. 1985). Lack of p53 mRNA expression has been observed in a number of myeloid leukemic cell lines (Koeffler et al. 1986) and in some cases elevated level of p53 expression has been observed (Baliant & Reisman 1996). A large proportion of spontaneous osteogenic sarcomas have rearrangements of one or both of their genes (Masuda et al. 1987) and the aberrant p53 protein level expressed in the cells of these tumours may reflect abnormal rates of transcription. It has been observed that malignant Friend Virus-induced erythroleukemias in mice frequently express unusually large or small amounts of p53, and this occurs at least in some cases because normal transcriptional control of the gene has been lost (Mowat et al. 1984). Transcription of p53 is observed to increase as cells enter S-phase and the components of this response have yet to be identified

(Reich & Levine 1984, Mosner et al. 1995). Recently, stage and tissue specific p53 regulation at the level of mRNA expression has been proposed (Gottlieb et al. 1997). In the present review the mRNA status has been correlated with the rearrangement in p53 gene, methylation of p53 promoter and NF1 transcription factors.

Rearrangement of p53 Gene

Rearrangement of p53 gene, another potential post-replicative mechanism is a very frequent event in virus transformed cell lines and chronic myelogenous leukemia (Mowat et al. 1984, Ahuja et al. 1989). It is a rare event in solid tumours and has been reported only in osteosarcomas (Masuda et al. 1987). Earlier reports had failed to detect any gross rearrangement in the p53 gene (Crawford et al. 1984, Rivkina et al. 1994, Yaginuma et al. 1995). Recently we have reported rearrangement of p53 gene for the first time in Indian population. Abnormalities of the p53 gene restriction pattern has been detected by us in about 35% of breast cancers (Nayak et al. 1998) and in 15% of oral cancers (Patnaik et al. 1999b). Earlier workers had demonstrated allelic loss of the p53 gene in chronic myelogenous leukemia (Ahuja et al. 1989). Reports have shown that rearrangement in the 5' region of the gene alters the functional activity in Burkitt lymphomas involving the first noncoding exon of myc (Croce et al. 1983, Rabbits et al. 1983) and in osteosarcomas involving the first noncoding exon of p53 (Masuda et al. 1987). However, earlier reports using 3' probe (covering exon 11) have indicated rearrangement in the 3' end of the gene (Ahuja et al. 1989). Rearrangement of p53 gene has been reported in dideoxy cytidine-induced lymphoma in mice and that too in one out of forty seven treated mice (Zhuang et al. 1997). The high frequency of p53 gene inactivation and rearrangement observed in friend erythroleukemic cells is intriguing and unexpected. Moreover, it suggests that inactivation of the p53 gene is not a random event, but is closely associated with the induction of erythroleukemia by Friend virus complex or its helper virus.

We have also correlated rearrangement of p53 gene with p53 expression and have shown that some patients having rearrangement show high p53 mRNA and high p53 protein expression (Nayak et al. 1998, Patnaik et al. 1999b). Some patients not having rearranged p53 gene also show very high p53 mRNA expression and this high p53 mRNA expression could be due to different transcriptional mechanisms as has been proposed by various groups (Furlong et al. 1996, Nayak & Das 1997, Schroeder & Mass 1997). Further, we have indicated that rearrangement of p53 does not lead to complete block of p53 protein expression as p53 protein is detected in the patients having rearranged p53 gene (Nayak et al. 1998). Thus the patients having rearrangement but not showing elevated p53 expression might have normal p53 function as has been indicated by earlier workers (Marasca et al. 1996) the other possibility is that they might have normal p53 RNA and p53 protein but the p53 protein might be defective or aberrant and this could lead to neoplastic transformation. Earlier reports have demonstrated that genomic rearrangements are responsible for complete p53 gene inactivation in Friend virus transformed erythroleukemic cell lines or in production of truncated p53 protein (Mowat et al. 1984, Wolf & Rotter 1984). High level of p53 protein is found in certain cell lines (Ruscetti & Scolnick 1983), but the protein is undetectable in others (Mowat et al. 1984). The exact explanation is not known why many human cells express high level of p53 protein whereas in some there is complete inactivation of the p53 gene. Future studies should bring about a solution to this paradigm.

Methylation and p53 Gene Regulation

DNA methylation plays two important roles in the progression of human cancers (Laird & Jaenisch 1994, Jones 1996). First, DNA methylation is a significant contributor of point mutations at CpG dinucleotides in a variety of growth regulatory genes. Second, methylation controls the regulation of gene expression. Methylation of DNA in mammals occurs at the cytosine residue of CpG dinucleotides by an enzymatic reaction that produces 5-methylcytosine (5-mc). This base modification is involved in the regulation of gene expression and its presence in DNA is correlated with gene silencing (Bird 1992). Alterations in the methylation pattern of DNA are common in cancer cells (Jones 1996). Evidences suggest that hypermethylation of tumour

suppressor genes like p16 (Gonzalez - Zulueta et al. 1995, Herman et al. 1996a, Zhang et al. 1998), retinoblastoma (Ohtani - Fujita et al. 1993), WT 1 suppressor (Kleymenova et al. 1998), p15 (Herman et al. 1996b) and BRCA1 gene (Mancini et al. 1998) and hypomethylation of oncogenes like ras (Feinberg & Vogelstein 1983) and myc (Cheah et al. 1984) are responsible for the process of tumorigenesis. Although 5-mc represents about 1% of the bases in the mammalian genome, it is believed to cause about one third of all transition mutations responsible for human genetic diseases and cancer (Tornaletti & Pfeifer 1995). High rate of C to T transitions have been found in coding regions of the p53 gene (Magewu & Jones 1994, Tornaletti & Pfeilfer 1995) and have been attributed to the spontaneous and/ or cytosine methyltransferase facilitated deamination of 5-methylcytosine (Tornaletti & Pfeifer 1995). It has been shown that all the CpG dinucleotides of exons 5-8 of the human p53 gene are methylated in all tissues and cell lines examined (Tornaletti & Pfeifer 1995). Five out of six p53 mutation hotspot codons contain CpG dinucleotides (175, 245, 248, 273 and 282); implicating methylation-driven deamination of 5-mC as a major source of G:C ® A:T transition mutations at CpG dinucleotides (Tornaletti & Pfeifer 1995). A tissue independent methylation of all cytosine residues in CpG dinucleotides of p53 exons 5-8 has been reported (Tornaletti & Pfeifer 1995). On the other hand, methylation status of the p53 promoter is not well known. Whereas DNA methylation studies on the promoter of tumour suppressor genes like p16 (Zhang et al. 1998) and Rb (Ohtani - Fujita et al. 1993) and BRCA1 (Mancini et al. 1998) have indicated an inverse correlation between gene expression and methylation. Studies have indicated an alteration in p53 mRNA level in many tumour tissues (Rogel et al. 1985, Baliant & Reisman 1996). It has been reported that CpG islands are commonly unmethylated in housekeeping genes (Bookstein et al. 1990). However, recently an in vitro study using African green monkey kidney CV-1 cells have shown that hypermethylation of p53 promoter inactivates the transcriptional activity of the gene (Schroeder & Mass 1997). But the exact CpG dinucleotides, which are essential for the drop of transcriptional activity of the p53 promoter was not determined. However our recent finding in breast cancer have demonstrated no change in the methylation status of the CCGG sites in p53 promoter when compared with its normal counterpart (Nayak & Das 1999a). It is essential to determine the methylation status of all cytosine residues in the p53 promoter to get a clear picture of the involvement of p53 gene promoter in p53 gene expression and p53 gene mutation.

Transcription Factors and p53 Gene Regulation

Transcription factors play a pivotal role in the determination and maintenance of cellular phenotypes. The activity of transcription factors is infact considered as the main switch to regulate gene expression (Mitchell & Tjian 1989). Transcription of p53 gene has been shown to be controlled by different *trans*-acting factors binding to specific *cis*-acting elements present in p53 promoter (figures 3a & 3b). Deffie et al. (1993) have shown that p53 binds to the site (+22 to +67) present in its own promoter and regulates its own transcription. Transcription factor USF binds to basic helix-loop-helix motif (CACGTG) present between +70 and +75 and enhances the p53 promoter activity (Reisman & Rotter 1993). Furthermore, the level of mutant forms of p53 and p53 mRNA correlate with cellular c-myc level in some B-Lymphoid cell lines; c-myc-max heterodimers bind to the b-HLH site in the p53 promoter and activate it (Roy et al. 1994). The p53 promoter region (-46 to -70) has been reported to be essential for p53 activation induced by genotoxic agents such as anticancer drugs and UV (Sun et al. 1995). It has been shown that pax protein binds to the 5' region (+181 to +209) of p53 gene in primary human diffuse astrocytomas and inhibits its expression (Stuart et al. 1995). Furlong et al. (1996) has shown that transcription factors NF1 and YY1 activate p53 promoter by alternatively binding to a composite element (-193 to -231). It has been shown that elevated level of USF (Reisman & Rotter, 1993), YY1 and NF1 (Furlong et al. 1996) activate p53, while tax oncoprotein (Uittenbogaard et al. 1995) and pax protein (Stuart et al. 1995) repress tanscription of p53. Therefore, both transcriptional activation and repression of the p53 gene are associated with tumorigenesis or neoplastic transformation. In principle, during oncogenic

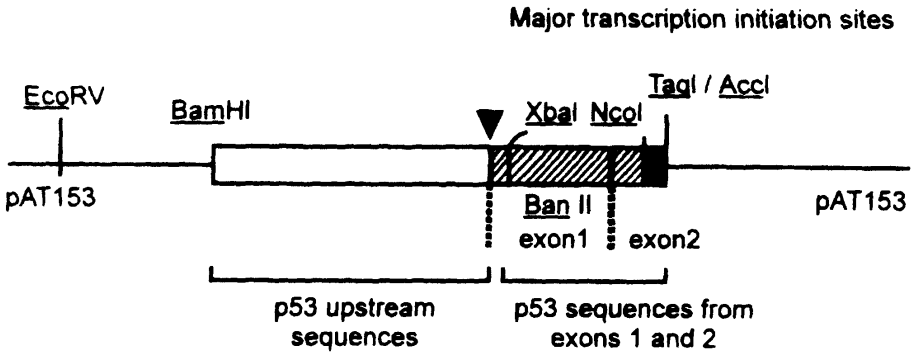

Figure 3a Restriction map of the human p53 gene promoter region, exon 1 and part of exon 2 contained in the plasmid pBT53

