# The p53 Tumor Suppressor Gene: From Molecular Biology to Clinical Investigation

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**ABSTRACT: The tumor suppressor p53 is a phosphoprotein barely detectable in the nucleus of normal cells. Upon cellular stress, particularly that induced by DNA damage, p53 can arrest cell cycle progression, thus allowing the DNA to be repaired; or it can lead to apoptosis. These functions are achieved, in part, by the transactivational properties of p53, which activate a series of genes involved in cell cycle regulation. In cancer cells bearing a mutant p53, this protein is no longer able to control cell proliferation, resulting in inefficient DNA repair and the emergence of genetically unstable cells. The most common changes of p53 in human cancers are point missense mutations within the coding sequences of the gene. Such mutations are found in all major histogenetic groups, including cancers of the colon (60%), stomach (60%), breast (20%), lung (70%), brain (40%), and esophagus (60%). It is estimated that p53 mutations are the most frequent genetic event in human cancers, accounting for more than 50% of cases. One of the most striking features of the inactive mutant p53 protein is its increased stability (half-life of several hours, compared to 20 min for wild-type p53) and its accumulation in the nucleus of neoplastic cells. Therefore, positive immunostaining is indicative of abnormalities of the p53 gene and its product. Several studies have shown that p53 mutations are associated with short survival in colorectal cancer, but the use of p53 as a tumoral marker is still a matter of debate.**

## THE p53 TUMOR SUPPRESSOR GENE

It is now clearly established that p53 belongs to the category of tumor suppressor genes. B. Vogelstein and J. Minna in 1989 were the first to report the presence of p53 mutations in colorectal and lung cancer cells.[1,2] These findings were subsequently confirmed by other groups, and there are currently more than 2,000 literature reports of p53 mutations in various types of human cancer.[3,4] p53 mutations are present in 40–45% of cancers, including all sites combined (FIG. 1). Indeed, p53 mutation is the most frequent genetic event demonstrated to date.[3,5] By way of comparison, ras gene mutations are found in only 10–20% of cancers. These mutations are usually accompanied by a loss of heterozygosity in the short arm of chromosome 17 (the gene encoding p53 is located on 17p13). In most cases, the mutations are point mu-

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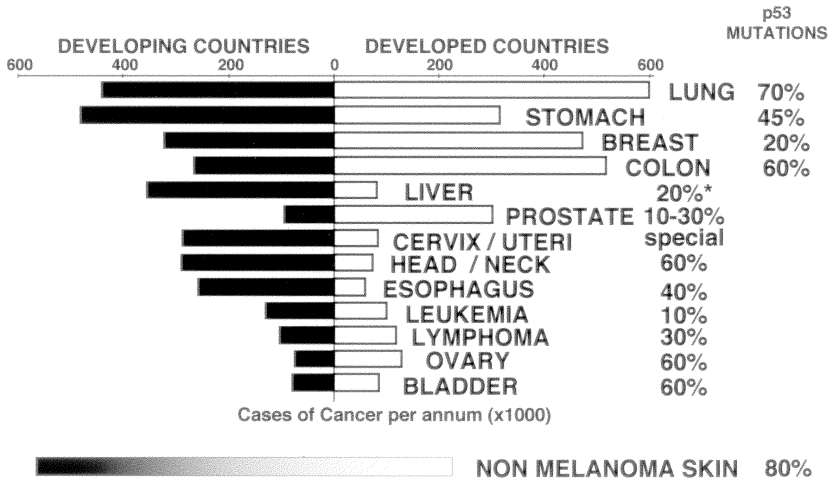


**FIGURE 1.** Worldwide distribution of cancers and p53 mutations: frequency of p53 mutation in human cancer. *The frequency of p53 mutations in liver cancer is higher in countries in which food is contaminated by aflatoxin B1. In cervix cancer, p53 is inactivated by the E6 protein expressed by human papilloma viruses.

tations located in four of the five evolutionary conserved domains (II to V) between amino acids 120 and 300.

These transforming mutations inactivate the negative regulatory function of p53 with respect to cell growth. However, in some cases the mutant gene also confers a dominant phenotype involved in maintenance and/or induction of transformation (see Ref. 6 for review).

