Review Article

RFP Indian Journal of Hospital Infection
Volume 1 Number 2, July - December 2024

TP53 as a Tumor Suppressor Gene

Mosin S Khan[1], Roohi Ashraf[2], Aaliya Shah[3], Syed Mudassar[4]

Abstract

Recognition of *TP53*'s prominent role in protection from cancer has boosted a huge amount of scientific reports (around 40000) describing the function of *TP53* as a tumour suppressor. Quite an unusual feature of a tumour suppressor was noted – *TP53* is point mutated rather than inactivated in cancers and is highly expressed in tumours. Mutation of *TP53* gene confers novel oncogenic properties on *TP53* protein.

Keywords: Cancer; *TP53*; Tumor suppressor gene; Polymorphism

History of discovery

TP53 was identified in 1979 as a protein in complex with large T-antigen oncoprotein of the SV40 DNA tumour virus (Linzer *et al*., 1979). Another study reported high levels of *TP53* in transformed, but not normal cells, with no history of viral infection, suggesting it was coded by cellular genes (DeLeo *et al*., 1979). *TP53* gene was cloned (Oren *et al*., 1983; Zakut-Houri *et al*., 1985) and originally described as an oncogene, due to its ability to transform cells in cooperation with other H-Ras oncogene (Eliyahu *et al*., 1984; Parada *et al*., 1984). In support of this notion, expression of *TP53* then was shown to immortalize the cells (Jenkins *et al*., 1984) and enhance tumorigenic potential of cells injected in mice (Wolf *et al*., 1984). Later it was realized that the originally studied *TP53* protein was the product of a mutated *TP53* gene, which indeed promoted tumour genesis. However, after the wild type *TP53* gene was cloned it became evident that wild type *TP53* protein blocked the ability of oncogenes to transform cells (Eliyahu *et al*., 1989; Finlay *et al*., 1988; Hinds *et al*., 1989). Wild type *TP53* was then reclassified as a tumour suppressor gene and numerous studies since then have demonstrated its key role in protecting cells from cancer (Vogelstein *et al*., 2000). It was also declared as the molecule of the year. The fact that *TP53* is mutated in at least half of all human cancers indicates a strong selection for its loss during tumour progression (Hollstein *et al*., 1991). Additional support for its crucial role in tumour genesis came from the study of Li-Fraumeni patients, who inherit one allele of mutant *TP53* gene and are extremely pre-disposed to cancer (Malkin *et al*., 1990).

Structure of *TP53*

The *TP53* gene contains eleven exons with two alternative translation start sites in exon 2 and 4 (Gen Bank Accession Number: NC_000077) (Murray-Zmijewski *et al*., 2006). The *TP53* protein contains three major functional domains: N-terminal transcriptional activation domain (TA), the central sequence-specific DNA-binding domain (DBD) and the oligomerization domain (OD) in the C-terminus. There is also an N-terminal proline rich domain involved in protein interactions and regulatory domain in the C-terminus (*Figure 1*).

TP53 is active as a tetramer, with four identical

Author's Affiliation:

[1,2]Department of Biochemistry, Govt. Medical College, Srinagar and Associated Hospitals, Karan Nagar, Srinagar, Jammu and Kashmir 190010, India. [3]Department of Biochemistry, SKIMS Medical College and Hospital, Bemina, Srinagar, Jammu and Kashmir 190018, India. [4]Professor and Head, Department of Clinical Biochemistry, Sher-I-Kashmir Institute of Medical Sciences (SKIMS), Soura, Srinagar, Jammu and Kashmir 190011, India.

Correspondence and Reprint Requests:

Syed Mudassar, Professor and Head, Department of Clinical Biochemistry, Sher-I-Kashmir Institute of Medical Sciences (SKIMS), Soura, Srinagar, Jammu and Kashmir 190011, India.

E-mail: syedmudassor@skims.ac.in

Received on: 28.07.2024 **Accepted on:** 15.10.2024

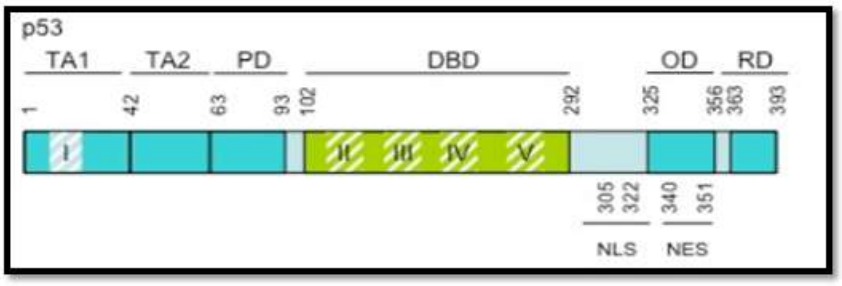

Fig. 1: Structure of *TP53* protein.

The main domains of TP53, nuclear export (NES), nuclear localization (NLS) signals and the location of the conserved boxes I, II, III, IV and V are shown. TA – transactivationdomain, PD - proline-rich domain, DBD - DNA binding domain, OD - oligomerization domain, RD – regulatory domain.

chains of 393 residues. The N-terminal region consists of an intrinsically disordered trans-activation domain (TAD) and a proline-rich region. It is followed by the central, folded DNA-binding core domain that is responsible for sequence-specific DNA binding. Via a flexible linker, this domain is connected to a short tetramerization domain that regulates the oligomerization state of *TP53 (Figure 2)*. At its C terminus, *TP53* contains the so-called regulatory domain. This natively unfolded region is rich in basic amino acids (mainly lysine) and binds DNA non-specifically.

TP53 Isoforms

The human *TP53* gene is composed of 19, 200 bp, spanning over 11 exons on chromosome 17p13.1 (NC_000017). Until recently only 3 mRNA splice variants of *TP53* have been known, which encode full-length *TP53*, *TP53*i9 and *TP53*Δ40 (Ghosh *et al.*, 2004). *TP53*i9 results from alternative splicing at exon 9 and encodes a protein truncated of the last 60 amino acids, which is defective in transcriptional activity. *TP53*Δ40 (other names p47 and Δ*NTP53*) protein is truncated of the first 40 amino acids and can be generated by two mechanisms: either by an alternative splicing of the intron 2 (Ghosh *et al*., 2004) or by alternative initiation of translation (Yin *et al*., 2002). *TP53*Δ40 contains the second transactivation domain and is capable of activating some of the *TP53* target genes. Interestingly, it can also inhibit transcriptional activity of the full-length *TP53* in a dominant-negative way (Ghosh *et al*., 2004). A recent study reports that the structure of the *TP53* gene is much more complex than previously thought and many more *TP53* isoforms exist (Bourdon *et al*., 2005). The structure of *TP53* gene and the currently known *TP53* isoforms are summarized in Figure 2. The *TP53* gene is transcribed from two distinct sites upstream of exon 1 and from an internal promoter located in intron 4. The alternative promoter leads to the expression of an N-terminally truncated *TP53* (Δ133*TP53*), which lacks the entire TA domain and part of the DNA binding domain. Usage of alternative promoter in intron 4 gives rise to Δ40*TP53* with truncation of N-terminal transactivation domain. In addition alternative splicing at intron 9 gives rise to α, β and γ isoforms. Therefore at least 9 different isoforms of *TP53* can be generated.