GGG AGA AAA CGT TAG GGT GTG GAT ATT ACG GAA AGC CTT C -493

CTA AAA AAT GAC ATT TAA CTG ATG AGA AGA AAG GAT CCA G -453

CTG AGA GCA AAC GCA AAA GCT TTC TTC CTT CCA CCC TTC A -413

TAT TTG ACA CAA TGC AGG ATT CCT CCA AAA TGA TTT CCA C -373

CAA TTC TGC CCT CAC AGC TCT GGC TTG CAG AAT TTT CCA C -333

CCC AAA ATG TTA GTA TCT ACG GCA CCA GGT CGG CGA GAA T -293

CCT GAC TCT GCA CCC TCC TCC CCA ACT CCA TTT CCT TTG C -253

TTC CTC CGG CAG GCG GAT TAC TTG CCC TTA CTT GTC ATG G -213

CGA CTG TCC AGC TTT GTG CCA GGA GCC TCG CAG GGG TTG A -173

NF1

-133

TGG GAT TGG GGT TTT CCC CTC CCA TGT GCT CAA GAC TGG C

-93

GCT AAA AGT TTT GAG CTT CTC AAA AGT CTA GAG CCA CCG T

-53

CCA GGG AGC AGG TAG CTG CTG GGC TCC GGG GAC ACT TTG C

-13

GTT CGG GCT GGG AGC GTG CTT TCC ACG ACG GTG ACA CGC T

TCC CTG GAT TGG

Figure 3b Sequence of the human p53 promoter showing the NF1 binding site

transformation either deregulated expression of transcription factors or mutations in the p53 promoter, resulting in enhanced protein binding, could lead to altered expression of p53 (Reisman & Rotter 1993). Recently, it has been shown that binding of transcription factors YY1 and NF1 to the p53 promoter (-193 to -231) varies in a tissue-specific manner. YY1 binds to this element in nuclear extracts of rat testis and spleen and NF1 in extracts of liver and prostate (Furlong et al. 1996). This may facilitate tissue-specific control of p53 expression (Furlong et al. 1996). However, we have clearly elucidated a differential binding of nuclear proteins to the p53 promoter (-101 to -457) in normal and tumour tissues (Nayak & Das 1997). Thus, different studies have indicated that the differential binding of nuclear factors to p53 promoter might play a critical role in the process of tumorigenesis.

NF1 Transcription Factors and p53 Gene Regulation

NF1 (Nuclear factor 1) proteins are a heterogeneous family of proteins encoded by multiple genes (Inoue et al. 1990, Apt et al. 1994). Cloning of cDNAs encoding NF1 proteins from a number of species (Meisternst et al. 1989, Rupp et al. 1990) has identified a family of four genes (NF1-A, NF1-B, NF1-C, and NF1-X) that are highly conserved from chicken to human. These proteins bind with high affinity to the palindromic sequence TGG(A/C)N5GCCAA (Gronostajski 1986) and with lower affinity to the half site TGGCA (Gounari et al. 1990). Binding sites for NF1 have been found in a wide variety of genes (Inoue et al. 1990, Zobras et al. 1992). NF1 proteins are expressed in many different tissues (Apt et al. 1994) suggesting that NF1s are crucial for cell function in many organs. It has been shown that NF1 regulates several cell-selective genes including liver-specific albumin (Cereghini et al. 1987, Jackson et al. 1993), retinol binding protein (Eskild et al. 1994), vitellogenin (Cardinaux et al. 1994), a-fetoprotein (Bois-Joyux & Danan 1994), adipocyte-specific aP2 (Graves et al. 1991), neuronal-specific peripherin (Adams et al. 1995), myelin basic proteins (Inoue et al. 1990), lung-specific surfactant protein-C (Bachurski et al. 1997) and mammary gland specific milk protein gene (Watson et al. 1991). Although NF1 is known as an ubiquitous transcription factor, interactions of specific NF1 isoforms in different cell types may contribute to cell-selective transcriptional activation or silencing of target genes (Inoue et al. 1990). It has been difficult to define which NF1 gene product function *in vivo* at specific promoters because the expression pattern of the NF1 multigene family is poorly understood. It has been shown that the four NF1 genes are differentially regulated during phorbol ester (TPA)-induced differentiation of human leukemic cells and may play an important role in hematopoietic development (Kulkarni & Gronostajski 1996). Recently, Chaudhry et al. (1997), have shown that the four NF1 genes are expressed in distinct patterns during mouse development and that the NF1 gene products differ in their transcriptional activation of an NF1-dependent promoter. Further, NF1 proteins have also been shown to suppress transformation of cells by nuclear oncogenes. Overexpression of avian NF1 proteins in chick embryo fibroblasts (CEF) reduces focus formation by the jun, fos, junD, myc, and qin oncogenes (Schuur et al. 1995). The mechanism of this suppression is unknown; however, overexpression of NF1 induces several morphologic changes including increased cell adherence and flattening the cell monolayer, which may be required for the suppression. But the precise role of NF1 in the process of tumorigenesis is not clearly defined.

Transcriptional deregulation of p53 has been proposed by different workers (Rogel et al. 1985, Koeffler et al. 1986, Mosner et al. 1995, Baliant & Reisman 1996, Gottlieb et al. 1997). The altered expression of p53 might be due to altered expression of trans-acting factors that are required for appropriate expression of the p53 gene. Transcription factors like NF1 (Furlong et al. 1996) and USF (Reisman & Rotter 1993) activate and tax (Uittenbogaard et al. 1995) and pax (Stuart et al. 1995) repress p53 gene expression. So both transcriptional activation and repression of p53 by different factors have been demonstrated. In the present review, the role of NF1 in p53 gene regulation has been discussed. Furlong et al. (1996) have demonstrated a tissue specific binding of NF1 and YY1 transcription factor to the same site on p53 promoter; NF1 predominantly binds in liver and YY1 predominantly binds in spleen and testes.

Recently we have demonstrated that NF1 predominantly binds to this site in breast tissue and have further shown a depletion or low level of NF1 factors in majority of breast tumours (Nayak & Das 1999b). Earlier reports have demonstrated a deregulation of NF1 in differentiating leukemic cells (Kulkarni & Gronostajski 1996) and in different cells of lung carcinoma (Wong & Bernal 1994). It has been shown that overexpression of NF1 proteins in chick embryo fibroblasts (CEF) reduces focus formation by the jun, fos, junD, myc and qin oncogenes (Schuur et al. 1995). Recently Geurts et al. (1998) have demonstrated that NF1B (a member of NF1 gene family) acts as a translocation partner gene of HMG1C in pleomorphic adenomas. Nayak & Das (1999b) have also demonstrated the presence of p53 mRNA in the tumour samples where there is a depletion or low level of NF1. So it is presumed that in normal tissues NF1 might have a role in p53 expression; but in tumour samples p53 gene expression is not dependent on NF1.

Studies have demonstrated that oncogenes like Ha-ras and myc repress the NF1 activity (Yang et al. 1993, Nebl et al. 1994). This inturn leads to deregulation of NF1-regulated genes. It has been reported that NF1 controls genes involved in differentiation and development (Watson et al. 1991, Kulkarni & Gronostajski 1996, Puzianowska-kuznicka & Shi 1996). For example, NF1 controls the expression of milk protein genes such as a-lactalbumin and whey acidic protein gene (Watson et al. 1991, Mink et al. 1992). On the other hand, differentiation of the mammary gland and milk protein gene expression is inhibited by oncogenic transformation of mammary epithelium with Ha-v-ras (Andres et al. 1988). Further, it has been shown that there is a down-regulation of NF1 level by Ha-v-ras (Nebl et al. 1994) and this might inhibit milk protein gene expression. As oncogenes influence the activity of NF1 and NF1-regulated differentiation-specific genes, it is speculated that oncogenes via NF1 and NF1-regulated genes might lead to malignant transformation (figure 4). So the depletion of NF1 in breast tumours as indicated by Nayak and Das (1999b) might result from activated oncogenes. However, the interaction of p53 and other tumour suppressor genes with NF1 can not be ruled out.

Translational Regulation

Translational control of p53 expression has already been inferred by earlier studies (Beinz-Tadmor et al. 1985, Steinmeyer et al. 1990) but not demonstrated directly. The absence of detectable p53 protein in cells expressing abundant level of wild type p53 mRNA has usually been attributed to the short half-life of p53 protein in normal cells (Rogel et al. 1985). Translational regulation of p53 could be another mechanism to account for the aberrant level of p53 protein.

Evidence for translational control of p53 protein biosynthesis comes from the observations of Mosner et al. (1995). First, after growth stimulation of resting Swiss 3T3 cells, the observed p53 mRNA production was not accompanied by a comparable induction of p53 protein expression. Rather, there was a time lag of 6 - 8 h between maximal expression of p53 mRNA and expression of p53 protein. This leads to a scenario in which p53 mRNA production peaks before or at the G1/S boundary of the cell cycle, while p53 protein is maximally expressed at mid-S phase. Second, upon γ-irradiation of Swiss 3T3 cells, there was an immediate response in p53 protein production that took no longer than 1 hour following irradiation. This time period is too short for making it likely that transcriptional processes (i.e., pre-mRNA production and maturation to mRNA, packaging into small nuclear particle proteins, mRNA transport through nuclear membrane and subsequent unpackaging (for translation) account for the observed rise in p53 protein expression. Furthermore, γ-ray induced upregulation of p53 biosynthesis was not inhibited by transcription inhibitor actinomycin D. Hence, translational control rather than transcriptional regulation of p53 production must be responsible for the rapid increase of p53 protein seen after DNA damage *in vivo*.