The tumor suppressor function of p53 was confirmed by S. Friend *et al.*, who demonstrated the existence of somatic and germ line p53 mutations in families with Li-Fraumeni syndrome, in which affected members are genetically predisposed to cancer.[7] In all the families studied, there was a close correlation between transmission of the mutant allele and development of a cancer.

## ROLE OF p53 IN MAINTAINING GENOME STABILITY

Gamma irradiation of human or animal cells induces stabilization of the p53 protein *in vivo*.[8] Accumulation of p53 protein results in a transient arrest of the cell cycle in G1, just before DNA replication, or in G2, just before mitosis. It is generally agreed that this arrest in cell division in response to DNA damage enables the cell to activate enzymatic DNA repair systems to repair the lesions.[9] On the other hand, in cells expressing a p53 mutation, cell division is not arrested despite the damage to DNA.

In some cases, wild-type p53 instead induces apoptosis in cells subjected to DNA damage.[10] The reasons for this differential activity of wild-type p53 either to induce

apoptosis in some cell types or to arrest cell division in others remain unclear. Nonetheless, introduction of wild-type p53 in cells lacking a functional p53 gene restores growth arrest or induction of apoptosis in response to irradiation.

This phenomenon is limited to gamma irradiation and applies to all types of DNA lesions.[11] In fact, agents such as ethidium bromide or vincristine, which do not induce DNA strand breaks, are incapable of inducing G1 arrest; whereas mitomycin C or cisplatin, which directly damages the DNA, induces accumulation of p53 protein and cell cycle arrest. Thus, all physical and chemical agents capable of causing DNA damage can induce this p53-dependent physiological response.[12]

Wild-type p53, therefore, acts as a "stop light," inducing reversible cell cycle arrest to allow time for DNA repair.[13] If, for reasons still unclear, the cell is no longer capable of DNA repair, p53 would then be involved in elimination of the cell through induction of intracellular signaling pathways leading to programmed cell death, or apoptosis. Tumor cells harboring a mutant p53 gene can no longer maintain the integrity of the genome because the cell no longer receives the signal for cell cycle arrest. The result is that the cell is less stable and can give rise to clones with greater malignant potential.

More recently it was shown that p53 does more than protect cells against DNA damage. Other types of cellular disturbances, such as cellular oncogene activation,[14] hypoxia,[15] or abnormal cellular ribonucleotide concentrations,[16] also lead to p53 activation and cell cycle arrest. These different types of cellular stress activate a number of signaling pathways leading to p53 activation.[12] Furthermore, the discovery of two genes homologous to p53—p73 and p63—suggests that this signaling mechanism is undoubtedly much more complex than was believed just a few years ago.[17] The biological functions of these proteins are not yet known, but p73 has been shown to be activated by the c-Abl protein in response to gamma irradiation or cisplatin-induced DNA damage. [18–20]

## p53, APOPTOSIS, AND DRUG SENSITIVITY

It has been known since the early 1980s that certain antineoplastic agents, such as fluorouracil and ionizing radiation, act by inducing apoptosis in tumor cells. Lowe *et al*. showed that mouse cells expressing a p53 mutation were resistant to apoptosis induced by these agents, in contrast to cells expressing wild-type p53, which were sensitive to their action.[21] Many studies in human tumor cell lines have attempted to find a correlation between the presence of a p53 mutation in the tumor and sensitivity of the tumor to different genotoxic agents. All the published results tend to show that there is a relationship between p53 mutation and resistance to chemo- or radioinduced DNA damage.[22–26] Though this relationship is still controversial, some authors have postulated that the role of p53 in chemoresistance is tissue specific.[27,28]

These studies are of major importance. First, if the role of p53 mutations in chemosensitivity is clearly established, knowledge of the p53 status of a tumor might help orient the treatment strategy accordingly—for example, by indicating the choice of microtubular inhibitors over cisplatin or mitomycin C. Conversely, detection of mutations in the tubulin gene that induce resistance to taxanes may someday make it possible to select candidates for taxane therapy.[29] It might be thought that reintroduction of wild-type p53 into tumor cells or conversion of mutant p53 to the