Function of *TP53*

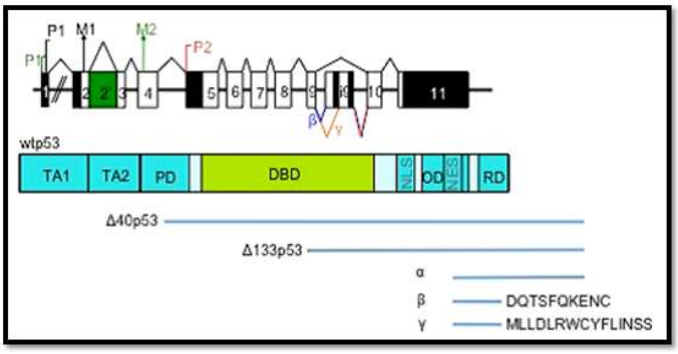

Fig. 2: Human *TP53* gene.

The structure of TP53 gene and the alternatively spliced TP53 isoforms are depicted.

TP53 is activated in response to oncogene activation, DNA damage and spindle damage, which can potentially increase the mutation occurrence in cells and increase the risk of becoming cancerous. *TP53* is also induced in response to other types of cellular stresses such as hypoxia, dNTP depletion and nutrient deprivation which can predispose cells to malignant transformation. Activated *TP53* can induce cell-cycle arrest, allowing DNA repair, or cause senescence, or promote apoptosis, eliminating the damaged cells (Vogelstein *et al*., 2000). Numerous studies have demonstrated that *TP53* can influence many other biological processes, such as invasion and motility, angiogenesis, differentiation, cell survival and more recently discovered glycolysis (Bensaad *et al*., 2006) and autophagy (Crighton *et al*., 2006) (Fig. 3).

TP53 is a Transcription Factor

The *TP53* gene encodes a transcription factor and mediates much of its biological activities by regulating the expression of numerous *TP53* target genes. *TP53* binds to the specific sequences– *TP53* responsive elements - in the regulatory region of its target genes and more than hundred different *TP53* target genes have been described with various biological functions and the list is likely to grow (Murray-Zmijewski *et al*., 2008). *TP53* activates transcription of most of its targets by recruiting general transcription factors (TATA-binding protein-associated factors) and histone acetyltransferases (HAT) CBP, p300 and PCAF to the promoter (Gu *et al*., 1997). One of the first discovered *TP53* target genes was the cyclin-dependent kinase inhibitor (CDK) p21, which induces a cell cycle arrest (el-Deiry *et al*., 1994). *TP53* induces apoptosis by activating genes mediating extrinsic and intrinsic apoptotic pathways (Chipuk *et al*., 2006). Such targets include genes encoding death receptors, Fas/CD95/Apo-1, Killer/R5, mitochondrial proteins Bax,Noxaand PUMA (Nakano *et al*., 2001). Activation of autophagy via induction of novel

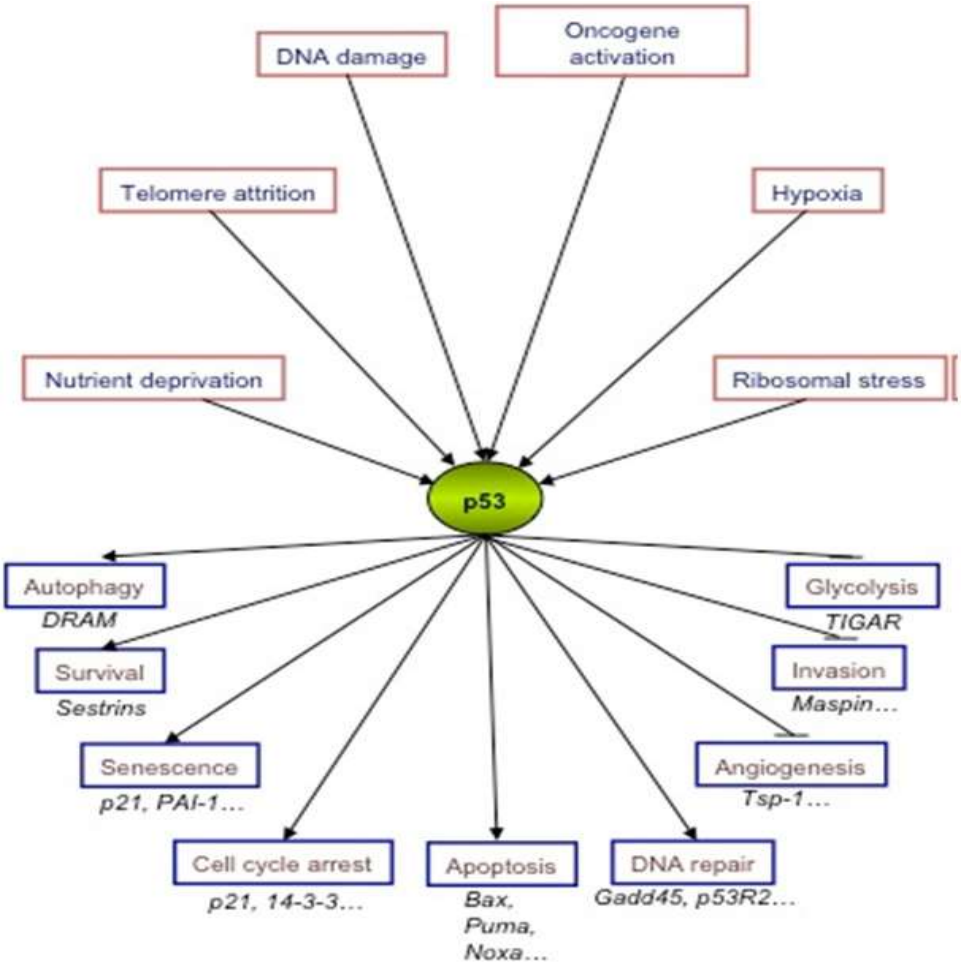

Fig. 3: Scheme of *TP53* response.

TP53 is activated by a number of cellular stresses (blue boxes) and regulates different biological processes (red boxes) via transcriptional activation of its target genes (marked in black).