Further, Mosner et al. (1995), have demonstrated an autoregulatory control of p53 expression by a tendency to form a stable stem-loop structure that involves the 5'-untranslated region (5'-UTR) plus some 280 nucleotides of the coding sequence (Beinz et al. 1995). p53 protein binds tightly to the 5'-UTR region and inhibits the translation of its own mRNA, most likely mediated by catalyzing of the re-annealing of the 5'-UTR stem-loop structures of its mRNA. Strong support for a catalytic rather than a mere binding mechanism for p53's inhibition of its own translation reaction came from the observation

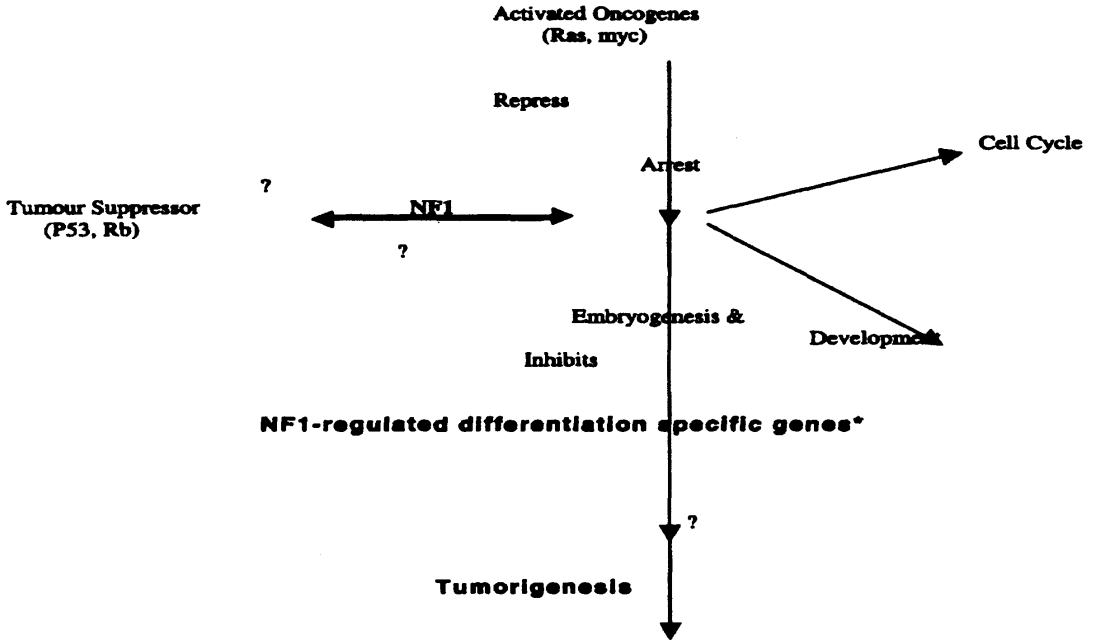

Figure 4 Model showing interrelationship between NF1, oncogenes and tumour suppressor genes. Activated oncogenes repress NF1, which in turn inhibits NF1-regulated differentiation specific genes and this might lead to tumorigenesis. The link between NF1 and tumour suppressors in cancers has not yet been established.

that mutant p53 protein binds to its own mRNA with a similar affinity as the wild-type form but, in spite of this, was not able to inhibit the translation reaction (Mosner et al. 1995).

Translation inhibition represents an efficient control mechanism that may be particularly suited for the fast and delicate regulation of expression of regulatory proteins (Rhoads 1988; Sonenberg 1988). Such a mechanism might enable a rapid rise of wild type p53 level after DNA damage under conditions where p53 biosynthesis is low, for example in cells stimulated to re-enter the cell-cycle, and even if the p53 gene has been damaged as well (provided the cell contains enough p53 mRNA). The autoregulation of p53 protein expression suggests a model according to which wt p53 interacts with its own mRNA in translational complexes to slow down p53 protein production in normal cells.

Translational regulation of p53 gene expression has been further strengthened by the finding of Fu et al. (1996) in blast cells obtained from patients with acute myelogenous leukemia (AML). Four experiments point towards translational control of human p53 gene expression. First there is no correlation between the level of p53 mRNA and the level of p53 protein expression in blast cells. Second, in two cell lines with similar level of p53 protein expression but with different level of p53 mRNA, there is a preferential association of p53 mRNA with large polysomes in the cells with less p53 RNA. Third, translation of synthetic human p53 transcripts in cell-free extracts is inhibited by the p53 3' UTR. Fourth, the p53 3'UTR, when present in cis, can repress translation of a heterologous transcript. These observations raise the possibility that human p53 mRNA translation may be regulated in vivo by RNA binding trans-acting factors on the p53 3'UTR (Fu et al. 1996). All these findings raise the possibility that translational regulation may provide an epigenetic mechanism to reduce or even eliminate wild-type p53 protein function in leukemic blasts.

P53 and Cancer Therapy: From Gene Mutations to Gene Therapy

Development of novel strategies is required for cancer treatment because most of the human tumours are

refractory to current conventional therapy. It is, therefore, a great challenge to develop novel strategies for tumour specific gene therapy. Nearly twenty years after the initial discovery of p53, we are in an ideal position to exploit our vast knowledge of p53 biology in the creation of novel cancer therapeutic strategies. Despite the current limitations, retroviral, adenoviral and liposomal vectors can, in certain circumstances, provide an effective means of delivering therapeutic genes to tumour cells (Zou et al. 1998, McPake et al. 1999, Ui et al. 1999). Another novel approach using mutant adenovirus is also employed in p53-deficient tumour cells. This is based on the principle that the mutant adenovirus not expressing E1B viral protein can replicate in and lyse p53-deficient human tumour cells but not cells with functional p53. Bischoff et al. (1996) have shown that injection of the mutant adenovirus into p53-deficient human cervical carcinoma grown in nude mice caused complete regression of 60 percent of the tumours. This work raised the possibility that mutant adenovirus could be used to treat certain human tumours. Recently Hay et al. (1999) explored the hypothesis in several lung cancer cell lines and evaluated the potential mechanisms that might regulate the replication of Ad 338, an E1B-deleted virus. They have shown that this virus replicates poorly in lung cancer cell lines with various p53 mutations. Thus, an E1B-deleted virus can not be used to specifically target viral replication in p53-mutated lung cancer. Moreover, this approach employing mutant adenovirus needs to be tested in different tumour types to evaluate its efficacy and future prospects. However, gene replacement therapy with wild-type p53 holds considerable potential for obtaining clinically relevant results (Kinzler & Vogelstein 1994, Wang & Harris 1996, Nielsen & Maneval 1998). Preclinical studies both *in vitro* and *in vivo* have shown that reintroduction of wild-type p53 can induce growth arrest, differentiation, apoptosis (Soddu & Sacchi 1998 Roth et al. 1999) and also can inhibit angiogenesis (Bouvet et al. 1998). Preclinical and phase I clinical trials using wild-type p53 are now being pursued in different types of tumours, such as, non-small cell lung cancer (Roth 1998, Swisher et al. 1999), head and neck (Clayman et al. 1999, Xu et al. 1999), liver (Habib et al. 1999), prostate (Boulikas et al. 1997), ovarian (Mujoo et al. 1996), breast (Li et al. 1998) and other type of cancers. Results from early clinical trials using p53 gene therapy by itself support optimism for the future of this therapeutic approach. However, some cancers are refractory and some are resistant to p53 gene therapy. For example, mdm2-overexpressing tumours are often resistant to p53 gene therapy (Yang et al. 1999). Further, Vinyals et al. (1999) have recently demonstrated a failure of wild-type p53 gene therapy in human cancer cells expressing a mutant p53 protein. The presence of mutant p53 may confer genome instability and mutator ability, which allows cells to escape the effects of the exogenous wt p53 and contributes to the failure of wt p53 gene therapy. So in order to obtain better results and greater efficacy, p53 gene therapy is now combined with other therapies, such as, chemotherapy (Gornani et al. 1999), radiotherapy (Badie et al. 1999, Xu et al. 1999) and other multiple gene therapy involving cytokines, such as, interleukin 2 (Putzer et al. 1998) and cell cycle control genes, such as, p16, p21, pRb (Strauss & Costanzi-Strauss 1999). However, cancer treatment strategies involving combined delivery of immunomodulatory and antiproliferative genes seem to be promising. So it is likely that many phase II and III trials will incorporate different combination therapies in treatment and management of cancers. Thus, p53 has attracted a great deal of interest as a prognostic factor, diagnostic tool and therapeutic target. However, despite many promising studies, its potential in practical cancer management is still to be realized. Although much research needs to be done, the possibility of specific gene targeting with high therapeutic index makes this a promising area of investigation. Future studies should be focussed on the following aspects: (1) The origin of cancers must be well understood so that a proper combination of therapeutic gene(s) can be chosen; (2) The nature of p53 and its downstream effector genes must be understood precisely in a particular type of cancer before its implementation as a therapeutic DNA drug; (3) Further, the current vectors and the gene delivery system must be tailored for optimum transfer of the therapeutic gene to the target tissue. However, the use of gene therapy continues to be promising, yet elusive, alternative for the treatment of cancer. The convergence and marriage between basic, clinical and epidemiological investigations may provide an

opportunity for the rapid transfer of research findings from the laboratory to clinic.

Conclusion and Future Prospects

As the tale of p53 unfolds, it becomes ever more intriguing. Although our understanding of the critical and complex roles played by p53 is progressing rapidly, new findings continue to pose new paradoxes. It is now clear that p53 tumour suppressor protein is involved in multiple central cellular processes including transcription, DNA repair, genomic stability, senescence, cell cycle control and apoptosis. P53 brings about these functions by activating and repressing a wide variety of genes. Transcriptional activity of p53 is different on various target genes even though they carry similar p53-binding sites. Although enormous amount of information on p53 function has accumulated over the past years, a crucial question yet to be answered is how p53 discriminately regulates transcription of various genes. Recently certain coactivators like p300/CBP (Avantaggiati et al. 1997, Gu et al. 1997, Lill et al. 1997) and JMY (Shikama et al. 1999) have been identified, which act synergistically with p53 in regulating the target genes. The coactivator-p53 complex binds to the basal transcriptional machinery to form transcriptosome and repressosome, which inactivates or represses the target genes respectively. Future studies in this direction might reveal cell or tissue -specific p53-interacting coactivators and their precise role in the p53 network/signaling pathways. Studies should also be focussed to identify and classify the p53-regulated genes in a cell-type-specific responses to p53 and their associated biologic effects. Another aspect in p53 biology is its metabolic stabilization. Untill now, mdm2 was the only molecule associated with degradation of p53 (Kubbutat et al. 1997). Recently Grossman et al. (1998) have shown that p300/ mdm2 complexes participate in mdm2-mediated p53 degradation. Further studies might reveal the mechanisms and the involvement of novel factors in p53 stabilization.