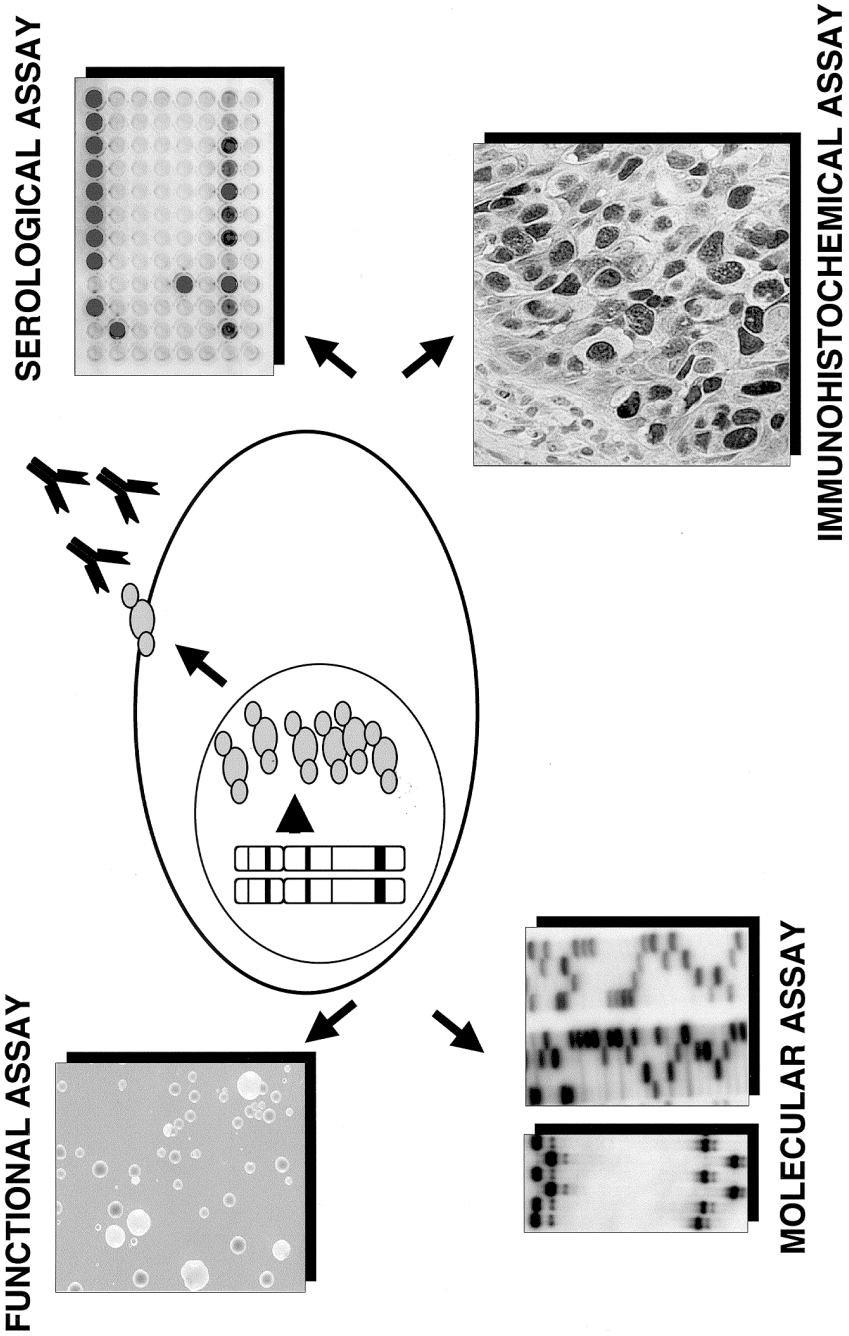


**FIGURE 2.** Multifactorial analysis of p53 alteration in human cancer (see text for details).

wild-type conformation might restore apoptosis in these cells. Indeed, this hypothesis was confirmed by Fujiwara *et al*., who showed that infection of a cisplatin-resistant tumor with a recombinant adenovirus expressing wild-type p53 restored tumor chemosensitivity, leading to the tumor's destruction by apoptosis.[30] Subsequent to this work, many studies on cell lines have shown that reintroduction of wild-type p53 to tumor cells expressing a mutant p53 restores sensitivity to agents as diverse as irradiation, cisplatin, and 5-FU.[31–33]