gene DRAM by *TP53* also contributes to cell death (Crighton *et al*., 2007). Recent studies have identified microRNA miR-34 as a *TP53* target gene, adding a new twist on regulation of *TP53* gene network (Chang *et al*., 2007). MiRNAs are a class of small regulatory RNAs that mediate post-transcriptional silencing of specific target mRNAs (Bartel *et al*., 2004). The miR-34 family is directly induced by *TP53* in response to DNA damage and oncogenic stress, which can lead to induction of growth arrest and apoptosis through inhibiting gene expression of proliferative and anti-apoptotic genes (Chang *et al*., 2007). *TP53* can contribute to cell survival by allowing DNA repair by activating genes such as Gadd45, *TP53*R2 (Tanaka *et al*., 2000). *TP53* has also been suggested to play a direct role in mediating DNA repair by interacting with components of the repair machinery (Gatz *et al*., 2006). In addition, *TP53* plays a survival role by protecting the genome from damage by reactive oxygen species (ROS). This activity of *TP53* is mediated by activation of TIGAR, sestrins, aldehyde dehydrogenase-4 and Sco2 (Matoba *et al*., 2006), which can decrease the levels of intracellular ROS. The current model suggests that at low levels of stress *TP53* plays a survival role and helps the cell to cope with stress, by decreasing ROS and allowing DNA repair. When stress is severe and/or DNA damage is irrepairable, *TP53* triggers irreversible growth arrest or apoptosis, to eliminate the damaged cells from the healthy pool (Vousden *et al*., 2007). In light of the current data, the role of *TP53* therefore emerges as a master regulator of cells well-being, which prevents cancer development. Several *TP53* target genes inhibit *TP53* activity in a negative feedback loop. *TP53* transcriptionally activates its major negative regulator Mdm2 (mouse double minute) (Wu *et al*., 1993), a ubiquitin ligase, which inactivates *TP53* mainly by targeting it for proteasomal degradation and promoting its nuclear export. Similarly, to Mdm2, *TP53* target genes Cop1 and Pirh2 encode ubiquitin ligases which can degrade *TP53* (Leng *et al*., 2003). In addition, *TP53* can directly interact with the transcription factors, such as Sp1 and AP1 and others, preventing their binding to the target genes. By this mechanism *TP53* leads to repression of genes such as cyclin B1and TERT (Kanaya *et al*., 2000). *TP53* also recruits histone deacetylases (HDACs) to the promoters which is mediated by the interaction with SIN3A (Murphy *et al*., 1999). By this mechanism, *TP53* represses transcription of genes such as MAP4 and stathmin (Murphy *et al*., 1999). One of the novel target genes CD44 is inhibited by *TP53* under conditions of basal stress. *TP53* plays a key role in mediating tumour progression in cells lacking *TP53*. CD44 encodes a cell-surface molecule and can block *TP53*-dependent stress-induced apoptotic signals. The repertoire of *TP53* target genes is extremely broad and in addition to genes mentioned above also includes secreted proteins regulating migration and angiogenesis (Teodoro *et al*., 2006). Though some of these biological responses have sometimes opposing roles, they all seem to contribute to the tumour suppressive function of *TP53*.The choice of *TP53* response depends on the type of the particular stress and cellular context and is the active area of research (Murray-Zmijewski *et al*., 2008), which has mostly focused on the choice between the fundamental *TP53* responses – cell cycle arrest and apoptosis. Posttranslational modifications are involved in dictating the choice of transcriptional target genes by *TP53*. Upon UV and DNA damage HIPK2 and DYRK2 phosphorylate *TP53* on S46 (Taira *et al*., 2007). This promotes induction of apoptosis by *TP53* via activation of pro-apoptotic *TP53*AIP1 gene (Oda *et al*., 2000). Acetylation of *TP53* on lysine 120 by MOF and TIP60 also promotes *TP53*-dependent apoptosis in response to DNA damage, via recruitment of *TP53* to pro-apoptotic target genes, PUMA and Bax (Tang *et al*., 2006). Ubiquitination of *TP53* on Lys320 by E3 ligase E4F1 promotes cell cycle arrest function of *TP53* via activation of p21, Gadd45 and cyclin G1, while not affecting the pro-apoptotic target genes (Le Cam *et al*., 2006). *TP53* family members p63 and p73 can also selectively enhance the apoptotic activity of *TP53* in some cell types, by promoting transactivation of PERP and BAX but not p21 (Flores *et al*., 2002). *TP53* interacting partners play an important role in the outcome of *TP53* response. The members of the ASPP (ankyrin-repeat-SH3-domain- and proline-rich-region-containing) family play an important role in regulating the apoptotic function of *TP53*. ASPPs act by selectively enhancing the *TP53* binding and trans-activating promoters of pro-apoptotic target genes such as Bax, PIG3 (*TP53*-induced gene 3) and PUMA, while not affecting the promoters of the CDKN1A and mdm2 genes.

Mutations of *TP53* in Cancer

Fraumeni syndrome

Li-Fraumeni syndrome (LFS) is a rare inherited cancer pre-disposition syndrome, affecting individuals before the age of 45 years. Unlike other inherited cancer syndromes, LFS is characterized by a variety of different cancers, predominantly sarcomas, breast cancers, brain tumours and adrenocortical carcinomas, though other cancers have also been reported. LFS is dominantly-inherited and is associated with high mortality. Analysis of the LFS families has shown that around

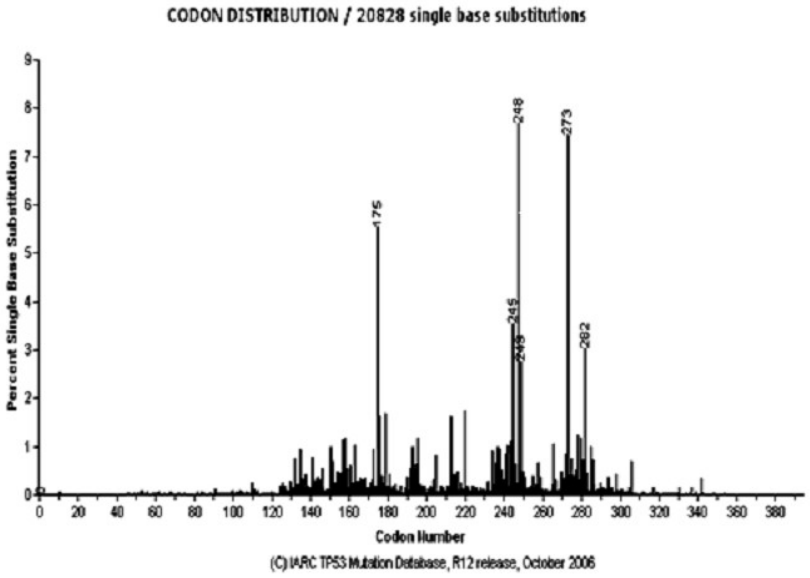

Fig. 4: Mutational frequency of *TP53*.

The frequency of the point mutations of each codon of TP53 found in tumors (IARC database, R12 release, 2006, www-TP53.iarc.fr).

70% of these families have a germline mutation in the *TP53* gene. Li-Fraumeni-like syndrome (LFL) describes a similar syndrome, which does not have all features of the classical LFS and similarly has been found to have germ-line mutations in *TP53* gene (Olivier *et al*., 2003). From the database information it is revealed that most of the *TP53* mutations are missense mutations (72%) and some are deletions (10%). About 46% of the mutations were located at the codons 175, 213, 245, 248, 273 and 282 in the DBD of *TP53*, which correspond to hotspot mutations in sporadic cancers (Soussi *et al*., 2007).

TP53 in sporadic cancers

As already mentioned, *TP53* gene is found mutated in nearly half of all human cancers analyzed. In many other types of cancers *TP53*pathway is inactivated by other ways, such as inactivation of ARF or over expression of Mdm2. Unlike most of the tumour suppressor genes, more than 80% of the *TP53* alterations are missense mutations which lead to generation of full-length *TP53* with single amino acid substitution (Petitjean *et al*., 2007). The initial observations, which showed that *TP53* mutations are a frequent event in many tumour types, were made some twenty years ago (Takahashi *et al*., 1989). Those studies demonstrated that most of the mutations are localized in the exons 5–8, which lead to a single amino acid substitution of the DNA binding domain. Therefore, most of the later studies (40% of all) have focused on the characterization of these mutations, which mostly affect the DNA binding domain. The database of *TP53* mutations has been updated and includes the analysis of some of the recent studies have found that mutations also occur outside exons 5–8 (about 10%) (Figure 4) (Bastien *et al*., 2008). The current version of the *TP53* mutation database reports about 24000 different mutations most of which occur as single amino acid substitutions in the DNA binding domain of *TP53*. Most of the mutations locate within the highly evolutionary conserved regions of the DBD of *TP53*.

