

REVIEW

p53 : an overview of over two decades of study

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Abstract

p53 is the most commonly mutated gene in human cancers. It encodes a 53 kilodalton protein with several evolutionarily conserved domains viz sequence-specific DNA binding, tetramerisation, SH³ molecule binding, C-terminal and N-terminal. Existing in the cell at a very low level and in a relatively inactive form, p53 protein is increased and activated during periods of cellular stress. Unlike other proteins, the increase in protein level and its activation result from modification of the protein rather than genetic transcriptional or translational upregulation. Normally, Mdm2 protein interacts with p53 protein and effectively targets it for ubiquitin proteolysis within an autoregulatory feedback loop. Phosphorylation at the N-terminus reduces p53 interaction with Mdm2 with a resultant increase in p53 protein level. Modification at the C and N termini via phosphorylation or acetylation upregulates binding to specific DNA targets increasing transcription of these downstream genes. The net effect of p53 protein increase and activation lies in arrest of the cell in cycle which allows time for repair of the incurred damage or apoptosis or death of the cell. Failure of these normal protective and adaptive mechanisms caused by mutation of the p53 gene with product of an abnormal protein, loss of p53 protein through interaction with and degradation by HPV E6 protein or overexpressed Mdm2 etc. permits DNA-damaged cells to continue replicating. Left unchecked, this frequently contributes to tumorigenesis. Various methods have been devised to screen for mutations of the p53 gene, still the most common source of failed p53 mechanism. These include immunohistochemical detection of mutated proteins or identification of altered electrophoretic mobility of mutated p53 sequences. Sequencing of the gene nonetheless remains the most accurate method for determination of mutation. Major advances have been made in p53 research but the most meaningful probably lies in the promising results achieved in tumour therapy where introduction of wild type p53 gene has resulted in regression of non-small-cell lung cancer (NSCLC). Many other notable developments in this field include description of p53 homologues, "gain of function" mutants, p53 polymorphisms, angiogenesis-inhibiting properties of wild type p53 protein etc.

Key words: p53, research, mutations, screening, gene therapy, review

INTRODUCTION

The tumour suppressor gene, p53, is the most commonly mutated gene in human cancers. To date, over 10,000 mutations have been recorded and p53 mutations are present in about 40% of human cancers.^{1,2} Located on chromosome 17p13, the gene encodes a 53 kilodalton nucleophosphoprotein which serves as a transcription factor with a pivotal role in regulation of the cell cycle. First identified in 1979 when antibodies to SV40 large T antigen from animal tumours induced by SV40-transformed cells simultaneously immunoprecipitated the 53 kilodalton protein, p53 was initially thought to be another of an array of oncogene products.^{3,4} This assumption was

supported further by (1) finding of the protein in chemically induced tumours, transformed cells, primary human tumour cell lines and sera from cancer patients and (2) recognising its ability to immortalise cells and transform primary rat embryo fibroblasts in cooperation with ras.³⁻¹⁰ Molecular cloning of the p53 cDNA¹¹ paved the way for further studies. When rearrangements and functional inactivation of the gene were found frequently in Friend erythro-leukaemia virus induced mouse spleen tumours it came to be realised that p53 was tumour-suppressing rather than tumour inducing.¹²⁻¹⁶ It also dawned that the cloned p53 cDNA used in early experiments which led to transformation were

mutated forms of the gene.^{17,18} Since then, marked interest has been generated in view of the important tumour suppressing potential of p53 in clinical oncology. In-depth research into the field has resulted in a plethora of publications with p53 earning the distinction of being selected as "molecule of the year" by "Science" in 1993 (Science 1993 Dec 24; 262(5142):1953). Not meant to be comprehensive, this review aims to provide an overview of facts currently known of p53 achieved through two decades of research in this field.

p53 PROTEIN

Figure 1 is a schematic representation of p53 protein and its conserved domains. With an open reading frame of 393 amino acids, the p53 gene has 11 exons with several conserved domains.¹⁹⁻²¹ A sequence-specific DNA binding domain spans the centre at amino acid positions 100-300. Encoded by exons 5-8, this domain recognises and interacts with specific target double-stranded DNA sequences made up of two copies of 5'-PuPuPu-C(A/T)(T/A)GPyPyPy-3' separated by 0-13 nucleotides.^{22,23} This forms the core of the protein and is essential for its transactivating properties. Majority of the p53 mutations which occur in human cancers involve this region and results in conformational change or loss of critical residues involved in DNA contact leading to inability to specifically bind to DNA. Binding to DNA is optimised when four p53 molecules interact with target DNA. This tetramerisation of the p53 protein is controlled by the tetramerisation domain which is another conserved domain next to the sequence-specific DNA-binding domain.²⁴ The basic C-terminal most likely influences sequence-specific DNA binding.^{25,26} The acidic N-terminal helps in expression of target genes following sequence-specific DNA binding to target genes and is also important for maintaining the stability of the p53 molecule through its interaction with Mdm2 protein.^{19,27,28} Adjacent to the N-terminal, lies a region that binds signal transduction molecules carrying SH³.²⁹

