

Demystified . . .

p53

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p53 is a critical controller of normal growth and homeostasis of cells and tissues. It is a tumour suppressor gene, the inappropriate function of which can lead to disordered growth and malignancy. Normal p53 acts as a "guardian of the genome" by preventing the proliferation of cells with damaged DNA. It does this by the production of normal, or wild-type, p53 protein (a 53 kDa nuclear phosphoprotein), which acts on downstream genes to arrest the cell cycle until the DNA damage is repaired, or to cause apoptosis—programmed cell death. p53 appears to have a pivotal function in human carcinogenesis because the gene is mutated in more than 50% of human cancers. The loss of wild-type function usually occurs by a two step mechanism, which comprises mutation of one copy of the p53 gene and deletion/inactivation of the remaining wild-type allele. Mutation results in the synthesis of a protein with a changed conformation, a longer half life, and disordered function in terms of cellular growth. Basic molecular research into, and understanding of, the role of p53 might lead to improved methods of diagnosis and treatment.

According to the hypothesis of Nowell, cancer develops through the stepwise accumulation of genetic events that leads to genetic instability and the progressive loss of growth regulation.¹ Neoplastic progression is a complex process which, in particular tissues, involves a particular order of genetic events. Thus, clinically evident cancer is the endpoint of a multistep process. One initial mutation will give rise to a genetically homogeneous clone with a growth advantage, but further mutations then occur giving rise to subclones, which are genetically heterogeneous. Experimental models suggest that there are at least three steps in tumour formation: initiation, promotion, and progression. In tumorigenesis, proto-oncogenes can be activated to produce oncogenic activity, and tumour suppressor genes can be lost or inactivated to release control of cellular proliferation. Many studies have focused on early events in the carcinogenetic sequence, because they might be detected in preneoplastic tissues as markers of malignant progression, and could be possible targets for preventive measures, or even for gene therapy. p53 has proved an ever expanding focus for research (with only three studies listed by Med Line in 1989 but 1027 listed in 1997). Since 1989, about 570 different mutations of p53 have been identified, in more than 8000 human

cancers,² with the listing data base being updated twice a year. p53 was designated "molecule of the year" by the journal *Science* in 1993.

p53 in normal cells

In normal cells, there is a balance between growth promotion, cellular differentiation, and growth restraint. Apoptosis, or programmed cell death, is an additional, normal mechanism for control of cellular numbers. However, escape from apoptosis is not essential for tumour growth and progression because tumour cell accumulation will occur if excessive proliferation offsets cellular loss by apoptosis. This might explain why high rates of mitosis and apoptosis are often seen together in human tumours. The central control of proliferation is via control of the cell cycle; wild-type p53 protein (the product of the normal form of the tumour suppressor gene, p53) is concerned critically in this control and in the control of apoptosis. The term "p53" was originally given to the phosphoprotein of molecular weight 53 kDa produced by the p53 gene (comprising 11 exons within a chromosomal domain of 20 kb), which is located on the short arm of chromosome 17. The protein was discovered before the gene, but both the gene and its protein are called p53. p53 is not essential for normal growth, differentiation, and function because transgenic mice, which have both copies of p53 deleted, develop normally but develop multiple tumours after a few months of life (thus proving that p53 normally acts as a tumour suppressor).³

p53 has been termed "the guardian of the genome"⁴ because it enables DNA repair to be carried out after damage, thus reducing the chance that cells with oncogenic mutations will survive. Summarising briefly, the concentration of the wild-type p53 gene product rises rapidly in cells after DNA damage, such as that caused by UV light, ionising radiation, or chemotherapeutic agents, or following cellular stress, such as hypoxia. The wild-type p53 protein binds to p53 binding sites on DNA as a tetramer and stimulates the expression of downstream genes that negatively control growth and/or invasion. The rise in wild-type protein ultimately causes arrest of the cell cycle in the G1 (the first gap) phase and blockage of entry into the S (DNA synthesis) phase via p21 protein inhibition of cyclin dependent kinases (CDKs) and proliferating cell nuclear antigen (PCNA).⁵⁻⁷ This arrest allows time for DNA