Properties of Mutant *TP53*

Loss-of-function

Biochemical studies have shown that *TP53* mutants exhibit certain heterogeneity in terms of structural alterations and loss of DNA-binding activity. The DNA-binding site recognized by *TP53* is highly degenerated and the affinity of *TP53* for target sites varies (Resnick *et al*., 2003). Though many *TP53* mutants exhibit total loss-of-function, some *TP53* mutants retain partial transactivation ability. Tumour-derived point mutants *TP53* 175Pro and *TP53* 181Lys retain the ability to activate p21 and induce cell cycle arrest, however fail to induce other target genes, which impairs their ability to induce apoptosis (Ludwig *et al*., 1996). In addition to loss-of-function, *TP53* mutants acquire cancer promoting properties (Finlay *et al*., 1988), which have been attributed to the ability of mutant *TP53* to inhibit wild type *TP53* in a dominant-negative manner and by gain-of-function effect.

Dominant-negative effect

Over expression studies in cells have shown that mutant *TP53* inhibits the function of wild type *TP53* acting in a dominant-negative manner (Willis *et al*., 2004). This results in interference with several *TP53*-mediated biological processes, such as: apoptosis (Gottlieb *et al*., 1994), growth arrest, differentiation, genetic stability and transformation suppression (Unger *et al*., 1993). One of the explanations was that mutant *TP53* can induce a conformational change in wild type *TP53* (Milner *et al*., 1991). However, structural studies suggest that contact mutants do not have a gross change to their structure, though are capable of inhibiting wild type *TP53* when over expressed (Chene *et al*., 1998). The current mechanism of the dominant-negative effect suggests the formation of mixed tetramers of mutant and wild type *TP53* proteins, which reduces the level of fully active homotetramers of wild type *TP53* (Willis *et al*., 2004). One report suggests that at least three mutant molecules are required per tetramer to inactivate the transactivation ability of *TP53* (Chan *et al*., 2004). This suggests that dominant-negative effects of mutant *TP53* can be biologically relevant only when the levels of mutant *TP53* are high. It is possible that in tumour cells, where mutant *TP53* accumulates to high levels; it might lead to inhibition of the wild type *TP53*. In the course of tumour progression the wild type allele is often lost (Olive *et al*., 2004). This might imply that wild type *TP53* retains its function to some extent in the presence of mutant *TP53*, as there is a selective pressure to lose it.

Gain of function

Experimental systems on a *TP53*-null background have demonstrated novel tumour promoting properties of mutant *TP53*, which is known as "gain-of-function" effect. One of the early studies showed that mutant *TP53* expression in cells lacking *TP53* enhanced their tumourigenic potential (Wolf *et al*., 1984). Mutant *TP53* can enhance the transformation potential of *TP53*-null cells as assessed by colony formation assay and leads to enhanced growth of the cells. Several studies have shown that exogenously expressed mutant *TP53* confers tumourigenic potential in several *TP53*-null cell types: murine fibroblasts, murine L-12 pre-B cells and human osteosarcoma cell line (Wolf *et al*., 1984; Lanyi *et al*., 1998). Another gain-of-function property of mutant *TP53* is the ability to interfere with the induction of apoptosis in response to various stress signals, such as DNA damage and growth factor deprivation when over expressed in cells (Zalcenstein *et al*., 2006). However, the most convincing evidence for the gain-of-function effect is provided by the study of knock-in mice with "hot-spot" mutations in *TP53*. *TP53* mutant mice with mutation at either Arg172His (equivalent to 175 in humans) or Arg270His (equivalent to 273 in humans), belonging to structural and contact class of hot-spot mutants respectively, have been generated (Olive *et al*., 2004). Both mutants*TP53* knock-in and *TP53*-null mice develop tumours, however, mutant *TP53* knock-in mice exhibit different spectra of tumour spectrum, with predisposition to carcinomas and endothelial tumours. Tumours in mutant *TP53* knock-in mice display more aggressive phenotypes and metastasize with higher frequency. These findings provide the most physiologically relevant evidence for the gain-of-function effect of certain *TP53* mutants (Olive *et al*., 2004). The mechanism of the gain-of-function effect of *TP53* mutants has been proposed to be mediated via their interaction with p63/p73. However, the exact mechanism of gain-of-function of mutant *TP53* is still unknown. Recent study has addressed the gain-of-function effects of *TP53* hot spot mutations (R248W and R273H) by introducing them into the HUPKI allele (Song *et al*., 2007). Another mechanism of the gain-of-function of mutant *TP53* involves regulation of the expression of a specific set of genes. One of the first genes shown to be up regulated by mutant *TP53* was MDR-1, which was suggested as a mechanism underlying chemo resistance promoted by mutant *TP53* (Chin *et al*., 1992). Mutation of L22 and W23, required for transcriptional activity of *TP53*, abrogated the ability of mutant *TP53* to transactivate MDR-1 and enhancement of tumourigenic potential of the cells by mutant *TP53* (Lin *et al*., 1995). This study has provided the evidence for transcriptional regulation mechanism of the gain-of-function of mutant *TP53*.

_TP53_ Polymorphisms

As is true of the human genome as a whole (in which over 3.1 million sequence variations have been mapped, which represent 25–35% of the total estimated SNPs (Frazer *et al*., 2007), numerous SNPs and other sequence variations are present at the *TP53*locus. Most of these variations are intronic and can be presumed to have no cancer-related biological consequences. Few of the many *TP53* polymorphisms have been assessed for altered biochemical and/or biological function, or for their effects on cancer risk in population studies.

Polymorphisms in non-coding sequences

More than 90% of the polymorphisms in *TP53*occur in the noncoding sequences. The well-characterized intronic*TP53* polymorphism is a 16 base pair insertion in intron 3 (Lazar *et al*., 1993). This is the only intronic polymorphism that has

been associated with an increase in the risk of several types of cancer (Costa *et al*., 2008).

*Synonymous polymorphisms in **TP53** coding sequences*

Of the 19 exonic polymorphisms that have been reported in *TP53*, eight are synonymous. Although these polymorphisms do not change the amino acid sequence or structure of the protein, in theory, changes in base sequence and codon use could modify protein expression, folding and function, or provoke new splicing events (Candeias *et al*., 2008).A silent mutation at codon 36 (CCG to CCT) was shown to reduce the ability of *TP53* to activate apoptosis by lowering the affinity of the *TP53*mRNA for MDM2; consequently, reducing *TP53* levels (Candeias *et al*., 2008). Three synonymous polymorphisms—D21D (GAC to GAT), Pro34Pro (CCC to CCA) and Pro36Pro (CCG to CCA) — are located in the region that is crucial for *TP53* mRNA binding to MDM2 and their roles await functional analysis.