Evidences indicate that p53 is functionally inactivated by structural mutations, interaction with viral proteins, rearrangement, deregulation of transcription factors, methylation in majority of tumours. This functional inactivation can, in some circumstances, produce resistance to DNA-damaging agents commonly used in cancer chemotherapy and radiotherapeutic approaches. Novel applications of the basic scientific knowledge of p53 could lead to an improvement in cancer treatment, hopefully in the not so distant future. The next few years promise to be exciting ones as we discover more about this multifaceted tumoursuppressor protein.

Acknowledgements

The financial support from the Department of Biotechnology, New Delhi and Council of Scientific and Industrial Research, New Delhi to BRD is highly acknowledged. The kind gift of p53 cDNA from Prof. A.J. Levine, USA and pBT53 (5' probe) from Prof. L. Crawford and Prof. S.P. Tuck, U.K, are highly acknowledged. The authors are grateful to the Director and his colleagues of A.H. Regional Cancer Centre, Cuttack, for their cooperation in getting tumour samples.

References

Adams A D, Choate D M and Thompson M A 1995 NF-1 is the DNA-binding component of the protein complex at the peripherin negative regulatory element; *J. Biol. Chem.* **270** 6975-6983

Agarwal M L, Taylor W R, Chernov M V, Chernova O B and Stark G R 1998 The p53 network; *J. Biol. Chem.* **273** 1-4

Ahuja H, Bar-Eli M, Advani S H, Benchimol S and Cline M J 1989 Alteration of p53 gene and the clonal evolution of the blast crisis of chronic myelocytic leukemia; *Proc. Natl. Acad. Sci. USA* **86** 6783-6787

Ambros R A, Vigna P A, Figge J, Kallakury B V S, Mastangelo A, Eastman A Y, Malfetano J, Figge H L and Ross J S 1994 Observations on tumour and metastatic suppressor gene status in endometrial carcinoma with particular emphasis on p53; **Cancer 73** 1686

Andres A C, Vander Valk M A, Schonenberger C A, Fluckiger F, LeMeur M, Gerlinger P and Groner B 1988 Ha-ras and c-myc oncogene expression interferes with morphological and functional differentiation of mammary epithelial cells in single and double transgenic mice; *Genes Dev.* **2** 1486-1495

Anelli A, Anelli F M, Yongson B, Rosen P P and Brogen P I 1995 Mutations of the p53 gene in male breast cancer; *Cancer* **75** 2233-2238

Apt D, Liu Y and Bernard H U 1994 Cloning and functional analysis spliced isoforms of human nuclear factor 1-X : Interference with transcriptional activation by NF1/CTF in a cell-type specific manner; *Nucleic Acids Res.* **22** 3825-3833

Avantaggiati M L, Ogryzko K G, Giordano A, Levine A S and Kelly K 1997 Recruitment of p300/CBP in p53-dependent signal pathways; *Cell* **89** 1175-1184

Bachurski C J, Kelly S E, Glasser SW and Currier T A 1997 Nuclear factor 1 family members regulate the transcription of surfactant protein-C; *J. Biol. Chem.* **272** 32759-32766

Baker S J, Markowitz S, Fearon E R, Willson J K V and Vogelstein B 1990 Suppression of human colorectal carcinoma cell growth by wild-type p53; *Science* **249** 912-915

Baliant E and Reisman D 1996 Increased rate of transcription contributes to elevated expression of the mutant p53 gene in Burkitt's lymphoma cells; *Cancer Res.* **56** 1648-1653

Barak Y, Juven T, Haffner R and Oren M 1993 MDM2 expression is induced by wild-type p53 activity; *EMBO J.* **12** 461-468

Baral R N, Patnaik S and Das B. R 1998 Co-overexpression of p53 and c-myc proteins linked with advanced stages of betel- and tobacco-related oral squamous cell carcinoma from eastern India; *Eur. J. Oral Sci.* **106** 907-913

Bartek J, Bartkova J, Lukas J, Staskova Z, Vojtesek B and Lane D P 1993 Immunohistochemical analysis of the p53 oncoprotein on paraffin sections using a series of novel monoclonal antibodies; *J. Pathol.* **169** 27-34

Battifora H 1994 p53 immunohistochemistry: a word of caution (editorial); *Hum. Pathol.* **25** 435

Beinz B, Zakut-Houri R, Givol D and Oren M 1984 Analysis of the gene coding for the murine cellular tumour antigen p53; *EMBO J.* **3** 2179-2183

Beinz-Tadmor B, Zakut-Houri R, Libresco S, Givol D and Oren M 1985 The 5' region of the p53 gene: evolutionary conservation and evidence for a negative regulatory element; *EMBO J.* **4** 3209-3213

Benchimol S, Lamb P, Crawford L V, Sheer D, Shours T B, Bruns G A P and Peacock J 1985 Transformation associated p53 protein is encoded by a gene on human chromosome 17; *Somatic Cell Mol. Genet.* **11** 505-509

Ben-David Y and Bernstein A 1991 Friend virus-induced erythroleukemia and the multi-stage nature of cancer; *Cell* **66** 831-834

Bird A 1992 The essentials of DNA methylation; *Cell* **70** 5-8

Bischoff J R, Kirn D H, Williams A, Heise C, Horn S, Muna M, Ng L, Nye J A, Sampson-Johannes A, Fattaey A, McCormick F 1996 An adenovirus mutant that replicates selectively in p53-deficient human tumour cells; *Science* **274** 373-376

Bois-Joyux B and Danan J-L 1994 Members of the CAAT/enhancer-binding protein, hepatocyte nuclear factor 1 and nuclear factor 1 families can differentially modulate the activities of the rat alpha-fetoprotein promoter and enhancer; *Biochem. J.* **301** 49-55

Bookstein R, Rio P, Madreperla S A, Hong F, Allred C, Grizzle W E and Lee W-H 1990 Promoter deletion and loss of retinoblastoma gene expression in human prostate cancer; *Proc. Natl. Acad. Sci. USA* **87** 7762-7766

Boulikas T 1997 Gene therapy of prostate cancer: p53, suicidal genes and other targets; *Anticancer Res.* **17** 1471-1505

Bouvet M, Ellis L M, Nishizaki M, Fujiwara T, Liu W, Bucana C D, Fang B, Lee J J and Roth J A 1998 Adenovirus-mediated wild-type p53 gene transfer down-regulates vascular endothelial growth factor expression and inhibits angiogenesis in human colon cancer; *Cancer Res.* **58** 2288-2292

Brennan J A, Boyle J O, Koch W M, Goodman S N, Hruban R H, Eby Y J, Couch M J, Forastiere A A and Sidransky D 1995 Association between cigarette smoking and mutation of the p53 gene in squamous cell carcinoma of the head and neck; *N. Engl. J. Med.* **332** 712-717

Buckbinder L, Talbott R, Velasco-Miguel S, Takenaka I, Faha B, Seizinger B R and Kdey N 1995 Induction of the growth inhibitor IGF-binding protein 3 by p53; *Nature* **377** 646-649

Burdie B, Goh C S, Klaver J, Herweijer H and Boothman D A 1999 Combined radiation and p53 gene therapy of malignant glioma cells; *Cancer Gene Ther.* **6** 155-162

Cairns C A and White R J 1998 p53 is a general repressor of RNA polymerase III transcription; *EMBO J.* **17** 3112-3123

Cardinaux J R, Chapel S and Wahli W 1994 Complex organization of CTF/NF1, C/EBP, and HNF3 binding sites within the promoter of the liver-specific vitellogenin gene; *J. Biol. Chem.* **269** 32947-32956

Caron de Fromentel C and Soussi T 1992 p53 tumour suppressor gene: a model for investigating human mutagenesis; *Genes Chromosomes Cancer* **4** 1-15

_____, Pakdel F, Chapus A, Baney C, May P, Soussi T 1992 Rainbow trout p53: cDNA cloning and biochemical characterization; *Gene* **112** 241-245

Cereghini S, Raymondjean M, Carranca A G, Herbomel P and Yaniv M 1987 Factors involved in control of tissue-specific expression of albumin gene; *Cell* **50** 627-638

Chaudhry A Z, Lyons G E and Gronostajski R M 1997 Expression patterns of the four nuclear factor 1 genes during mouse embryogenesis indicate a potential role in development; *Developmental Dynamics* **208** 313-325

Cheah M S C, Wallace C D and Hoffman R M 1984 Hypomethylation of DNA in human cancer cells: a site-specific change in the c-myc oncogene; *J. Natl. Cancer Inst.* **73** 1057-1061

Chernova O B, Chernov M V, Ishizaka Y, Agarwal M L and Stark G R 1998 Myc abrogates p53-mediated cell cycle arrest in N-(phosphonacetyl)- L-aspartate-treated cells, permitting CAD gene amplification; *Mol. Cell. Biol.* **18** 536-545

Cho Y, Gorina S, Jeffrey P D and Pavletich N P 1994 Crystal structure of a p53 tumour suppressor-DNA complex: understanding tumorigenic mutations; *Science* **265** 346-355

Clayman G L, Frank D K, Bruso P A and Goepfert H 1999 Adenovirus-mediated wild-type p53 gene transfer as a surgical adjuvant in advanced head and neck cancers; *Clin. Cancer Res.* **5** 1715-1722

Coles C 1992 p53 mutations in breast cancer; *Cancer Res.* **52** 5291-5298

Crawford L V, Pim D and Lamb P 1984 The cellular protein p53 in human tumours; *Mol. Biol. Med.* **2** 261-272