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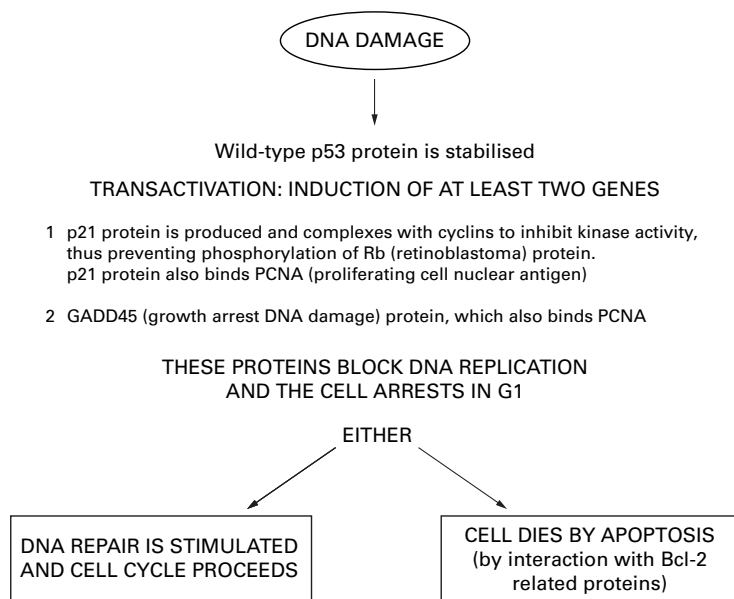


Figure 1 Cellular interactions of p53.

repair by interaction of p53 with downstream activators. If DNA arrest and repair fail, then wild-type p53 can trigger apoptosis.⁸ p53 acts as an extra defence mechanism by selectively destroying aberrant cells by promoting apoptosis. A large number of apoptotic regulating proteins are related to, or interact with, the apoptotic blocking protein, Bcl-2 (the gene was localised in B-cell follicular lymphomas).⁹ Deregulation of Bcl-2 will remove suppression of apoptosis and promote tumour growth. At least seven Bcl-2 related proteins are known (including Bax-1, which promotes apoptosis). These proteins form stable homodimers or heterodimers, which have various effects in promoting or blocking apoptosis. Bcl-2 blocks apoptosis by interaction with Bax-1; some viral proteins resemble Bcl-2 and might function by interacting with this system.

Knowledge of the details of the wild-type p53 response is becoming ever more complex. It is known to act as a cell cycle checkpoint and to induce apoptosis by downstream transactivation of a multiplicity of genes.¹⁰ A simplified summary of these cellular interactions is given in fig 1.

Cell cycle arrest and apoptosis represent distinct and independent responses to wild-type p53 activation.¹¹ Such modulation by the p21 protein (product of the WAF1 growth suppressor gene, also known as CIP1), the downstream effector of p53, is also controlled by p53 independent pathways.¹²⁻¹⁴ p53 is not always essential for apoptosis; there are both p53 dependent and p53 independent mechanisms.¹⁵

p53 in cancer

p53 is the most commonly mutated gene in human cancers, with probable involvement in the development of at least 50% of clinical tumours.¹⁶ Mutations of genes associated with the cell cycle, such as p53, lead to the loss of the guardianship of the genome, with progression of cells with damaged DNA through the cycle. p53 loss is believed to lead to widespread

karyotypic instability, resulting in aneuploidy, with multiple genetic amplifications and deletions such as loss of heterozygosity (LOH). Inactivation of p53 can occur through several mechanisms, including loss of p53 alleles, or by deletions, insertions, or point mutations. Most mutations in the DNA are missense base substitutions in the p53 coding sequence that change a single amino acid in the core domain, resulting in conformational change and stabilisation of the translated protein in the central region of the molecule.⁸ One aberrant allele can be sufficient to compromise tumour suppressor function and will thus be selected for.