## ANALYSIS OF p53 MUTATIONS: HOW?

Four analytical methods may be used to investigate p53 gene status in human tumors:[34] (1) Molecular analysis can be used to identify the type of mutation in the p53 gene. (2) Immunohistochemical analysis can be used to demonstrate accumulation of mutant p53 protein in tumor cells. p53 mutations change the conformation of the protein, rendering it more stable: although wild-type p53 protein has a half-life of 15–20 minutes, most of the p53 mutants (regardless of the location of the mutation) have half-lives of 5–10 hours. Thus, inactive mutant p53 protein accumulates in tumor cell nuclei. (3) Serological analysis can be used to detect anti-p53 antibodies in the serum of patients. It has been shown that these antibodies result from autoimmunization following accumulation of p53 in tumor cells. Their presence is therefore an indirect result of p53 mutation. (4) Functional analysis can be used to measure the transactivating activity of p53 (FIG. 2).

## MOLECULAR ANALYSIS

Polymerase chain reaction (PCR) followed by sequence analysis can be used to directly determine the type of mutational event that altered the gene. More than 90% of the time these are point mutations that alter only a single nucleotide of the 23,000 comprising the gene. Unlike the *ras* oncogene, for which only 3 of 189 codons are targets of mutation, p53 mutations have been found in 90 of the 393 codons required for synthesis of the protein. This considerable heterogeneity makes diagnosis more difficult, because the region to be analyzed extends over virtually the entire gene. Many groups have focused their investigations on exons 5 to 8, which are the target of 80–90% of all mutations. We shall see below that this strategy might lead to erroneous prognosis, because it overlooks a considerable fraction of mutations.

Molecular analysis of the p53 gene is complex and poorly suited to routine diagnostic use. Nonetheless, for retrospective molecular epidemiology analyses, it remains the only means to accurately determine the type of mutation that altered the gene. Semidirect detection methods, such as SSCP (single-strand conformation polymorphism), which is the most frequently used because it is the least complex; or DGGE (denaturant gradient gel electrophoresis), more complex but also more sensitive, may be used to select a region of the genome for sequencing. These approaches allow rapid detection of mutations in DNA fragments but give no information on the precise location or type of the mutation in the absence of a second, sequencing step.

Several new molecular methods have been developed in recent years. In particular, DNA chips appear very promising for the detection of mutations. Affymetrix® has recently marketed a DNA chip that, in a single experiment, can test a DNA sample for all the known p53 mutations. Indeed, the advantage of these DNA chips is to be able to combine, in a precise and predetermined arrangement, a large number of oligonucleotides (50,000), corresponding to as many mutations, on a small area of approximately 1 $cm^2$. Specific hybridization of one of these nucleotides with the DNA sample is detected by fluorimetry and indicates the presence of the same mutation in the DNA sample.[35] Other methods, such as high-pressure chromatography using heteroduplex methods to detect mutations, are also highly sensitive and can be automated; they might therefore also be used in the near future for routine diagnostic testing. Semiindustrial-scale production is expected to lower the cost of these technologies, following the example of computer chips in the early 1980s.

## IMMUNOHISTOCHEMICAL ANALYSIS

An important feature of mutant p53 proteins is their longer half-life. This makes it possible to perform an immunocytochemical diagnosis (coupled with histological analysis) on tumor tissue to directly visualize the accumulation of inactive mutant p53 protein.[36] This approach has been used in many types of cancer, generally with good correlation between the molecular results (presence of mutation) and the immunohistochemical findings (accumulation of mutant protein). However, 100% agreement is never attained,[37,38] because 10–15% of p53 mutations consist of nonsense mutations or microdeletions that do not produce detectable levels of protein. Despite this drawback, this approach has the advantage that it can be used for routine testing in anatomopathology laboratories.