Croce C M, Thierfelder W and Erikson J 1983 Transcriptional inactivation of an unrearranged and untranslated c-myc oncogene by translocation of a C lambada locus in Burkitt; *Proc. Natl. Acad. Sci. USA* **80** 6922-6926

Czosnek H H, Beinz B, Givol D, Zakut-Houri R, Pravtcheva D D, Ruddle R H and Oren M 1984 The gene and the pseudogene for mouse p53 cellular tumour antigen are located on different chromosomes; *Mol. Cell. Biol.* **4** 1638-1640

Davidoff A M, Herndon J E, Glover N S, Kerns B M, Pence J C, Iglehart J D and Marks J R 1991b Genetic basis of p53 overexpression in human breast cancer; *Surgery* **110** 259-264

_____, Kerns B J M, Igleharj J D and Marks J R 1991a Maintenance of p53 alterations throughout breast cancer progression; *Cancer Res* **51** 2605-2610

Deffie A, Wu H, Reinke V and Lozano G 1993 The tumour suppressor p53 regulates its own transcription; *Mol. Cell. Biol.* **13** 3415-3423

Denissenko M F, Pao A, Tang M and Pfeifer G P 1996 Preferential formation of benzo(a)pyrene adducts at lung cancer mutational hotspots in p53; *Science* **274** 430-432

Donehower L A, Harvey M, Slagle B L, McArthur M J, Montgomery C A, Butel J S and Bradley A 1992 Mice deficient for p53 are developmentally normal but susceptible to spontaneous tumours; *Nature* **356** 215-221

Dugimont T, Montpellier C, Adriaenssens E, Lotin S, Dumont L, Iotsova V, Lagrou C, Stehelin D, Coll J and Curgy J 1998 The H19 TATA-less promoter is efficiently repressed by wild-type tumour suppressor p53; *Oncogene* **16** 2395-2401

El-Deiry W S, Tokino T, Velculescu V E, Levy D B, Parsons R, Trent J M, Lin D, Mercer W E, Kinzler K W and Vogelstein B 1993 WAF1, a potential mediator of p53 tumour suppression; *Cell* **75** 817-825

Eliyahu D, Michalovitz D, Eliyahu S, Pinhas-Kimhi O and Oren M 1989 Wild type p53 can inhibit oncogene-mediated focus formation; *Proc. Natl. Acad. Sci. USA* **86** 8763-8767

_____, Raz A, Gruss P, Givol D and Oren M 1984 Participation of p53 cellular tumour antigen in transformation of normal embryonic cells; *Nature* **312** 646-649

Elledge R M and Allred D C 1994 The p53 tumour suppressor gene in breast cancer; *Breast Cancer Res. Treat.* **32** 39-47

Eskild W, Simard J, Hansson V and Guerin S L 1994 Binding of member of the NF1 family of transcription factors to two distinct cis-acting elements in the promoter and 5'-flanking region of the human cellular retinol binding protein 1 gene; *Mol. Endocrinol.* **8** 732-745

Esrig D, Spruck C H, Nichols P W, Chaiwun B, Steven K, Groshen S, Chen S C, Skinner D G and Jones P A 1993 p53 nuclear protein accumulation correlate with mutations in the p53 gene, tumour grade and stage in bladder cancer; *Am. J. Pathol.* 143 1389

Falette N, Paperin M P, Treilleux I, Gratadour A-C, Peloux N, Mignotte H, Tooke N, Lofman E, Inganas M, Bremond A, Ozturk M and Puisieux A 1998 Prognostic value of p53 gene mutations in a large series of node-negative breast cancer patients; *Cancer Res.* **58** 1451-1455

Feinberg A P and Vogelstein B 1983 Hypomethylation of ras oncogenes in primary human cancers; *Biochem. Biophys. Res. Commun.* **111** 47-54

Field J K, Spandidos D A, Malliri A, Yiagnisis M, Gosney J R and Stell P M 1991 Elevated p53 expression correlates with a history of heavy smoking in squamous cell carcinoma of the head and neck; *Br. J. Cancer* **64** 573-577

Finlay C A, Hinds P W and Levine A J 1989 The p53 protooncogene can act as a suppressor of transformation; *Cell* **57** 1083-1093

Finlay C A, Hinds P W, Tan T H, Eliyahu D, Oren M and Levine A J 1988 Activating mutations for transformation by p53 produce a gene product that forms an hsp70-p53 complex with an altered half life; *Mol. Cell. Biol.* **8** 531-539

Fu L, Minden M D and Benchimol S 1996 Transcriptional regulation of human p53 gene expression; *EMBO J.* **15** 4392-4401

Furlong E M, Rein T and Martin F 1996 YY1 and NF1 both activate the human p53 promoter by alternatively binding to a composite element and YY1 and E1A cooperate to amplify p53 promoter activity; *Mol. Cell. Biol.* **16** 5933-5945

Gannon J V, Greaves T, Iggo R, Lane D P 1990 Activating mutations in p53 produce a common conformational effect: a monoclonal antibody specific for the mutant form; *EMBO J.* **9** 1595

Geurts J M W, Schoenmakers E F P M, Roijer E, Astrom A K, Stenman G and Vanden Ven Win J M 1998 Identification of NF1B as recurrent translocation partner gene of HMG1C in pleomorphic adenomas; *Oncogene* **16** 865-872

Gire V and Wynford-Thomas D 1998 Reinitiation of DNA synthesis cell division in senescent human fibroblasts by microinjection of anti-p53 antibodies; *Mol. Cell. Biol.* **18** 1611-1621

Goga A, Liu X, Hambuch T M, Senechal K, Major E, Berk A J, Witte O N and Sawyers C L 1995 P53-dependent growth suppression by the c-Abl nuclear tyrosine kinase; *Oncogene.* **11** 791-799

Gonzalez-Zulueta M, Bender C M, Yang A S, Nguyen T, Beart R W, Van Tornout J M and Jones PA 1995 Methylation of 5' CpG island of p16/CDKN2 tumour suppressor gene in normal and transformed human tissues correlates with gene silencing; *Cancer Res.* **55** 4551-4555

Gopalkrishnan R V, Lam E W F and Claude K 1998 The p53 tumour suppressor inhibits transcription of the TATA-less mouse DP1 promoter; *J. Biol. Chem.* **273** 10972-10978

Gottlieb E, Haffner R, King A, Asher G, Gruss P, Lonai P and Oren M 1997 Transgenic mouse model for studying the transcriptional activity of p53; *EMBO J.* **16** 1381-1390

_____, _____, Von Ruden T, Wagner E F and Oren M 1994 Down regulation of wt p53 activity interfere with apoptosis of IL3-dependent hematopoietic cells following IL3 withdrawl; *EMBO J.* **13** 1368-1374

Gottlieb T M and Oren M 1996 p53 in growth control and neoplasia; *Biochem. Biophys. Acta* **1155** 181-205

Gounari F, De Francesco R, Schmitt J, Vander V P C, Cortese R and Stunnenberg H 1990 Amino terminal domain of NF1 binds to DNA as a dimer and activates adenovirus DNA replication; *EMBO J.* **9** 559-566

Gournani M, Lipari P, Dell J, Shi B and Nielsen L L 1999 Adenovirus-mediated p53 gene therapy has greater efficacy when combined with chemotherapy against human head and neck, ovarian, prostate and breast cancer; *Cancer Chemother. Pharmacol.* **44** 143-151

Graves R A, Tontonoz P, Ross S R and Spiegelman B M 1991 Identification of a potent adipocyte-specific enhancer: involvement of an NF1-like factor; *Genes Dev.* **5** 428-437

Greenblatt M S, Bennet W P, Hollstein M and Harris C C 1994 Mutations in the p53 tumour suppressor gene: Clues to cancer etiology and molecular pathogenesis; *Cancer Res.* **54** 4855-4878

Gronostajski R M 1987 Site-specific DNA binding of nuclear factor 1: effect of the spacer region; *Nucleic Acids Res.* **15** 5545-5559

Grossman S R, Perez M, Kung A L, Joseph M, Mansur C, Xiao Z-X, Kumar S, Howley P M and Livingston D M 1998 p300/MDM2 complexes participate in MDM2 mediated p53 degradation; *Molecular Cell* **2** 405-415

Grubb G R, Yun K, Reeve A E and Eccles M R 1995 Exclusion of the Wilms tumour gene (WT1) promoter as a site of frequent mutation in Wilms tumour; *Oncogene* **10** 1677-1681

Gu W, Shi X-L and Roeder R G 1997 Synergistic activation of transcription by CBP and p53; *Nature* **387** 819-823

Habib N A, Hodgson H J, Lemoine N and Pignatelli M 1999 A phase I/II study of hepatic artery infusion with wt p53-CMV-Ad in metastatic malignant liver tumours; *Hum. Gene Ther.* **10** 2019-2034

Hainaut P, Hernandez T, Rodriguez-Tome P, Flores T, Hollstein M, Harris C C and Montesano R 1998 IARC database of p53 gene mutations in human tumours and cell lines : updated compilation, revised formats and new visualization tools; *Nucleic Acids Res.* **26** 205-213

Hall P A and Lane D P 1997 Tumour suppressors: a developing role for p53; *Curr. Biol.* **7** R144-R147

Harris C C 1996a Structural and function of the p53 tumour suppressor gene: clue for rational cancer therapeutic strategies; *J. Natl. Cancer Inst.* **88** 1442-1455

_____ 1996b p53 tumour suppressor gene: from the basic research laboratory to the clinic : an abridged historical perspective; *Carcinogenesis (London)* **17** 1187-1198

Harris H, Miller O J, Klein G, Worst P and Tachibana T 1969 Suppression of malignancy by cell fusion; *Nature* **223** 363-368

Hartmann A, Blaszyk H, McGovern R M, Schroeder J J, Cunningham J, De Vries E M G, Kovach J S and Sommer S S 1995 p53 gene mutations inside and outside of exons 5 - 8: the pattern differ in breast and other cancers; *Oncogene* **10** 681-688