p53 is unusual because dominant (activating) as well as passive (causing loss of function) mutations can contribute to cancer progression. Inactivation of the p53 gene can also occur because the transcribed protein is silenced by complex formation. This can be through interaction with viral products, such as the simian virus 40 (SV40) large T antigen, adenovirus E1b protein, or the E6 protein from the high risk types of the human papillomavirus (HPV),¹⁷ or by interactions with other cellular proteins, such as murine double minute 2 (MDM2).¹⁸ Inactivation of p53 leads to a reduction in WAF1 levels, phosphorylation of the retinoblastoma gene product (Rb) and progression from G1 through the S phase of the cell cycle.

Mutations in human cancers are often found in conjunction with the loss of the wild-type allele. Loss of normal activity of p53 is seen in both hereditary and sporadic cancers, in both of which there is frequent loss of chromosome 17p (and therefore presumably, of p53). Patients with the rare Li-Fraumeni syndrome inherit germline p53 mutations, with subsequent loss of the wild-type allele leading to multiple tumours (in adrenal cortex, brain, breast, and also sarcomas and leukaemias). A carrier has a 90% chance of developing cancer by the age of 70. It is a puzzle why common sporadic cancers that frequently have p53 mutations, such as colorectal cancer, do not appear with increased frequency in Li-Fraumeni families. In hereditary and somatic cancers, at least two genetic hits on p53 are needed for cancer to develop; loss of one allele occurs, with subsequent inactivation of the other. A missense mutation of one p53 allele is often accompanied by a deletion of the other allele (usually through mitotic recombination), resulting in the absence of any wild-type p53 tetramers. This occurs in many tumours, including those of bladder, brain, colon, liver, and lung. In most cervical cancers, the HPV protein, E6, binds and degrades p53, and in some soft tissue sarcomas, MDM2 amplification binds p53, but in other cervical carcinomas and soft tissue sarcomas, p53 mutations are involved in tumorigenesis.⁸ Mutated p53 is thought to produce a selective growth advantage, even in the presence of the wild-type p53 produced by the normal allele, thus allowing a reasonable chance for inactivation of the normal allele to occur. The mutant p53 gene might inactivate the wild-type gene product by binding to it and preventing its normal cellular

Region	AMINO TERMINAL	CORE DOMAIN	CARBOXYL TERMINAL
Structure	Contains many amino acids	Highly conserved between species. Majority of mutations found here in human tumours	Hydrophilic, with charged residues
Function	Transactivation	DNA binding Interaction with target sequences of genes	Modulation of DNA binding Oligomerisation (tetramer formation)

Figure 2 The p53 protein molecule.

interactions.¹⁹ Co-translation of mutant and wild-type proteins results in a change in conformation of the wild-type protein that mimics the conformation of the mutant and thus causes inactivation.²⁰

Wild-type p53 might also act to suppress angiogenesis, a critical step in the early growth of primary tumours. Mutant p53 cannot suppress angiogenesis in gliomas,²¹ and in cultured fibroblasts from patients with Li-Fraumeni syndrome, loss of p53 function results in decreased expression of thrombospondin 1 (which is a powerful inhibitor of angiogenesis)²²; both these situations can be reversed by wild-type p53.