Several groups have produced new monoclonal antibodies directed against human p53.[39–41] These antibodies have been used for immunohistochemical diagnosis under a wide variety of conditions such as p53 detection in paraffin-embedded sections after formol fixation or Bouin staining.

## SEROLOGICAL ANALYSIS

Anti-p53 antibodies (p53-Ab) are present in 10% of breast cancer patients[42] and up to 20% of children with B lymphoma but are absent in patients with T cell lymphomas. This observation is closely correlated with the frequency of p53 mutations in hematologic malignancies, which is high for B cell types but nil for T cell malignancies.[43] These serological investigations have been extended in the past five years, revealing that anti-p53 antibodies are detected only in the sera of patients with cancer or precancerous lesions.[44] p53-Ab have been found in the sera of 20% of patients with colon cancer.[45–51] Several studies have found an association with the presence of these p53-Abs and poor prognosis.[45,51] Appearance of the anti-p53 Ab is linked to an immune response to p53 protein accumulation within the tumor, but the precise mechanisms governing this autoimmunization phenomenon have not yet been elucidated.[44] Only 30–50% of tumors harboring a p53 mutation give rise to the presence of anti-p53 Ab in serum.

p53 serological studies have certain advantages over other approaches: (1) tumor material is not required; (2) they give an overall idea of p53 mutations and expression and do not depend on tumor heterogeneity or necrosis; (3) they can be used as a marker during therapy;[52] (4) anti-p53 Ab are very stable, making it possible to perform retrospective analyses on frozen serum samples; and (5) they can be used for early diagnosis. Indeed, anti-p53 Ab have been detected in patients at high risk for developing cancer (smokers, Barrett's syndrome) several years before clinical expression of the tumor.[53–55] Yet because only a fraction of patients with p53 mutation produce anti-p53 Ab, serological analysis will not supplant molecular analysis, although it can serve as a valuable complementary approach.

## FUNCTIONAL ANALYSIS

All mutations in the p53 gene lead to loss of the transactivating function of the p53 protein. This observation led the groups of R. Iggo and S. Friend to design a diagnostic test based on this biological activity.[56,57] To this end, they developed marker yeast harboring a reporter gene under the control of p53 expression. Transfection of wild-type p53 into the yeast results in white colonies, whereas mutant p53 gives rise to red colonies. For diagnosis of mutations in tumor specimens, tumor mRNA is simply amplified by PCR, and the product is introduced into the yeast. The advantage of this approach is that it can test the entire p53 coding sequence.[58]

## p53 MUTATIONAL ANALYSIS

An analysis of all the point mutations in the p53 gene reveals that 51% of these mutations are G:C $\rightarrow$ A:T transitions, of which 59% are located at a CpG dinucleotide.[3,5] The cytosine in this dinucleotide is very frequently methylated in mammalian cells. Spontaneous deamination of a cytosine leads to formation of a U:G mismatch, which can be efficiently repaired by a uracil DNA glycosylase. On the other hand, deamination of a 5-methylcytosine leads to a T:G mismatch, the nonspecific repair of which can result in a C $\rightarrow$ T transition. Moreover, the spontaneous deamination rate has been shown to be greater for 5-methylcytosine than for cytosine. All of the 42 CpG dinucleotides in the p53 coding sequence are methylated, regardless of the tissue. In addition, the five hot spot codons, 175, 248, 273, 245, and 282, harbor a CpG dinucleotide. Over 90% of the mutational events in these codons are consistent with a deamination phenomenon, with the exception of smoking-related cancers. Analysis of somatic mutations in colorectal cancer shows that the CpG dinucleotide mutation rate is very high, suggesting that most of the p53 mutations in these cancers are due to endogenous processes linked to deamination of 5-methylcytosine. It will be of interest if the change of methylation pattern observed in sporadic colon cancer can be linked to p53 mutation either qualitatively or quantitatively. The incidence of colorectal carcinoma is rising in Asian urban societies. Hong Kong showed a much higher incidence of colorectal carcinoma among the young age groups. Yuen *et al*. defined the mutation spectrum of p53 and Ki-ras in 67 unselected cases by direct DNA sequencing.[59] Interestingly, insertion/deletion mutations in p53 from colorectal carcinoma in Hong Kong showed a significantly higher frequency (17.2%) than the Scot-