*Non-synonymous polymorphisms in **TP53** coding sequences*

The remaining 11 polymorphisms in *TP53* are non-synonymous, resulting in an amino acid change in the protein. Only four of these polymorphisms have been validated by multiple submissions of the polymorphism to *TP53* databases, reports on the frequency of the polymorphism, or inclusion of the polymorphism in the Hap Map database. In addition, they have not been reported as somatic mutations in tumours. Changes in the amino acid sequence can alter the ability of *TP53* to bind to response elements of target genes (as shown by tumour-associated *TP53* mutations alter recognition motifs for post-translational modifications, or alter the protein stability and interactions with other proteins (Li *et al*., 2007). For two of the polymorphisms, there is sufficient molecular evidence to suggest that the polymorphisms cause a functional change in the *TP53* pathway (Pro47Ser and Arg72Pro). The remaining two validated non-synonymous polymorphisms have not been associated with an altered cancer risk to date (Val217Met and Gly360Ala).

Codon 47 (Pro47Ser)

Pro47Ser, a rare polymorphism in the N-terminal transactivation domain of *TP53*, results from a C→T base substitution at position 1 of codon 47. It has only been reported in populations of African origin, in which it is found at an allele frequency of approximately 5% (Felley-Bosco *et al*., 1993). Phosphorylation of the N-terminal domain of *TP53* has been shown to regulate its transactivation properties (Kruse *et al*., 2008). P38 and homeodomain-interacting protein kinase 2 (HIPK2) phosphorylate Ser46, which enhances the transcription of apoptosis-related genes and hence promotes *TP53*-mediated apoptosis (Kruse *et al*., 2008). These two kinases are directed to phosphorylation sites by a proline residue adjacent to Ser46. Thus, replacement of Pro47, as occurs with the Per47Ser polymorphism, would be expected to decrease phosphorylation at Ser46, decrease transactivation of pro-apoptotic target genes and thus potentially increase cancer risk (Kurihara *et al*., 2007).

Codons 217 and 360 (Val217Met and Gly360Ala)

Val217Met (resulting from a G>A transition) is the only validated coding polymorphism that is located in the DBD of *TP53*; thus, in principle, it could dramatically affect the activity of *TP53*.Functional studies have been limited to transactivation assays in yeast (Kato *et al*., 2003), which indicate that this polymorphism results in little loss of activity The genes that show the most variation in activation are CDKN1A, BAX and PMAIP1 (also known as NoXA), but the *TP53*-Met217 variant leads to greater expression of these genes than the more common *TP53*-Val217 variant; extrapolating from this result, one can speculate that the Val217Met polymorphism might be protective against cancer.

Gly360Ala is located in the linker region adjacent to the tetramerization domain of *TP53*. Again, the functional data for this polymorphic variant have been provided by transactivation studies in yeast (Kato *et al*., 2003), which showed a slight decrease in the transactivation of BAX, MDM2 and *TP53*AIP, and a more marked decrease in stratifin (SFN, also known as 14-3-3 sigma) and GADD45 (growth arrest and DNA damage-inducible (Gemignani, *et al*., 2004). Codon 72 (Arg72Pro) polymorphism in *TP53*

The codon 72 polymorphism

This common SNP results in a non-conservative changeof an arginine (R72) to a proline (P72) at amino acid72 that results in a structural change of the proteingiving rise to variants of distinct electrophoretic mobility (Matlashewski *et al*., 1987). This polymorphism occurs in a proline-richregion of *TP53*, which is known to be important forthe growth suppression and apoptotic functions ofthis protein (Sakamuro *et al*., 1997). Beckman and co-

workers were the first todemonstrate a significant difference in the allelic distribution of the R72 and P72 variants. They first noted a significant difference in the P72 allele frequency between a Nigerian population (African Black) and a Swedish population (Western Europe), which were17 and 63%, respectively; in contrast, they did notnote any differences between populations living on the same geographical latitude (Beckman *et al*., 1994). The authors went on to demonstrate that the frequencyof the P72 allele differs with latitude, increasing ina linear manner as populations near the equator (Sjalander *et al*., 1995). These observations led the authors to suggest that the codon 72 variants differed inbiological activity, and further that these differences inactivity might be subject to selection in areas of high ultraviolet light exposure.

Banks and co-workers subsequently demonstrated the existence of biochemical and biological differences between the R72 and P72 isoforms of *TP53*. Noted are the conserved functional domains of *TP53*, with amino-acid residues for each functional domain listed below. Thelocations of the two coding region polymorphic variants (codon 47 and codon 72) are denoted with an asterisk. Figure 2 Amino-acid sequences of the *TP53* polymorphism atresidue 47. The two p38 MAPK sites of phosporylation (serines 33 and 46), adjacent to proline residues at amino acids 34 and 47, aredenoted. Figure 3 Amino-acid sequences of the *TP53* polymorphism atresidue 72. This region contains several SH3-binding motifs (PXXP), which are postulated to be important for the ability of *TP53* to induce apoptosis. In a subsequent study, the authors went on to demonstrate that the P72 form of *TP53* had enhanced ability to function as a sequence specific trans-activator, owing, in part, to its strongerinteraction with two TFIID-associated factors, TAFII32 and TAFII70 (Thomas *et al*., 1999). In contrast, the authors found that the R72 variant of *TP53* was amarkedly better suppressor of cellular transformation, an activity commonly associated with *TP53*'s apoptotic function. Differences in the biological activity of R72 and P72 proteins have also been described for certain tumor-derived mutant forms of *TP53*. Specifically, the *TP53*-homolog p73 has been reported to physically interactwith certain tumor-derived mutant forms of *TP53* (butnot wild-type *TP53*). More to the point, the authors demonstrated that these mutant forms of *TP53* interacted with p73 preferentially when they occurred in cis with the R72 *TP53* polymorphism (Marin *et al*., 2000). This study went on to show that, in tumours from individuals heterozygous for the codon 72 polymorphism (R72/P72), the R72 allele was most commonly subject tomutation, while the other allele (P72) was morefrequently lost by deletion (Marin *et al*., 2000). Thesedata suggested that the R72 variant of *TP53*, when in ciswith certain tumor-derived mutations, might haveenhanced tumor suppressive function owing to increased ability to inactivate p73. Subsequent studies suggest thatthe ability of R72 to target and inhibit p73 may be celltype dependent (Vikhanskaya *et al*., 2005). Specifically, these authors demonstrated that some of the *TP53* tumorderived mutants that are unable to bind and inhibit p73 are still able to confer resistance to drug treatment, suggesting that R72-containing mutants may possess other mechanisms to disrupt chemotherapy-induced apoptosis. Two groups found that, for non-mutated forms of *TP53*, the R72 variant has a significantly increased abilityto induce programmed cell death, in cells containinginducible versions of *TP53*, as well as in cells homozygousfor R72 and P72 (Dumont *et al*., 2003). The absence of differences in specific DNAbinding or transcriptional ability of these two polymorphic variants led our group to discover that theenhanced apoptotic potential of the R72 variant wasowing to increased trafficking to the mitochondria, resulting from enhanced interaction with, and ubiquitylation by, the MDM2 ubiquitin ligase (Dumont *et al*., 2003). Such mitochondrial localization of *TP53*, leading to cytochrome c release, was first described by Moll andco-workers, and later confirmed by our group (Dumont *et al*., 2003). Our group inassociation with the group of George has identified thepro-apoptotic protein BAK, an important member fromthe Bcl-2 family involved in cytochrome c release frommitochondria, as a mitochondrial *TP53*-interacting protein (Leu *et al*., 2004). Interestingly, we found that thetwo *TP53* isoforms R72 and P72 demonstrate the same affinity for BAK, suggesting that the enhanced ubiquitylation and nuclear export of the R72 under liesits enhanced mitochondrial function in cell death. Insum, the combined data from several groups hasconfirmed the altered apoptotic potential of the codon72 polymorphic variants, with the R72 variant demonstrating enhanced apoptotic ability, and the P72 variant demonstrating enhanced growth arrest (Pim and Banks, 2004). Based on these findings, a number of studies have tried to establish a correlation between the *TP53*codon 72 polymorphism and the risk to develop certain types of cancer. In general, these studies have notyielded consistent results; this may be accounted for by the fact that the R72 variant, when found in mutantforms of *TP53*, might be predicted to enhance tumor development (increased inactivation of p73), but whenfound in the context of wild-type *TP53*, might be predicted