Mutations of p53 occurring early in the neoplastic sequence are found in those tumours caused by chronic exposure to external carcinogens or inflammation, such as squamous cancers of the skin,²³ oral cancers,²⁴ and tumours of Barrett's oesophagus.²⁵ However, p53 loss can occur late in tumour progression; it is found at the late adenoma/carcinoma interface in sporadic colorectal tumorigenesis, after Ki-ras oncogene activation, and the loss of the deleted in colorectal cancer (DCC) tumour suppressor gene.²⁶

Molecular structure of p53

p53 activity is governed both by changes in the three dimensional conformation of the molecule and also by changes in the concentration of the protein. Normal p53 protein is termed "wild-type" and abnormal p53 is termed "mutated". p53 protein contains three domains, each of which has a distinct function (fig 2). The N-terminal region mainly controls transactivation²⁷ and the C-terminal region controls oligomerisation, the assembly of active tetramers. Mutations in this region can also lead to relocation of the protein from the nucleus, its primary site, to the cytoplasm. Within the C-terminal region is an additional regulatory region controlling the allosteric switch from a latent form of the protein to a form that is active for sequence specific binding.²⁸ The intervening region of the p53 molecule is highly conserved between species, and most mutations found in human tumours occur here. This is the region with DNA binding activity that interacts with target sequences of transcriptionally activated genes. Mutations in this region interfere with the three dimensional folding of the protein and therefore its interaction with DNA. p53 binds in a sequence specific manner via its central domain as a tetrameric protein.²⁹

PATTERNS OF MUTATION

The typical mutation is a missense mutation in the core region of p53, which will disrupt specific DNA binding and the transcriptional activation of downstream genes. Different cancer types have a different pattern of DNA base changes. These changes arise either spontaneously during DNA production or they can be caused by external carcinogens that affect DNA directly. Different carcinogens cause characteristic changes. In skin cancer, CC→TT base substitution is characteristic of UV damage to DNA.³⁰ In internal cancers (and in Li-Fraumeni families) DNA transition changes are more commonly spontaneous, with C→T mutations at 5-meCpG dinucleotides, involving amino group hydrolysis at 5-meC (5-methyl cytosine), producing thymidine.³¹ This results in the substitution of a C:G bp to a T:A bp—thus, there is a change in the genetic code. For example, oesophageal squamous carcinoma and adenocarcinoma have different profiles. The large predominance of transitions of CpG dinucleotides in oesophageal adenocarcinomas suggests a spontaneous, endogenous mechanism, whereas the high percentage of transversions (a change from one nucleotide to another (for example, from a G:C base pair to a T:A bp)) seen in oesophageal squamous cell carcinoma from high risk areas and in lung cancers suggests a direct effect of carcinogens present in tobacco.³²⁻³⁴ Geographically, differences in p53 tumour mutation patterns suggest the importance of different environmental factors in the epidemiology of a particular cancer. In high risk areas for hepatocellular cancer, the risk factors are known to be hepatitis B infection and dietary, mould derived aflatoxin. In these areas, there is one hot spot for a specific p53 gene mutation, but in low risk areas the site of mutation is variable.³³

Detection of alterations in p53

MOLECULAR DETECTION OF CHANGES IN THE p53 GENE

RNA or DNA can be extracted from frozen or formalin fixed, paraffin wax embedded tissues and p53 can be amplified with the polymerase chain reaction (PCR). Exons 5–8 (the conserved region of the p53 molecule) are most commonly screened for mutations. Non-isotopic single strand conformation polymorphism (SSCP) is a simple method for screening for mutations. It recognises sequence variations because of differences in the migration properties of single stranded DNA in non-denaturing

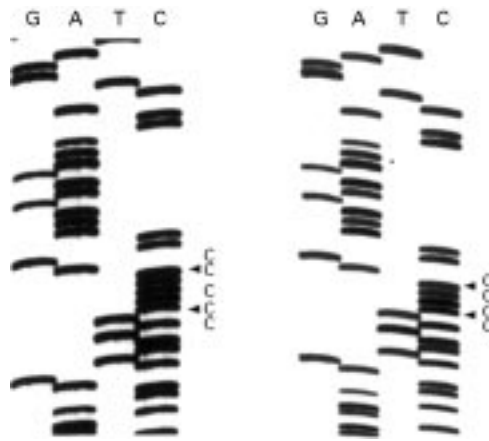


Figure 3 p53 sequence analysis of an oesophageal carcinoma showing the addition of a cytosine nucleotide (on the left), compared with normal tissue (photograph kindly supplied by Professor A G Casson).