tish data (0%) and the world database (6.6%), although the overall frequency of p53 mutation (43%) in Hong Kong was similar to others. The high incidence of colorectal carcinoma in young people and the raised proportion of frameshift mutations in p53 suggest a different etiology for p53 mutation in Hong Kong.

Analysis of p53 mutations in lung cancer reveals that 30% are G:C → T:A transversions, in contrast to other cancers.[3,5] Furthermore, these mutations occur at a specific hot spot that is absent in other cancers (region A' identified in 1992, codons 150–160 with preference for codons 157 and 158). There are many lines of evidence indicating that the transversions found in the p53 gene are caused by tobacco carcinogens: (1) only lung cancer (and to a lesser extent head and neck and esophageal cancers, which are also smoking related) has such a high rate of G:C → T:A transversions;[60] (2) there is a linear relationship between the transversion rate and cigarette consumption;[61] (3) lung cancer patients who do not smoke do not have this high transversion rate;[62] (4) experimental treatment of human bronchial cells with benzo(a)pyrene specifically induces adducts in codon 157 of p53,[63] a guanine-rich region that is undoubtedly a hot spot for this type of adduct.[64] It is interesting that this high transversion rate has not been found in bladder cancer, which is also smoking related. On the other hand, bladder cancer is associated with a high rate of G:C → A:T transitions outside of CpG dinucleotides, suggesting that another tobacco carcinogen is involved.

In 1991, two reports showed that of all the mutations in the p53 gene in hepatocellular carcinoma there was a predominance of the GC-to-TA transversions at the third base of codon 249 (Arg to Ser) in patients from Mozambique and China.[65,66] Worldwide epidemiological studies showed that the mutation in codon 249 was strictly specific to countries in which food was contaminated by aflatoxin B1.[4,67] In Mozambique, for example, more than 50% of the mutations were found in codon 249, and aflatoxin B1 is a common contaminant. In Transkei, which borders on Mozambique and has a similar rate of chronic HBV infection but less aflatoxin B1 contamination, the mutation rate at codon 249 was less than 10 percent. A similar situation has been observed in various parts of China, which differ in their levels of aflatoxin exposure. In countries that do not consume contaminated food (including Europe and the USA), the rate of p53 mutations in hepatocellular carcinoma is low, and the mutations are scattered along the central part of p53, as they are for the other types of cancer. It has been demonstrated in human cells *in vitro* and *in vivo* that this phenomenon is due to the very high sensitivity of p53 codon 249 to the action of aflatoxin B1.[68,69] This observation, along with the fact that this 249 mutation is deleterious for p53 function, explains the existence of this mutational hot spot.

Skin tumors, including melanomas, basal cell carcinomas, and squamous cell carcinomas, are the most frequently encountered human cancers. Their number has increased dramatically in the last 10 years, especially for melanoma. Several lines of evidence indicate that the incidence of these skin tumors is linked to sun exposure. Their frequency increases with the amount of exposure or with decrease in latitude, and they are usually found on sun-exposed parts of the body. UV radiation–induced mutations have been studied in various animal models. The majority of the mutations are found to be located at dipyrimidine sites—that is, T-T, C-C, C-T, or T-C—and correspond to a C-to-T transition. More than 20% correspond to tandem mutations involving the two adjacent nucleotides of the dipyrimidine sites (C-C to T-T).