FUNCTIONAL ASPECTS OF p53

Regulation of p53

Under normal circumstances, p53 protein is present in the cell at extremely low levels with the protein being relatively inactive and inefficient at binding target DNA.³⁰⁻³² This is mainly due to its interaction with Mdm2 protein. The Mdm2 oncogene was first amplified from a double minute chromosome in a derivative of tumourigenic mouse 3T3 cells.³³ The human homologue, Hdm2, has also been found amplified in 30-40% of human sarcomas.³⁴ Mdm2 protein interacts with p53 protein at its N-terminus and regulates p53 by at least two mechanisms. Firstly, the interaction of the two proteins inhibits the ability of p53 to activate transcription of downstream genes.^{35,36} More recently, it has also been shown that the short half-life of p53 in the unstressed cell is predominantly due to ubiquitin-dependent proteasome-mediated degradation of p53 on interaction with Mdm2.^{27,28,37-40} Thus, disruption of Mdm2/p53 interaction would counter the negative regulatory effect of Mdm2 on p53, allowing protein level to increase as well as making p53 more effective in transcribing downstream target genes. Interestingly, the promoter of the Mdm2 gene has a p53 binding site and is transcribed in a p53-dependent manner.⁴¹⁻⁴³ This normally provides for an autoregulatory feedback loop where activation of p53 upregulates Mdm2 expression.

Effects of stress on p53 protein

Various types of stress increase p53 protein levels in the cell and these include DNA damage, heat shock, hypoxia, hyperoxia, cytokines, growth factors, metabolic changes, oncogenes etc.¹⁹ Although some transcriptional and translational controls exist for p53 protein, they appear not to be important mechanisms for increasing protein levels or activating p53 during stress. In fact, p53 protein levels have been noted to increase in the presence of inhibitors of transcription and protein synthesis.^{44,45} In contrast, post-translational modification of

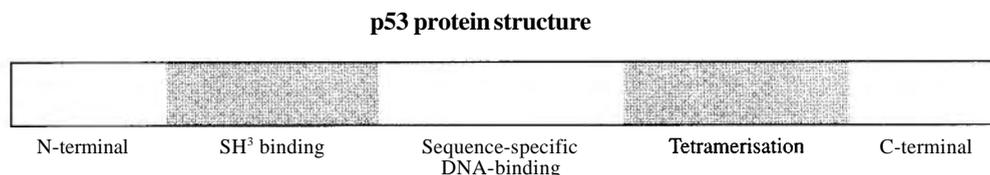


FIG. 1: Schematic representation of p53 protein structure with conserved domains indicated

the protein appears to be the most important mechanism for increasing protein level as well as transcriptional activity of p53 during stress. Modification of p53 protein commonly occurs via phosphorylation or acetylation although other mechanisms including O-glycosylation have also been shown to cause alteration of p53 protein. DNA-activated protein kinase (DNA-PK), protein kinase C (PKC), casein kinase II (CKII), ataxia telangiectasia gene product (ATM) are examples of protein kinases known to be involved in phosphorylation of p53.¹⁹ while histone acetylases, p300/CBP and PCAF appear to be responsible for acetylation of some p53 residues.^{46,47} Phosphorylation of p53 protein at the N-terminus leads to reduced interaction with Mdm2 protein hence decreasing Mdm2 targeted ubiquitin proteolysis with subsequent rise in p53 protein level.^{19,30-32} Several sites where phosphorylation can occur have been identified and these include serine 15 and 37.⁴⁸⁻⁵⁰ Nevertheless, so far there appears to be no individual site of phosphorylation responsible for stabilisation of p53 to all signals.⁵¹ Interestingly, apart from phosphorylation at the N-terminus of p53 protein, p53/Mdm2 interaction has recently been shown to be breached by another mechanism involving an alternate reading frame (ARF) encoded by a gene overlapping a negative regulator of pRb, namely p16INK4a.⁵² ARF binds Mdm2 and prevents its binding to p53 hence protecting p53 from proteolytic destruction.⁵³⁻⁵⁶ Phosphorylation at the C-terminus of p53 protein has been shown to activate p53 sequence-specific DNA binding.^{25,57-59} Acetylation which has also been demonstrated to occur at the N and C-terminals of p53 modifies the protein such that its sequence-specific DNA binding and influence on transcriptional activity of downstream genes are upregulated.^{47,60-62}

In general, p53 protein level and sequence-specific binding to target DNA increase with resultant increased transcriptional activity of downstream target genes during cellular stress. Typically, p53 induced response to cellular stress can be either in the form of arrest of growth or apoptosis of the cell. The reason for the opting of one response over another is still unclear but appears to be influenced by cell and tissue type as well as the type of insult.^{63,64} Notwithstanding, both responses can be seen as adaptive for the cell i.e. the cell is either allowed time for repair through a process of cell cycle arrest or undergoes ablation and dies when somehow the insult is viewed as irreparable. p53 arrests the damaged cells mainly at 2 cell cycle check-

points, G1 or G2.