Figure 4 A routinely processed section of squamous cell carcinoma of the oesophagus stained with the monoclonal antibody Ab-6 (clone DO-1) with no form of antigen retrieval. Ninety eight per cent of the tumour nuclei are stained positive (brown) for p53 protein.

polyacrylamide gels.³⁵ Mutations identified by SSCP can then be sequenced, using PCR amplified DNA strands recovered from the detecting gel (fig 3).

IMMUNOHISTOCHEMICAL DETECTION OF THE p53 PROTEIN

Wild-type p53 protein is virtually undetectable in normal cells because it has a half life of about 20 minutes. Mutant p53 has a relatively long half life (a single mutation characteristically increases its half life to ~ 12 hours³⁶ and, therefore, can more be detected more readily.³⁷ Positive immunoreactivity has been considered to equate with altered protein structure or function, as a result of mutation or viral oncogene involvement.^{38, 39} Several anti-p53 monoclonal antibodies (directed against different domains of the protein) are available commercially for use on frozen or archival material (fig 4). Monoclonal antibody 240 is believed to be mutant form specific,⁴⁰ but is only effective on frozen sections and is therefore unsuitable for archival studies. All other antibodies recognise both wild-type and mutant forms: detection relies on accumulation of either. If antigen retrieval is used (microwave or pressure cooker) both wild-type and mutant protein can be detected,⁴¹ or the threshold for detection may be lowered.⁴²

METHODOLOGICAL ADVANTAGES, DISADVANTAGES, AND DISCREPANCIES

Molecular techniques, such as SSCP, allow for quick screening (but immunohistochemistry is still quicker, more widely available, and cheaper), followed by precise identification of mutations. Such techniques might be inaccurate because a given sample of a tumour will also include a variety of other cell types, and there can be no localisation of p53 protein to cells or compartments of cells. Cell lines derived from tumours form a more homogeneous source of tumour cells but might acquire mutations not present in the original tumour.⁴³

The main advantage of immunohistochemical detection is that it is a simple, rapid technique that localises p53 protein directly in specific cells. The main disadvantage is non-specificity: an immunohistochemical positive result could be the result of an accumulation of the wild-type protein (accumulated for example after cytotoxic exposure to light,⁴⁴ inflammation, or hypoxia), particularly if there has been antigen retrieval, or of mutant-type protein. Nonsense point mutations that do not cause p53 protein stabilisation, frame shift mutations, and deletions that are usually associated with loss of p53 protein expression will not be detected: immunohistochemistry cannot detect loss. An immunohistochemically positive but molecularly negative tumour could be explained by the presence of a mutation outside the exons screened by molecular means or by non-mutant protein stabilisation detected immunohistochemically. Some studies show good correlation between immunohistochemical and molecular methods but some do not.⁴⁵ Standardised methodologies with improved sensitivity and specificity for the p53 protein are required but, at present, simultaneous use of molecular methods (such as PCR followed by SSCP and/or sequencing) and immunohistochemistry increases the number of p53 abnormalities detected and allows for spatial identification.

Clinical importance of p53

Translational research seeks to apply knowledge gained from the basic molecular sciences to clinically relevant problems. The clinical challenges are to prevent disease, to improve diagnosis, to improve on prognostication, and to introduce more effective treatments.