to better inhibit tumor development (increased apoptotic ability)

One of the first studies to demonstrate a correlation between the codon 72 polymorphism of *TP53* and the riskto develop cancer was by Banks and co-workers, who reported that women with the R72 variant of *TP53* (better targeted for degradation by HPV E6 protein) had aseven-fold increased risk to develop cervical cancer (Storey *et al*., 1998). To date, dozens of studies have failed to confirm these results, possibly because of differences in subtypes of HPV, so an association between cervical cancer and the codon 72 polymorphism of *TP53* is not currently accepted.Several groups have reported an association betweenthe R72 *TP53* variant (binds and inactivates p73 better)and increased risk for epithelial cancer, including gastric cancer (Shen *et al*., 2004) and cancer of the breast, ovary oesophagus, skin (DeOliveira *et al*., 2004), lung, bladder, prostate and larynx (Sourvinos *et al*., 2001). In other studies, however, authors have found the opposite correlation,instead demonstrating an association between the P72 (lesser apoptotic) variant and increased risk forother cancer types, including cancer of the thyroid, nasopharynx, prostate, skin, urogenital region and lung (Zhang *et al*., 2003). Still other groups have failed to demonstrate any association between codon 72variants of *TP53* and cancer risk. Again, these discrepancies may be influenced by a failure to determine the mutational status of *TP53* in these tumours. Other researchers suggest that these discrepancies may beaccounted for by a failure to conduct meta-analyses, orowing to poorly controlled 'normal' populations that donot take into account the latitudinal differences in allele *TP53* polymorphisms. Oncogene frequency (Koushik *et al*., 2004). While correlations between cancer risk and the codon 72 polymorphism have been in consistent, more consistent have been the correlations between these polymorphic variants and cancer progression, survival, andage of on set of cancer. In particular, several groups havefound that patients homozygous for P72 (lesser apoptotic allele) were diagnosed at an earlier median ageof onset for their cancer. The median age varied from 6 years earlier for squamous cell carcinoma of thehead and neck, to 13 years earlier for non-polyposiscolorectal cancer, and between 10 and 11 years earlier for oral cancer (Jones *et al*., 2004). These data are consistent with the hypothesis that the R72 allele, which has greater apoptotic ability, consequently possesses enhanced tumor suppression function. Also consistent with thishypothesis are findings that individuals with the R72 genotype have higher response rates and better survival after receiving chemo- and radiation therapy foradvanced head and neck cancer and for cancers of the breast and lung (Xu *et al*., 2005). Therefore, while correlations between cancer risk and *TP53* polymorphic variants havenot been clear, more consistent correlations exist for cancer progression, survival, age of onset, and response to therapy.

In human populations, codon 72 of *TP53* has either the sequence CCC, which encodes proline, or CGC, which encodes arginine. The variants are hereafter abbreviated *TP53*-Pro72 and *TP53*-Arg 72. Comparative sequence analyses in non-human primates suggest that *TP53*-Pro72 is the ancestral form, although *TP53*-Arg 72 occurs at a high frequency (>50%) in some populations. A latitude gradient in variant frequency (an increasing frequency of the *TP53*–72 variant towards the equator (Sjalander *et al*., 1996) invited early speculation that *TP53*-Pro72 might protect against adverse consequences of sunlight or other environmental cancer risk factors.

The NIH genetic association database, which is not comprehensive, has records on over 230 studies evaluating the effect of the codon 72 polymorphism on susceptibility to a wide variety of cancers. Many of these studies have reported 'statistically significant' associations. Several formal meta-analyses combining data from multiple studies have been published on breast, gastric and lung cancer, and these do not support a role for this polymorphism in the risk of developing these cancers (Matakidou *et al*., 2003).

References

1. Bartel DP. MicroRNAs: genomics, biogenesis, mechanism, and function. Cell. 2004.2:281-97.
2. Bastien R, Lewis TB, Hawkes JE, *et al*. High-throughput amplicon scanning of the *TP53* gene in breast cancer using highresolution fluorescent melting curve analyses and automatic mutation calling. Hum Mutat. 2008;5:757–64.
3. Beckman G, Birgander R, Sjalander A, *et al*. Is p53 polymorphism maintained by natural selection? Hum Hered. 1994;44:266–70.
4. Bensaad K, Tsuruta A, Selak MA, *et al*. TIGAR, a p53-inducible regulator of glycolysis and apoptosis. Cell. 2006;1:107–20.
5. Bourdon JC, Fernandes K, Murray-Zmijewski F, *et al*. P53 isoforms can regulate p53 transcriptional activity. Genes Dev. 2005;19(18):2122–37.
6. Candeias, M. M. *et al*. p53 mRNA controls p53 activity by managing Mdm2 functions. Nature Cell

Biol. 2008;10:1098–1105.