Hay J G, Shapiro N, Sauthoff H, Heitner S, Phupakdi W and Rom W N Targeting the replication of adenoviral gene therapy vectors to lung cancer cells: the importance of adenoviral E1b-55Kd genes; *Hum Gene. Ther.* **10** 579-590

Herman J G, Graff J R, Myohanen S, Nelkin B d and Baylin S B 1996a Methylation specific PCR: a novel PCR assay for methylation status of CpG islands; *Proc. Natl. Acad. Sci. USA* **93** 9821-9826

_____, Jen G, Merlo A and Baylin S B 1996b Hypermethylation associated inactivation induces a tumour suppressor role for p15INK4B; *Cancer Res.* **56** 722-727

_____, Merlo A, Mao L, Lapidus R G, Issa J P J, Davidson N E, Sidransky D and Baylin S B 1995 Inactivation of the CDKNN2/p16/MTS1 gene is frequently associated with aberrant DNA methylation in all common human cancers; Cancer Res **22** 4525-4530

Hinds P W, Finlay C A and Levine A J 1989 Mutation is required to activate the p53 gene for cooperation with the ras oncogene and transformation; *J. Virol.* **63** 739-746

Hollstein M, Shomer B, Greenblatt M, Sousssi T, Hovig E, Montesano R and Harris C C 1996 Somatic point mutations in the p53 gene of human tumours and cell lines: updated compilation; *Nucleic Acids Res.* **241** 141-146

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

Horikoshi N, Usheva A, Chen J, Levine A J, Weinmann R and Shenk T 1995 Two domains of p53 interact with the TATA-binding protein, and the adeno virus 13S E1A protein disrupts the association, relieving p53 mediated transcriptional repression; *Mol. Cell. Biol.* **15** 227-234

Hupp T R, Sparks A and Lane D P 1995 Small peptides activated the latent sequence-specific DNA binding function of p53; *Cell* **83** 237-245

Hurlimann J, Chaubert P and Benhatter J 1994 p53 gene alteration and p53 protein accumulation in infiltrating ductal breast carcinomas: correlation between immunohistochemical and molecular biology techniques; *Mod. Pathol.* **7** 423

Iacopetta B, Grieu F, Powell B, Soong R, McCaul K and Seshadri R 1998 Analysis of p53 gene mutation by Polymerase Chain Reaction-Single Strand Conformation Polymorphism provides independent prognostic information in node negative breast cancer; *Clin. Cancer Res.* **4** 1597-1602

Iggo R, Gatter K, Bartek J, Lane D P and Harris A L 1990 Increased expression of mutant forms of p53 oncogene in primary lung cancer; *Lancet* **335** 675-679

Inoue T, Tamura T, Furuichi T and Mikoshiba K 1990 Isolation of complementary DNAs encoding a cerebellum-enriched nuclear factor 1 family that activates transcription from the mouse myelin basic protein promoter; *J. Biol. Chem.* **265** 19065-19070

Jackson D A, Rowander K E, Stevens K, Jiang C, Milos P and Zaret K S 1993 Modulation of liver-specific transcription by interaction between hepatocyte nuclear factor 3 and nuclear factor 1 binding in close apposition; *Mol. Cell. Biol.* **13** 2401-2410

Jones P A 1996 DNA methylation errors and cancer; *Cancer Res.* **56** 2463-2467

Kastan M B, Zhan Q, El-Deiry W S, Carrier F, Jacks T, Walsh W V, Plunkett B S, Vogelstein B and Fornace Jr. A J 1992 A mammalian cell cycle check point pathway utilizing p53 and GADD45 is defective in ataxia-telangiectasia; *Cell* **71** 587-597

Kaur J, Srivastava A and Ralhan R 1994 Overexpression of p53 protein in betel-related human dysplasia and squamous cell carcinoma in India; *Int. J. Cancer* **58** 340-345

Kennedy S M, Macgeogh C, Jaffe R and Spurr N K 1994 Overexpression of the oncoprotein p53 in primary hepatic tumours in childhood does not correlate with gene mutations; *Hum. Pathol.* **25** 438

Kinzler K W and Vogelstein B 1994 Cancer therapymeets p53; *N. Eng. J. Med.* **331** 49-50

Kleymenova E V, Yuan X, La Bate M E and Walker C L 1998 Identification of tumour-specific methylation site in the Wilms tumour suppressor gene; *Oncogene* **16** 713-720

Ko L J and Prives C 1996 p53: puzzle and paradigm; *Genes Dev.* **10** 1054-1072

Koeffler H P, Miller C, Nicolson M A, Ranyard J and Bosselman R A 1986 Increased expression of p53 protein in human leukemia cells; *Proc. Natl. Acad. Sci. USA* **83** 4035-4039

Kubbutat M H G, Jones S N and Vousden K H 1997 Regulation of p53 stability by Mdm 2: *Nature* **387** 299-303

Kulkarni S and Gronostajski R M 1996 Altered expression of the developmentally regulated NF1 gene family during phorbol ester-induced differentiation of human leukemic cells; *Cell Growth Diff.* **7** 501-510

Laird P W and Jaenisch R 1994 DNA methylation and cancer; *Hum. Mol. Genet.* **3** 1487-1495

Lamb P and Crawford L 1986 Characterization of the human p53 gene; *Mol. Cell. Biol.* **6** 1379-1385

Landers J E, Haines D S, Strauss J F, George D L 1994 Enhanced translation: a novel mechanism of mdm2 oncogene overexpression identified in human tumour cells; *Oncogene* **9** 2745-2750

Lane D P 1992 p53, guardian of the genome; *Nature* **358** 15-16

_____ and Crawford L V 1979 T antigen is bound to a host protein in SV-40-transformed cells; *Nature* **278** 261-263

Langdon J D and Partridge M 1992 Expression of the tumour suppressor gene p53 in oral cancer; *Br. J. Oral Maxillofac. Surg.* **30** 214-220

Lehman T A, Bennett W P, Metcalf R A, Welsh J A, Ecker J, Modali R A, Ullrich S, Romano J W, Appella E, Testa J R, Gerwin B L and Harris C C 1991 p53 mutations, ras mutations and p53-heat shock 70 protein complexes in human lung carcinoma cell lines; *Cancer Res.* **51** 4090-4096

Levine A J 1997 p53, the cellular gatekeeper in growth and division; *Cell* **88** 323-331

_____, Momand J and Finlay C A 1991 The p53 tumour suppressor gene; *Nature* **351** 453-456

Li P, Bui T, Gray D and Klamut H J 1998 Therapeutic potential of recombinant p53 overexpression in breast cancer cells expressing endogenous wild-type p53; *Breast Cancer Res. Treat.* **48** 273-286

Li X, Tsuji T, Wen S, Sobhan F, Wang Z and Shinozaki F 1995 Cytoplasmic expression of p53 protein and its morphological features in salivary gland lesions; *J. Oral Pathol. Med.* **24** 201-205

Lill N L, Grossman S R, Ginsberg D, Decarpio J and Livingston D M 1997 Binding and modulation of p53 by p300/CBP coactivators; *Nature* **387** 823-827

Linzer D I H and Levine A J 1979 Characterization of a 54 K dalton cellular SV40 tumour antigen in SV40 transformed cells; *Cell* **17** 43-52

Louis J M, McFarland V W, May P and Mora P T 1988 The phosphoprotein p53 is down-regulated post-transcriptionally during embryogenesis in vertebrates; *Biochim. Biphys. Acta* **950** 395-402

Lozano G and Levine A J 1991 Tissue-specific expression of p53 in transgenic mice is regulated by intron sequences; *Mol. Carcinog.* **4** 3-9

Magewu A N and Jones P A 1994 Ubiquitous and tenacious methylation of CpG site in codon 248 of the p53 gene may explain its frequent appearance as a mutation hotspot in human cancer; *Mol. Cell. Biol.* **14** 4225-4232

Maheswaran S, Englert C, Bennett P, Heinrich G and Habar D A 1995 The Wt1 gene product stabilizes p53 and inhibits p53-mediated apoptosis; *Genes Dev.* **9** 2143-2156

Malkin D, Li F P, Strong L C, Fraumeni J F Jr., Nelson C E, Kim D H, Kassel J, Gryka M A, Bischoff F Z, Tainsky M A and Friend S H 1990 Germline p53 mutations in a familial syndrome of sarcomas, breast cancer and other neoplasms; *Science* **250** 1233-1238

Mancini D N, Rodenhiser D I, Ainsworth P J, O'Malley F P, Singh S M, Xing W and Archer T K 1998 CpG methylation within the 5' regulatory region of the BRCA1 gene is tumour specific and includes a putative CREB binding site; *Oncogene* **16** 1161-1169

Marasca R, Luppi M, Barozzi P, Ferrai M G, Morselli M and Torelli G 1996 p53 gene mutations in chronic myelogenous leukemia, medullary and extramedullary blast crisis; *Leuk. Lymph.* **24** 175-182

Masuda H, Miller C, Koeffler H P, Battifora H and Cline M J 1987 Rearrangement of p53 gene in human osteosarcomas; *Proc. Natl. Acad. Sci. USA* **84** 7716-7719

Matlashewski G, Lamb P, Pim D, Peacock J, Crawford L and Benchimol S 1984 Isolation and characterization of a human p53 cDNA clone : expression of the human p53 gene; *EMBO J.* **13** 3257-3262

McPake C R, Shetty S, Kitchingman G R and Harris L C 1999 Wild-type p53 induction mediated by replication -deficient adenoviral vectors; *Cancer Res.* **59** 4247-4251

Meisternst M, Rogge L, Foeckler R, Karaghiosoff M and Winnacker E L 1989 Structural and functional characterization of a porcine gene coding for nuclear factor 1; *Biochemistry* **28** 8191-8200

Mercer W E, Amin M, Sauve G J, Appella E, Ullrich S J and Romano J 1990 Wild-type human p53 is antiproliferative in SV40-transformed hamster cells; *Oncogene* **5** 973-980

Midgely C A, Fisher C J, Bartek J, Vojtesk B L and Bames D M 1993 Analysis of p53 expression in human tumours: an antibody raised against human p53 expressed in Escherchia coli; *J. Cell Sci.* **101** 183-189