PREVENTION

The detection of different spectra of changes to p53 might allow for a better knowledge of the epidemiology of cancers and enable the institution of preventative measures. One obvious option is avoidance of smoking because of the mutagenic effects of cigarette smoke in various human cancers.⁴⁶

DIAGNOSIS

If p53 changes are found to occur early in the neoplastic sequence for a particular tumour, detection of p53 abnormalities might aid early detection (as in the preneoplastic Barrett's oesophagus).⁴⁷ p53 mutations have been detected in sputum samples taken up to 13

months before the emergence of a clinically evident lung cancer, and tumours resected subsequently had an identical p53 mutational profile to that detected previously in sputum cells.⁴⁸ PCR has been used to demonstrate rare p53 mutations in exfoliated cells in the urine of patients with bladder cancer several years before the clinical diagnosis, one famous case being that of Hubert Humphrey, the Vice President of the USA.⁴⁹ High risk HPV needs additional genetic alterations to induce cervical carcinogenesis (this might explain why not all carriers of high risk HPV types will go on to develop tumours). A recent study⁵⁰ has shown that the p53 gene can cause polymorphism at one amino acid residue on the protein, which can contain either proline or arginine. The arginine form is more susceptible to the HPV E6 protein than the proline form. Therefore, this polymorphism might be used for screening high risk HPV carriers who are at a greater risk for developing cancer. A percentage of people (44% of patients with head and neck cancer)⁵¹ can mount an immune response to mutant p53 and, in these, serum anti-p53 antibodies can be detected by enzyme linked immunosorbent assay. This technique might have a potential in the screening of asymptomatic patients in high risk groups.

DISEASE SPREAD

p53 mutations can be analysed in the primary tumour and used to detect cells with the same mutation in apparently clear resection margins and lymph nodes.⁵²

MONITORING OF DISEASE

In cases with anti-p53 serum antibodies, monitoring of serum concentrations could be used to check the response/resistance to treatment.⁵³

PROGNOSIS

p53 mutations are associated with poor prognosis in a variety of tumour types,⁵⁴ but the association is not consistent for all tumours. Discrepancies in results could arise owing to differing methods of detection of p53 changes: molecular analysis might be too restricted in terms of the exons examined, or immunohistochemistry might be inaccurate. However, prognosis might be dependent ultimately on other clinicopathological factors.

TREATMENT

Gene therapy and immunotherapy might be the setting for future advances in treatment but, presently, cancer treatment is based on surgery, irradiation, and chemotherapy. Knowledge of molecular factors leading to the response and resistance to the last two treatments is limited. p53 modulation might allow for manipulation of treatment in the future. Cytotoxic agents that kill by apoptosis perhaps require wild-type p53.⁵⁵ This could help to explain why several common carcinomas that harbour p53 mutations are relatively resistant to chemotherapy, and why treatment can select for the survival of aggressive clones, resistant to apoptotic agents.¹⁰ As a corollary, some tumours with a favourable prognosis,

such as testicular cancers, which respond readily to cisplatin, seldom contain p53 mutations.⁵⁶ p53 mutations might also influence resistance to cytotoxic drugs via the induction of the multidrug resistance gene (MDR1).⁵⁷ Effectiveness of treatment in the future could perhaps be increased by induction or introduction of wild-type p53 to the tumour, as has already been trialled in human non-small cell lung cancer.⁵⁸ The combination of p53 as a neoadjuvant or adjuvant with chemotherapy or radiation treatment might improve control of non-resectable or resistant tumours.

Innovatory treatments might include prevention of progression of preneoplastic lesions (such as Barrett's oesophagus) by the local introduction of a wild-type p53 vector. New therapeutic agents might be blocking chemicals for p53 oligomerisation or for the linkage of p53 to MDM2 or viral proteins, or agents that could induce a steric change from the mutant to the wild-type p53 protein configuration.

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Fourth European Forum on Quality Improvement in Health Care, and Fourth Swedish QUL Conference

A three day conference to be held in Stockholm, Sweden, Tuesday 25 May–Thursday 27 May 1999.

Aims of the Forum

- To provide education on how to improve health care
- To exchange sound, practical ideas on improving health care
- To provide a setting for deep discussion and shared learning among those charged with leading improvements in health care
- To build the scientific base of methods to improve health care
- To accelerate the improvements of health care
- To make change happen

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