7. Chan WM, Siu WY, Lau A, Poon RY. How many mutant p53 molecules are needed to inactivate a tetramer? Mol Cell Biol. 2004;8:3536–51.
8. Chang TC, Wentzel EA, Kent OA, *et al*. Transactivation of miR-34a by p53 broadly influences gene expression and promotes apoptosis. Mol Cell. 2007;5:745–52.
9. Chene P. In vitro analysis of the dominant negative effect of p53 mutants. J M Biol. 1998;2:205–09.
10. Chin KV, Ueda K, Pastan I, Gottesman MM. Modulation of activity of the promoter of the human MDR1 gene by Ras and p53. Science. 1992;5043:459–62.
11. Chipuk JE, Green DR. Dissecting p53-dependent apoptosis. Cell Death Differ. 2006;6:994–1002.
12. Costa, S. *et al*. Importance of *TP53*codon 72 and intron 3 duplication 16bp polymorphisms in prediction of susceptibility on breast cancer. BMC Cancer. 2008;8:32.
13. Crighton D, Wilkinson S, Ryan KM. DRAM links autophagy to p53 and programmed cell death. Autophagy. 2007;1:72–74.
14. De Oliveira WR, Rady PL, Grady J, *et al*. Association of p53 arginine polymorphism with skin cancer. Int J Dermatol. 2004;43:489–493.
15. DeLeo AB, Jay G, Appella E, *et al*. Detection of a transformation-related antigen in chemically induced sarcomas and other transformed cells of the mouse. PNAS, USA. 1979;5:2420–24.
16. Dumont P, Leu JI, Della Pietra A III, *et al*. The codon 72 polymorphic variants of p53 have markedly different apoptotic potential. Nat Genet 2003;33:357 65.
17. El-Deiry WS, Harper JW, O'Connor PM, *et al*. WAF1/CIP1 is induced in p53-mediated G1 arrest and apoptosis. Cancer Res. 1994;5:1169–74.
18. Eliyahu D, Michalovitz D, Eliyahu S, *et al*. Wild-type p53 can inhibit oncogene-mediated focus formation. PNAS, USA. 1989;22:8763–67.
19. Eliyahu D, Raz A, Gruss P, *et al*. Participation of p53 cellular tumour antigen in transformation of normal embryonic cells. Nature. 1984;5995:646–49.
20. Felley-Bosco E, Weston A, Cawley HM, *et al*. unctional studies of a germ-line polymorphism at codon 47 within the p53 gene. Am. J. Hum. Genet. 1993;53:752–59.
21. Finlay CA, Hinds PW, Tan TH, *et al*. Activating mutations for transformation by p53 produce a gene product that forms an hsc70-p53 complex with an altered half-life. Mol Cell Biol. 1988;2:531–39.
22. Flores ER, Tsai KY, Crowley D, *et al*. (2002). p63 and p73 are required for p53-dependent apoptosis in response to DNA damage. Nature. 6880:560–64.
23. Frazer KA, *et al*. A second generation human haplotype map of over 3.1 million SNPs. Nature. 2007;449:851–61.
24. Gatz SA & Wiesmuller L. p53 in recombination and repair. Cell Death Differ. 2006;6:1003–16.
25. Gemignani F, *et al*. A *TP53* polymorphism is associated with increased risk of colorectal cancer and with reduced levels of *TP53* mRNA. Oncogene. 2004;23:1954–56.
26. Ghosh A, Stewart D, Matlashewski G. Regulation of human p53 activity and cell localization by alternative splicing. Mol Cell Biol. 2004;18:7987–97.
27. Godar S, Ince TA, Bell GW, Feldser D, *et al*. Growth inhibitory and tumor-suppressive functions of p53 depend on its repression of CD44 expression. Cell. 2008;1:62–73.
28. Gottlieb E, Haffner R, von Ruden T, *et al*. Down-regulation of wild-type p53 activity interferes with apoptosis of IL-3-dependent hematopoietic cells following IL-3 withdrawal. EMBO J. 1994;6:1368–74.
29. Gu W & Roeder RG. Activation of p53 sequence-specific DNA binding by acetylation of the p53 C-terminal domain. Cell. 1997;4:595–606.
30. Hollstein M, Sidransky D, Vogelstein B, *et al*. P53 mutations in human cancers. Science. 1991;253: 49–5.
31. Jones JS, Chi X, Gu X, *et al*. Modifiers of expression in mutations of mismatch repair gene carriers in hereditary nonpolyposis colorectal cancer. Clin Cancer Res. 2004;10:5845–5849.
32. Kanaya T, Kyo S, Hamada K, Takakura M, *et al*. Adenoviral expression of p53 represses telomerase activity through down-regulation of human telomerase reverse transcriptase transcription. Clin Cancer Res. 2000;6(4):1239–47.
33. Kato S, *et al*. Understanding the function-structure and function-mutation relationships of p53 tumor suppressor protein by high-resolution missense mutation analysis. PNAS, USA. 2003;100: 8424–29.
34. Koushik A, Platt RW, Franco EL. Cancer Epidemiol Biomarkers Prev, 2004;13:11–22. Review.
35. Kruse JP & Gu W. SnapShot: p53 posttranslational modifications. Cell. 2008;133: 930–31.
36. Kurihara A, *et al*. Ser46 phosphorylation of p53 is not always sufficient to induce apoptosis: multiple mechanisms of regulation of p53-dependent. Genes Cells. 2007 Jul;12(7):853-61.
37. Lanyi A, Deb D, Seymour RC, *et al*. Gain of function' phenotype of tumor-derived mutant p53 requires the oligomerization/nonsequence-specific nucleic acid-binding domain. Oncogene. 1998;16(24):3169–76.
38. Lazar V, *et al*. Simple sequence repeats polymorphism within the p53 gene.Oncogene. 1993;8:1703–05.
39. Le Cam L, Linares LK, Paul C, Julien E, *et al*. E4F1 is an atypical ubiquitin ligase that modulates p53 effector functions independently of degradation. Cell. 2006;4:775–88.