Mink S, Hartig E, Jennewein P, Doppler W and Cato A C B 1992 A mammary cell-specific enhancer in MMTV DNA is composed of multiple regulatory elements including binding sites for CTF/NF1 and a novel transcription factor, mammary cell activating factor; *Mol. Cell. Biol.* **12** 4960-4978

Mitchell P J and Tjian R 1989 Transcriptional regulation in mammalian cells by sequence-specific DNA binding protein; *Science* **245** 371-378

Miyashita T and Reed J C 1995 Tumour suppressor p53 is a direct transcriptional activator of the human bax gene; *Cell* **80** 293-299

Moll U M, LaQueglia M, Benard J and Riou G 1995 Wild-type p53 protein undergoes cytoplasmic sequestration in undifferentiated tumours; *Proc. Natl. Acad. Sci. USA* **92** 4407-4411

_____, Ostermeyer A G, Haladay R, Winkfield B, Frazier M and Zambetti G 1996 Cytoplasmic sequestration of wild-type p53 protein impairs the G1 checkpoint after DNA damage; *Mol. Cell. Biol.* 16 1126-1137

_____, Rion G and Levine A J 1992 Two distinct mechanisms alter p53 in breast cancer; *Proc. Natl. Acad. Sci. USA* **89** 7262-7266

Montesano R, Hollstein M and Hainut P 1996 Genetic alterations in esophageal cancer and their relevance to etiology and pathogenesis: a review; *Int. J. Cancer* **69** 225-235

Mosner J, Mummenbraner T, Bauer C, Sczakiel G, Grosse F and Deppert W 1995 Negative feedback regulation of wild-type p53 biosynthesis; *EMBO J.* **14** 4442-4449

Mowat M, Cheng A, Kimura N, Bernstein A and Benchimol S 1984 Rearrangement of the cellular p53 gene in erythroleukemic cells transformed by friend virus; *Nature* **314** 633-636

Mujoo K, Maneval D C, Anderson S C and Gutterman J V 1996 Adenoviral-mediated p53 tumour suppressor gene therapy of human ovarian carcinoma; *Oncogene* **12** 1617-1623

Munirajan A K, Tutsumi-Ishii Y, Mohanprasad B K C, Hirano Y, Munakata N, Shanmugam G and Tsuchida N 1996 p53 gene mutations in oral carcinomas from India; *Int. J. Cancer* **66** 297-300

Nakazawa H, English D, Randell P L, Nakazawa K, Martel N, Armstrong B K and Yamasaki H 1994 UV and skin cancer: specific p53 gene mutation in normal skin as a biologically relevant exposure measurement; *Proc. Natl. Acad. Sci. USA* **91** 360-364

Nayak B K and Das B R 1997 Differential binding of nuclear proteins to p53 gene promoter in male breast tumour; *Eur. J. Cancer* **33** 1484-1487

_____ and Das B R 1999a Mutation and methylation status of p53 promoter in human breast tumours; *Tumor Biol.* **20** 341-346

_____ and Das B R 1999b Differential binding of NF1 transcription factor to p53 gene promoter and its depletion in human breast tumours; *Mol. Biol. Reports* **26** 223-230

_____ , Baral R N and Das B R 1996 p53 gene mutation in relation to p53 protein accumulation in male and female breast cancer; *Neoplasma* **43** 305-310

_____ , Patnaik S and Das B R 1998 Rearrangement of p53 gene in human breast tumours; *Biochem. Biophys. Res. Commun.* **245** 388-391

Nebl G, Mermod N and Cato Andrew C B 1994 Post-transcriptional down-regulation of expression of transcription factor NF1 by Ha-ras oncogene; *J. Biol. Chem.* **269** 7371-7378

Nielsen L L and Maneval D C 1998 P53 tumour suppressor gene therapy for cancer; *Cancer Gene Ther.* **5** 52-63

Nigro J M, Baker S J, Preisinger 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 tumour types; *Nature* **342** 705-708

Ohtani-Fujita N, Fujita T, Aoike A, Osifchin N E, Robbins P D and Sakai T 1993 CpG methylation inactivates the promoter activity of the human retinoblastoma tumour suppressor gene; *Oncogene* **8** 1063-1067

Oren M and Levine A J 1983 Molecular cloning of a cDNA specific for the murine p53 cellular tumour antigen; *Proc. Natl. Acad. Sci. USA* **80** 56-59

Oren M, Maltzman W and Levine A J 1981 Post-translational regulation of the 54K cellular tumour antigen in normal and transformed cells; *Mol. Cell. Biol.* **1** 101-110

_____, Beinz B, Givol D, Rechavi G, Zakut R 1983 Analysis of recombinant DNA clones specific for murine p53 cellular tumour antigen; *EMBO J.* **2** 1633-1639

Ori A, Zauberman A, Doitsh G, Paran N, Oren M and Shaul Y 1998 p53 binds and represses the HBV enhancer: an adjacent enhancer element can reverse the transcription effect of p53; *EMBO J.* **17** 544-553

Paonessa G, Gounari F, Frank R and Cortese R 1988 Purification of a NF1-like DNA binding protein from rat liver and cloning of the corresponding cDNA; *EMBO J.* **7** 3115-3123

Parada L F, Land H, Weinberg R A, Wolf D and Rotter V 1984 Cooperation between gene encoding p53 tumour antigen and ras in cellular transformation; *Nature* **312** 649-651

Patnaik S, Nayak B K and Das B R 1999a Alterations in p53 gene in human oral tumours; *Oral Oncol.* Vol. VI pp 87-92 ed. A K Varma

Patnaik S, Nayak B K and Das B R 1999b Rearrangement in the coding and 5' region of p53 gene in human oral tumours; *IUBMB Life* **48** 305-310

Pinhasi-Kimhi O, Michalovitz D, Benzeev A and Oren M 1986 Specific interaction between the p53 tumour antigen and major antigen and major heat shock proteins; *Nature* **320** 182-184

Polyak K, Xia Y, Zewier J L, Kinzler K W and Vogelstein B 1997 A model for p53-induced apoptosis; *Nature* **6648** 300-310

Purdie C A, O'Cardy J, Piris J, Wyllie A H and Bird C C 1991 p53 expression in colorectal tumours; *Am. J. Pathol.* **138** 807-813

Putzer B M, Bramson J L, Addison C L, Hitt M, Siegel P M, Muller W J and Graham F L 1998 Combination therapy with interleukin-2 and wild-type p53 expressed by adenoviral vectors potentiates tumour regression in a murine model of breast cancer; *Hum. Gene Ther.* **9** 707-718

Puzianowska-Kuznicka M and Shi Y-B 1996 Nuclear factor 1 as a potential regulator during post-embryonic organ development; *J. Biol. Chem.* **271** 6273-6282

Rabbits T H, Hamlyn P H and Baer R 1983 Transcriptional enhancer identified near the human C mu immunoglobulin heavy chain gene is unavailable to the translocated c-myc gene in Burkitt's lymphoma; *Nature* **306** 760-765

Ranasinghe A, Macheoch C, Dyer S, Spurr N and Johnson N W 1993 Some oral carcinomas from Srilankan betel/tobacco chewers overexpress p53 oncoprotein but lack mutation in exons 5-9; *Anticancer Res.* **13** 2065-2068

Ray R B, Steele R, Meyer K and Ray R 1998 Transcriptional repression of p53 promoter by hepatitis C virus core protein; *J. Biol. Chem.* **272** 10983-10986

Reich N C and Levine A 1984 Growth regulation of a cellular tumour antigen, p53, in non-transformed cells; *Nature* **308** 199-201

Reisman D and Rotter V 1993 The helix-loop-helix containing transcriptional factor USF binds to and transactivates the promoter of the p53 tumour suppressor gene; *Nucleic Acids Res.* **21** 345-350

_____, Greenberg M and Rotter V 1988 Human p53 oncogene contains one promoter upstream of exon 1 and a second stronger promoter within intron 1; *Proc. Natl. Acad. Sci. USA* **85** 5146-5150

Rhoads R E 1988 Cap recognition and the entry of mRNA into the protein synthesis initiation cycle; *Trends Biochem. Sci.* **13** 52-56

Rigaudy P and Eckhart W 1989 Nucleotide sequence of a cDNA encoding the monkey cellular phosphoprotein p53; *Nucleic Acids Res.* **17** 8375

Rivkina M B, Cullen J M, Robinson W S and Marison P L 1994 State of the p53 gene in hepatocellular carcinomas of ground squirrels and woodchucks with and ongoing infection with hepadnaviruses; *Cancer Res.* **54** 5430-5437

Rogel A, Popliker M, Webb C G and Oren M 1985 p53 cellular tumour antigen: Analysis of mRNA levels in normal adult tissues, embryos and tumours; *Mol. Cell. Biol.* **5** 2851-2855

Roth J A 1998 Gene replacement strategies for lung cancer; *Curr. Opin. Oncol.* **10** 127-132

Roth J A, Swisher S G and Meyn R E 1999 P53 tumour suppressor gene therapy for cancer; *Oncology* **13** 148-154

Rotter V, Aloni G R, Schwartz D, Elkind N B, Simons A, Wolkowics R, Lavigne M, Beserman P, Kapon A and Goldfinger N 1994 Does wild-type p53 play a role in normal cell differentiation; *Sem. Cancer Biol.* **5** 229-236

Rovinski B and Benchimol S 1988 Immortalization of rat embryo fibroblasts by the cellular p53 oncogene; *Oncogene* **2** 445-452

Roy B, Beamon J, Baliant E and Reisman D 1994 Transactivation of the human p53 tumour suppressor gene by c-myc/max contributes to elevated mutant p53 expression in some tumours; *Mol. Cell. Biol.* **14** 7805-7815

Runnebaum I B, Nagarajan M, Bowman M, Soto D, Sukuman S 1991 Mutations in p53 gene as potential molecular markers for human breast cancer; *Proc. Natl. Acad. Sci. USA* **88** 10657-10666