G1 arrest

Under normal circumstances, exit from G1 and entry into the S phase requires phosphorylation of pRb (retinoblastoma protein) by G1-specific cyclin/cdk complexes. This releases E2F, a transcription factor which is bound to hypophosphorylated pRb and which is required for entry into the S phase.^{65,66} In the presence of damaged DNA there is an increase of wild type p53 which induces p21^{CIP1/WAF1}. p21^{CIP1/WAF1} inactivates the G1-specific cyclin/cdk complex, disallowing pRb to be phosphorylated. E2F is not released and progress to S phase is interrupted.⁶⁷

G2 arrest

Although earlier studies on p53 induced cell cycle arrest focussed mainly on G1 arrest, it has become increasingly evident that p53 protein can also lead to arrest at G2 of the cell cycle. The cyclin B1/Cdc2 complex appears to be the major regulatory factor required for entry into the M phase of the cell cycle.^{68,69} Overexpression of p53 is known to induce Gadd45 protein which inhibits activity of cyclin B1/Cdc2 complex. With this disruption of cyclin B1/Cdc2, cells are prevented from entry to the mitotic phase.⁷⁰⁻⁷³ p21^{CIP1/WAF1}, responsible for G1 arrest has also been implicated in disruption of cyclin B1/Cdc2 and may also be involved in G2 arrest.⁷⁴

Apoptosis

p53 induced genes associated with apoptosis of the damaged cell are being increasingly recognised but the best known so far is Bax. Under certain conditions of cellular damage p53 induces expression of Bax, a member of the Bcl-2 family and this leads to cell suicide.^{75,76} Besides Bax, other downstream genes activated by p53 and which are involved in the apoptotic process have been identified and these include PAG608 and Fas/Apo-1.^{77,78} It is still unclear how these various genes and their respective products act when a cell opts to undergo apoptosis directed by an increase in p53 protein.

p53 IN ONCOGENESIS

Under normal circumstances, cells respond to stress by increasing p53 level. This subsequently results in adaptive cum protective responses. The cell is thus provided increased time to repair damage to the cellular DNA or undergoes apoptosis if the damaged DNA is not to be repaired. It is thus conceivable that loss of normal p53

function is potentially harmful when cells with damaged DNA are left unchecked and permitted to continue replicating.

Loss of p53 function can arise from several causes but the most common is still that due to mutation in the p53 gene. Majority of the mutations lead to inactivation of the normal protective p53 function. Among the mutational changes, about 85% are missense and of these about 95% lie within the sequence-specific DNA binding domain i.e. exons 5-8.^{1,79} Deletions and insertions of the gene also occur but are less frequent. Besides mutations, infection by high-risk human papillomavirus (HPV) has a unique mechanism for inactivating p53 protein. E6 protein of high-risk HPV binds p53 protein and promotes degradation of p53 via the ubiquitin pathway.^{80,81} This leads to loss of wild type p53 protein and abrogation of normal p53 function. The amplification of Mdm2 gene in some human sarcomas with the consequent over-expression of Mdm2 protein naturally targets p53 protein for increased ubiquitination.⁴⁸ Currently another interesting mechanism has been described whereby there is functional inactivation of p53. Being a transcriptional factor, p53 protein's main activity takes place within the cell nucleus where it is normally located. However it has recently been shown that nearly 40% of inflammatory breast carcinomas and 95% undifferentiated neuroblastomas show an abnormal sequestration of p53 protein in the cellular cytoplasm.⁸²⁻⁸⁴ This physical translocation of the protein makes it impotent with regards to its normal function.

Most p53 mutations are sporadic and acquired in somatic cells. Nevertheless, in the Li-Fraumeni syndrome, a mutant p53 allele is inherited with cancers occurring on a second hit mutation.⁸⁵ With an approximately 25 times increased risk of developing malignancy patients with Li-Fraumeni syndrome often develop multiple tumours at a younger age. A multitude of cancers have been associated with inactivation of p53 and include sarcomas e.g. osteosarcoma, rhabdomyosarcoma, glioblastoma multiforme, anaplastic astrocytoma, malignant ependymoma, leukaemias, lymphomas and carcinomas of colorectal, breast, lung, liver, oesophageal, bladder, skin and cervical origin.