40. Leng RP, Lin Y, Ma W, *et al*. Pirh2, a p53-induced ubiquitin-protein ligase, promotes p53 degradation. Cell. 2003;6:779–91.
41. Leu JI, Dumont P, Hafey M, Murphy ME, *et al*. Mitochondrial p53 activates Bak and causes disruption of a Bak-Mcl1 complex. Nat Cell Biol 2004;6:443e50.
42. Li Y & Prives C. Are interactions with p63 and p73 involved in mutant p53 gain of oncogenic function? Oncogene. 2007;26:2220–25.
43. Lin J, Teresky AK, Levine AJ. Two critical hydrophobic amino acids in the Nterminal domain of the p53 protein are required for the gain of function phenotypes of human p53 mutants. Oncogene. 1995;12:2387–90.
44. Linzer DI & Levine AJ. Characterization of a 54K Dalton cellular SV40 tumour antigen present in SV40-transformed cells and uninfected embryonal carcinoma cells. Cell. 1979;17:43–52.
45. Ludwig RL, Bates S, Vousden KH *et al*. Differential activation of target cellular Inhibition of cell migration, spreading, and focal adhesions by tumor suppressor PTEN. Science. 1996;280 (1998) 1614–1617.
46. Malkin D, Li FP, Strong LC, *et al*. Germ line p53 mutations in a familial syndrome of breast cancer, sarcomas, and other neoplasms. Science. 1990;4985:1233–38.
47. Marin MC, Jost CA, Brooks LA, Irwin MS, *et al*. A common polymorphism acts as an intragenic modifier of mutant p53 behaviour. Nat Genet. 2000; 25:47e54.
48. Matakidou A, Eisen T, Houlston RS. *TP53* polymorphisms and lung cancer risk: a systematic review and meta-analysis. Mutagenesis. 2003;18:377–85.
49. Matlashewski GJ, Tuck S, Pim D, *et al.* Primary structure polymorphism at amino acid residue 72 of human p53. Mol Cell Biol. 1987;7:961–963.
50. Matoba S, Kang JG, Patino WD, *et al*. p53 regulates mitochondrial respiration. Science. 2006;312(5780):1650–53.
51. Milner J, Medcalf EA. Cotranslation of activated mutant p53 with wild type drives the wild-type p53 protein into the mutant conformation. Cell. 1991;65(5):765–74.
52. Murphy M, Ahn J, Walker KK, *et al*. Transcriptional repression by wild-type p53 utilizes histone deacetylases, mediated by interaction with mSin3a. Genes Dev. 1999;13:2490–501.
53. Murray-Zmijewski F, Lane DP, *et al.* P53/p63/p73 isoforms: an orchestra of isoforms to harmonise cell differentiation and response to stress. Cell Death Differ. 2006;13:962–72.
54. Murray-Zmijewski F, Slee EA, Lu X. A complex barcode underlies the heterogeneous response of p53 to stress. Nat Rev Mol Cell Biol. 2008;9(9):702–12.
55. Nakano K, Vousden KH. PUMA, a novel proapoptotic gene, is induced by p53. Mol Cell. 2001;7(3):683–94.
56. Oda E, Ohki R, Murasawa H, Nemoto J, Shibue T, *et al*. Noxa, a BH3-only member of the Bcl-2 family and candidate mediator of p53-induced apoptosis. Science. 2000;288(5468):1053–58.
57. Ohayon T, Gershoni-Baruch R, Papa MZ, *et al.* The R72P P53 mutation is associated with familial breast cancer in Jewish women. Br J Cancer. 2005;92:1144–8.
58. Olive KP, Tuveson DA, Ruhe ZC, *et al*. Mutant p53 gain of function in two mouse models of Li-Fraumeni syndrome. Cell. 2004;119(6):847–60.
59. Olivier M, Goldgar DE, Sodha N, *et al*. Li-Fraumeni and related syndromes: correlation between tumor type, family structure, and *TP53* genotype. Cancer Res. 2003;63:6643–50.
60. Oren M & Levine AJ. Molecular cloning of a cDNA specific for the murine p53 cellular tumor antigen. PNAS, USA. 1983;80(1):56–9.
61. Petitjean A, Achatz MI, Borresen-Dale AL, *et al*. *TP53* mutations in human cancers: functional selection and impact on cancer prognosis and outcomes. Oncogene. 2007;26:2157–65.
62. Pim D, Banks L. p53 polymorphic variants at codon 72 exerts different effects on cell cycle progression. Int J Cancer 2004;108:196-9.
63. Pim D, Banks L. promoters by p53 mutants with impaired apoptotic function. Mol Cell Biol. 2004;9:4952–60.
64. Resnick MA & Inga A. Functional mutants of the sequence-specific transcription factor p53 and implications for master genes of diversity. Proc Natl AcadSci U S A. 2003;100(17):9934–39.
65. Sakamuro D, Sabbatini P, White E, Prendergast GC. The polyproline region of p53 is required to activate apoptosis but not growth arrest. Oncogene 1997;15:887–98.
66. Shen H, Solari A, Wang X, *et al*. P53 codon 72 polymorphism and risk of gastric cancer in a Chinese population. Oncol Rep. 2004;11:1115–20.
67. Sjalander A, Birgander R, Athlin L, *et al*. P53 germ line haplotypes associated with increased risk for colorectal cancer. Carcinogenesis. 1995;16:1461e4.
68. Sjalander A, Birgander R, Saha N, *et al.* p53 polymorphisms and haplotypes show distinct differences between major ethnic groups. Hum Hered. 1996;46:41–48.
69. Song H, Hollstein M, Xu Y. p53 gain-of-function cancer mutants induce genetic instability by inactivating ATM. Nat Cell Biol. 2007;9(5):573–80.
70. Sourvinos G, Kazanis I, Delakas D, *et al.* Genetic detection of bladder cancer by microsatellite analysis of p16, RB1 and p53 tumor suppressor genes. J. Urol. 2001;165:249e52.
71. Soussi T &Wiman KG. Shaping genetic alterations

in human cancer: the p53 mutation paradigm. Cancer Cell. 2007;12(4):303–12.

72. Storey A, Thomas M, Kalita A, *et al*. Role of a p53 polymorphism in the development of human papillomavirus-associated cancer. Nature 1998;393:229–234.
73. Taira N, Nihira K, Yamaguchi T, Miki Y. DYRK2 is targeted to the nucleus and controls p53 via Ser46 phosphorylation in the apoptotic response to DNA damage.Mol Cell. 2007;25:725–38.
74. Takahashi T, Nau MM, Chiba I, *et al*. p53: a frequent target for genetic abnormalities in lung cancer. Science. 1989;246(4929):491–94.
75. Tanaka H, Arakawa H, Yamaguchi T, *et al*. A ribonucleotide reductase gene involved in a p53-dependent cell-cycle checkpoint for DNA damage. Nature. 2000;404(6773):42–9.
76. Tang Y, Luo J, Zhang W, Gu W. Tip60-dependent acetylation of p53 modulates the decision between cell-cycle arrest and apoptosis. Mol Cell. 2006;24(6):827–39.
77. Teodoro JG, Parker AE, Zhu X, *et al.* p53-mediated inhibition of angiogenesis through up-regulation of a collagen prolyl hydroxylase. Science. 2006;313(5789):968–71.
78. Thomas M, Kalita A, Labrecque S, *et al.* Two polymorphic variants of wild-type p53 differ biochemically and biologically. Mol Cell Biol. 1999;19:1092–100.
79. Unger T, Mietz JA, Scheffner M, *et al.* Functional domains of wild-type and mutant p53 proteins involved in transcriptional regulation, transdominant inhibition, and transformation suppression. Mol Cell Biol. 1993;13(9):5186–94.
80. Vikhanskaya F, Siddique MM, Kei Lee M, *et al*. Evaluation of the combined effect of p53 codon 72 polymorphism and hotspot mutations in response to anticancer drugs. Clin Cancer Res 2005;11:4348–56.
81. Vogelstein B, Lane D, Levine AJ. Surfing the p53 network. Nature. 2000;408(6810):307–10.
82. Vousden KH & Lane DP. p53 in health and disease. Nat Rev Mol Cell Biol. 2007;8(4):275–83.
83. Willis A, Jung EJ, Wakefield T. Mutant p53 exerts a dominant negative effect by preventing wild-type p53 from binding to the promoter of its target genes. Oncogene. 2004;23:2330–38.
84. Wolf D, Harris N, Rotter V. Reconstitution of p53 expression in a nonproducer Ab-MuLV-transformed cell line by transfection of a functional p53 gene. Cell. 1984;38(1):119–26.
85. Wu X, Bayle JH, Olson D, Levine AJ. The p53-mdm-2 autoregulatory feedback loop. Genes Dev. 1993;7(7A):1126–32.
86. Xu Y, Yao L, Ouyang T, *et al*. p53 Codon 72 polymorphism predicts the pathologic response to neoadjuvant chemotherapy in patients with breast cancer. Clin Cancer Res. 2005;11:7328–7333.
87. Yin Y, Stephen CW, Luciani MG, *et al*. p53 Stability and activity is regulated by Mdm2-mediated induction of alternative p53 translation products. Nat Cell Biol. 2002;4:462–67.
88. Zakut-Houri R, Bienz-Tadmor B, Givol D, *et al*. Human p53 cellular tumor antigen: cDNA sequence and expression in COS cells. EMBO J. 1985;4:1251–55.
89. Zalcenstein A, Weisz L, Stambolsky P, *et al*. Repression of the MSP/MST-1 gene contributes to the antiapoptotic gain of function of mutant p53. Oncogene. 2006;25:359–69.
90. Zhang JH, Li Y, Wang R, *et al*. p53 gene polymorphism with susceptibility to esophageal cancer . Zhonghua Zhong Liu ZaZhi. 2003;25: 365–367.