Rupp R, Kruse U, Multhaup G, Gobel U, Beyreuther K and Sippel A 1990 Chcken NF1/TGGCA proteins are encoded by at least three independent genes, NF1-A, NF1-B and NF1-C with homologues in mammalian genomes; *Nucleic Acids Res.* **18** 2607-2616

Ruscetti S K and Scolnick E M 1983 Expression of transformation-related protein (p53) in the malignant stage of Friend virus-induced disease; *J. Virol.* **46** 1022-1026

Sakai T, Ohtani N, McGee T L, Robbins P D and Dryja T P 1991 Oncogenic germ-line mutations in sp1 and ATF sites in the human retinoblastoma gene; *Nature* **353** 83-86

Santoro C, Mermod N, Andrews P and Tjian R 1988 A family of human CCAAT box-binding proteins active in transcription and DNA replication : cloning and expression of multiple cDNAs; *Nature* **334** 218-224

Sarnow P, Ho Y S, Williams J and Levine A J 1982 Adenovirus E1b-58kd tumour antigen and SV40 large tumour antigens are physically associated with the same 54kd cellular protein in transformed cells; *Cell* **28** 387-394

Schmid P, Lorenz A, Hameister H and Montenarh M 1991 Expression of p53 during mouse embryogenesis; *Development* **113** 857-865

Schroeder M and Mass M J 1997 CpG methylation inactivates the transcriptional activity of the p53 promoter of the human p53 tumour suppressor gene; *Biochem. Biophys. Res. Commun.* **235** 403-406

Schuur E R, Kruse U, Iacovoni J S and Vogt P K 1995 Nuclear factor 1 intereferes with transformation induced by nuclear oncogenes; *Cell Growth Diff.* **6** 219-227

Shikama N, Lee C-W, France S, Delavaine L, Lypn J, Krstic- Demonacos M and Thangue N B 1999 A novel cofactor for p300 that regulates the p53 response; *Molecular Cell* **4** 365-376

Smith M L and Fornace Jr. A J 1997 p53-mediated protective responses to UV irradiation; *Proc. Natl. Acad. Sci. USA* **94** 12255-12257

Soddu S and Sacchi A 1998 p53: prospects for cancer gene therapy; *Cytokines Cell Mol. Ther.* **4** 177-185

Sonenberg N 1988 Cap-binding proteins of eukaryotic messenger RNA: functions in initiation and control of translation; *Progr. Nucleic Acid Res. Mol. Biol.* **35** 174-207

Soussi T, Caron de Fromentel C and May P 1990 Structural aspects of p53 protein in relation to gene evolution; *Oncogene* **5** 945-952

Soussi T, DeFromentel C C, Breugnount C and May E 1988 Nucleotide sequence of a cDNA encoding the rat p53 cellular oncoprotein; *Nucleic Acids Res.* **16** 11384

Soussi T, _____, Mechali M and Kress M 1987 Cloning and characterization of a cDNA from Xenopus laevis coding for a protein homologous to human and murine p53; *Oncogene* **1** 71-78

Srivastava S, Zou Z, Pirollo K, Blattner W and Chang E H 1990 Germline transmission of a mutated p53 gene in a cancer prone family with Li-Fraumeni syndrome; *Nature* **348** 747-749

Steinmeyer K, Maacke H and Deppert W 1990 Cell cycle control by p53 in normal mouse cells; *Oncogene* **5** 1691-1699

Strauss B E and Costanzi-Staruss E 1999 Efficient retrovirus-mediated transfer of cell-cycle control genes to transformed cells; *Braz. J. Med. Biol. Res.* **32** 905-914

Stuart E T, Haffner R, Oren M and Gruss P 1995 Loss of p53 function through pax-mediated transcriptional repression; *EMBO J.* **14** 5638-5645

Sun X, Shimuzu H and Yamamoto J 1995 Identification of a novel p53 promoter element involved in genotoxic stress-inducible p53 gene expression; *Mol. Cell. Biol.* **15** 4489-4496

Swisher S G, Roth J A, Nemunaitis J, Lawrence D D, Kemp B L, Carrasco C H, Connors D G, El-naggar A K, Fossella F, Glisson B S, Hong W K, Khuri F R, Kurie J M, Lee J J, Lee J S, Mack M, Merritt J A, Ngugen D M, Nesbitt J C, Perez-Soler R, Pisters K M, Putnam J B Jr, Richli W R, Savin M and Waugh M K 1999 Adenovirus- mediated p53 gene transfer in advanced non-small-cell lung cancer; *J. Natl. Cancer Inst.* **91** 763-771

Takagi Y, Koo L C, Osada H, Ueda R, Kyaw K, Ma C-C, Suyama M, Saji S, Takahashi T, Tominaga S and Takahashi T 1995 Distinct mutational spectrum of the p53 gene in lung cancer from Chinese women in Hong Kong; *Cancer Res.* **55** 5354-5357

Tandle A T, Saranath D and Deo M G 1994 p53 tumour suppressor gene in oral cancers and putative premalignant lesions; *XVI International Cancer Congress*, New Delhi pp 913-917

Taylor J A, Li Y, He M, Mason T, Mettlin C, Vogler W J, Maygarden S and Liu E 1996 p53 mutations in bladder tumours from arylamine-exposed workers; *Cancer Res.* **56** 294-298

Tornaletti S and Pfeifer G 1995 Complete and tissue independent methylation of CpG sites in the p53 gene: implications for mutations in human cancers; *Oncogene* **10** 1493-1499

Tuck S P and Crawford L 1989 Characterization of the human p53 gene promoter; *Mol. Cell. Biol.* **9** 2163-2172

Ui M, Takada M, Arai T, Matsumoto K, Yamada K, Nakahata T, Nishiwaki T, Furukawa Y, Tokino T, Nakamura Y and Iba H 1999 Retrovirus vectors designed for efficient transduction of cytotoxic or cytostatic genes; *Gene Ther.* **6** 1670-1678

Uittenbogaard M N, Giebler H A, Reisman D and Nybord J K 1995 Transcriptional repression of p53 by human T-cell leukemia virus type 1 tax protein; *J. Biol. Chem.* **270** 28503-28506

Velculescu V E and El Deiry W S 1996 Biological and clinical importance of the p53 tumour suppressor gene; *Clin. Chem.* **42** 858-868

Vinyals A, Peinado M A, Gonzalez-Garrigues M, Monzo M, Bonfil R D and Fabra A 1999 Failure of wild-type p53 gene therapy in human cancer cell lines expressing a mutant p53 protein; *Gene Ther.* **6** 22-23

Wang X W and Harris C C 1996 TP53 tumour suppressor gene: clues to molecular carcinogenesis and cancer therapy; *Cancer Surv.* **28** 169-196

Wang Y-C, Chen C-Y, Chen S-K, Cherng S-H, Ho W L and Lee H 1998 High frequency of deletion of mutations in p53 gene from Squamous cell lung cancer patients in Taiwan; *Cancer Res.* **58** 328-333

Watson C J, Gordon K E, Robertson M and Clark A J 1991 Interaction of DNA-binding proteins with milk protein gene promoter in vitro : Identification of a mammary gland-specific factor; *Nucleic Acids Res.* **19** 6603-6610

Werner H, Karnieli E, Rauscher F J and Le Roith 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** 8318-8323

Wolf D and Rotter V 1984 Inactivation of p53 gene expression by an insertion of moloney murine leukemia virus-like DNA sequence; *Mol. Cell. Biol.* **4** 1402-1410

Wong Y C and Bernal S D 1994 Multiple NF1 site binding factors associated with different cell types of human lung carcinomas; *Anti Cancer Res.* **14** 593-596

Wu X, Bayle J H, Olson D and Levine A J 1993 The p53-mdm2 autoregulatory feedback loop; *Genes Dev.* **7** 1126-1132

Wynford-Thomas D 1992 p53 in tumour pathology : can we trust immunohistochemistry (editorial) *J. Pathol.* **166** 329

Xu L, Chen Y-T, Huvos A G, Zlotolow I M, Rettig W, Old L J and Garin-Chesa P 1994 Overexpression of p53 in squamous cell carcinomas of head and neck without apparent gene mutations; *Diagn. Mol. Pathol.* **3** 83

_____, Pirrollo K F, Tang W H, Rait A and Chang E F 1999 Transferrin-liposome-mediated systemic p53 gene therapy in combination with radiation results inregression of human head and neck cancer xenografts; *Hum. Gene Ther.* **10** 2941-2952

Yaginuma Y, Yamashita T, Takuma N, Katayama H and Ishikawa M 1995 Analysis of the p53 gene in human choriocarcinoma cell lines; *British Jour. Cancer* **71** 9-12

Yang B S, Gilbert J D and Freytag S O 1993 Overexpression of myc suppresses CCAAT transcription factor/nuclear factor 1-dependent promoters in vivo; *Mol. Cell. Biol.* **13** 3093-3102

Zauberman A, Lubp A and Oren M 1995 A functional p53-responsive intronic promoter is contained within the human mdm2 gene; *Oncogene* **10** 2361-2366

Zhang Q, Qutsch D and Kenney S 1998 Induction of cellular p53 activity by DNA damaging agents and growth arrest; *Mol. Cell. Biol.* **14** 1929-1938

Zhang W, Hu G, Estey E, Hester J and Deissertoh A 1992 Altered conformation of the p53 protein in myeloid leukemia cells and mitogen stimulated normal cells; *Oncogene* **7** 1645-1647

Zhuang S M, Cochran C, Goodrow T, Wiseman R W and Soderkvist P 1997 Genetic alteration of p53 and ras genes in 1,3-butadine and 2', 3'-dideoxycytidine-induced lymphomas; *Cancer Res.* **57** 2710-2714

Zobras H, Rein T, Krause A, Hoffmann K and Winnacker E L 1992 Nuclear factor 1 (NF1) binds to an NF1-type site but to the CCAAT site in the human ?-globin gene promoter; *J. BIol. Chem.* **267** 8478-8484

Zou Y, Zong G, Ling Y H, Hao M M, Lozano G, Hong W K and Perez-Soler R 1998 Effective treatment of early endobronchial cancer with regional administration of liposome-p53 complexes; *J. Natl. Cancer Inst.* **90** 1130-1137