METHODS FOR DETECTION OF p53 ABNORMALITIES

Several methods have been devised for detection of p53 mutations. Among the simplest and

most easily applicable in a routine Histo-pathology Laboratory would be tests based on immunohistochemical methods. Immunohistochemistry provides a simple method for studying the protein product of the p53 gene in-situ. This method of detection is basically founded on the fact that wild type p53 protein normally has a short half-life, ranging up to 30 min, and does not accumulate to immunohistochemically detectable levels⁸⁶⁻⁸⁸ while mutant proteins have longer half-lives.⁸⁹ Immunohistochemical detection has provided a surrogate method for screening p53 genomic alterations in many tumours. Apart from technical shortcomings of any immunohistochemical method, mutations which cause deletion of the p53 gene, nonsense mutations, abnormalities of p53 protein due to high risk HPV E6 protein or Mdm2 degradation will give rise to false negative results. On the contrary, accumulation of wild-type p53 protein level during cellular damage, not due to mutations, are known to reach immunohistochemically detectable levels.

More precise screening methods are currently available for identifying p53 mutations based on altered electrophoretic mobilities of mutated DNA sequences. Nonetheless, techniques based on electrophoretic mobility changes e.g. single strand conformation polymorphisms (SSCP) analyses, denaturant gradient gel electrophoresis (DGGE), constant denaturant gel electrophoresis (CDGE) and temperature gradient gel electrophoresis (TGGE) etc.⁹⁰⁻⁹³ are still only about 90% accurate in determining mutations confirmed by DNA sequencing.⁷⁹ Technical artifacts and some germline polymorphisms can often lead to erroneous results.

DNA sequencing is finally required for the precise identification of mutations in the p53 gene. Yet, results from studies based on sequencing of the p53 gene must be interpreted in the light of the design of the study. Most are confined to analyses of exons 5-8, being the region affected by majority of mutations leading to human cancers. In Greenblatt *et al.*'s review of the results of 50 studies in which the entire coding region of the p53 gene was analysed, 87% of the 560 mutations reported occurred in exons 5-8, 8% occurred in exon 4 and 4% in exon 10. Mutations occurring in introns and promoter regions were also noted. Thus, the most common practice of study of exons 5-8 probably still underestimates mutations in the p53 gene by about 10-15%.⁷⁹

MAJOR IMPACTS ON CANCER MANAGEMENT

Among the various aspects of p53 advances, probably the most meaningful would lie in tumour therapy. The more promising so far includes the induction of tumour regression following injection of wild type p53 into human lung carcinoma." Foster *et al.* have recently identified multiple small molecules of 300-500 daltons molecular weight which stabilise the sequence-specific DNA-binding domain of both wild and mutant p53 protein. This enables mutant p53 protein to continue activating transcription and has been shown to slow tumour growth in mice.⁹⁵

RECENT DEVELOPMENTS

Research in the p53 area is continuing at a rapid pace and understanding of this very important cell-cycle regulator is increasing all the time with new discoveries. For example, once believed that mutations of the p53 gene mainly led to loss of function of p53 protein, some workers have recently shown that some mutations can give rise to new functions or commonly referred to as "gain of function mutants". One example is the recently described R175H mutant form of p53 which has been shown to have its own oncogenic potential and is not merely a mutant with a loss of ability to be tumour-suppressive.⁹⁶

Another new function of p53 in tumorigenesis seems to be emerging with the discovery that wild type p53 protein normally stimulates production of endogenous thrombospondin-1 (TSP-1), a potent inhibitor of angiogenesis. The switch to the malignant phenotype in cultured fibroblasts from patients with Li-Fraumeni syndrome seems to be accompanied by loss of wild-type p53 protein and downregulation of TSP-1.⁹⁷ Hence, it appears that in malignancy, loss of p53 function may be important for the switch to the angiogenic phenotype which encourages growth of the tumour.

Polymorphisms of the p53 gene have also been noted and some appear to be associated with enhanced risk of particular tumours. Recently, it was reported that arginine/proline polymorphism occurs at codon 72 of p53 with the arginine form appearing to be more prone to HPV E6 degradation than the proline form.⁹⁸⁻¹⁰⁰

Dosage effects of p53 protein in the production of tumours are also being investigated. Although it may be expected that obliteration of both alleles of p53 is necessary for development of cancers, this being in line with the Knudson's

two-hit phenomenon,¹⁰¹ it has been shown in mice that loss of one p53 allele can be associated with neoplastic change.¹⁰² This sublimates the theory that the level of p53 protein is important for cellular protection and loss of one p53 allele has a potentially increased risk of neoplastic transformation.

Homologues of p53 are also being described although it was originally thought that p53, unlike many other cell regulators, did not belong to a superfamily. Of the human p53 homologues, p63 and p73 have been better studied but their actual functions still require further clarification.^{103,104}

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