Analysis of several types of human skin cancer shows that there is a significant predominance of C-to-T transitions at dipyrimidine sites.[70,71] Although such events at dipyrimidine sites are found in 43% of cancers of other than the skin, the frequency reaches 95% in non-XP skin cancer and 100% in XP skin cancer. Furthermore, analysis of more than 11,000 p53 mutations in cancers of other than the skin shows that less than 1% are tandem mutations, whereas this number reaches 14% in non-XP skin cancer and 55% in XP skin cancer, which is typical of UV exposure.[72]

The field of molecular epidemiology has been developing at a remarkable speed. There is no longer any doubt that many human cancers are caused by mutations induced by environmental carcinogens. p53 mutational analysis is a striking example of this molecular archeology approach, which allows the origin of mutations to be defined. Other genes will also be used as probes for this type of analysis in the near future. A better understanding of the molecular signatures associated with carcinogen exposure should help advance these investigations.

## p53 AND GENETIC CHANGE IN COLORECTAL CANCER

The development of colorectal cancer is a multistep process involving a series of genetic changes in the colonic mucosa that lead sequentially to hyperplasia, adenoma, carcinoma, and metastasis.[73] Numerous genes, including protooncogenes, tumor suppressor genes, and DNA mismatch repair genes, have been implicated in the genesis of colon cancer. Furthermore, some evidence suggests that there are several pathways from normal cells to colorectal cancer. The archetypal pathway described in a majority of sporadic colorectal cancers involves alteration of the APC-β-catenin-Tcf pathway as an early event.[74] It is followed by mutation of K-ras, p53, Smad2, and Smad4 and changes in the methylation pattern. p53 mutation is a late event and can be found in 50–60% of sporadic colorectal carcinomas. This pathway is similar to that found in FAP patients with an inherited APC mutation. Tumors originating from this pathway are usually aneuploid, with a huge chromosome number instability. A different pathway was discovered in patients with HNPCC. In 1993, comparative analysis at microsatellite loci revealed in the DNA of some tumors the frequent presence of alleles that were not observed in the matched normal DNA. These new alleles are possibly generated as the result of errors occurring during replication (replication error, RER). The tumors that exhibit the highest frequency of RER at microsatellite loci, thus termed RER+, were shown to be impaired in the DNA mismatch repair pathway. Among the genes involved in this pathway, *MSH2*, *MLH1*, *PMS2*, *GTBP*/*MSH6*, *MSH3*, and perhaps *PMS1* and DNA polymerase are the sites of somatic mutations.[75] RER+ tumors account for 10–15% of all colorectal cancers. They arise preferentially from the proximal colon, a site where tumors rarely exhibit losses of 17p (p53), 18q (smad4), and 5q (APC). Mismatch repair deficiency may be an early event in tumorigenesis. This deficiency is expected in part to determine the subsequent genetic events associated with tumor progression. This high rate of mutation eventually alleviates the requirement of chromosomal loss in the biallelic inactivation of tumor suppressor genes. In support of the latter hypothesis are the observations that RER+ tumors or cell lines infrequently demonstrate allelic losses, exhibit normal or quasinormal karyotypes, and are the site of

biallelic frameshift mutations in the tumor growth factor receptor type II (TGF-RII) gene and in the *BAX* gene. The frequency of p53 mutation is lower in RER+ tumors than in RER– tumors.[76]

## CLINICAL VALUE OF p53 GENETIC CHANGES IN COLORECTAL CANCER

### *Methodological Difficulties*

There are a number of biases that can explain both the differences in the prevalence of p53 mutations reported in different studies and also the conflicting findings from prognostic studies. Molecular studies are fewer in number than immunohistochemical studies because the former are technically more difficult and costly. Semidirect detection methods have been used in most molecular studies. As the sensitivity of these methods is not greater than 90% in the case of SSCP, the problem of false negatives arises. Another bias of molecular analyses concerns the portion of the p53 gene analyzed. Most studies have focused only on exons 5–8. It is generally agreed that 10% of mutations can be overlooked by this approach, an approximation that was confirmed experimentally by several authors in an analysis of the entire p53 gene in various types of cancer.[38,51,77,78] Finally, many studies do not take into account the heterogeneity of the tumor samples, extracting the DNA from a tumor homogenate without separating out stromal cells from actual tumor cells. This may be a critical factor in tumors, which often have a large stromal component. If a mixture of tumor and stromal DNA is amplified by PCR, the mutational signal may be diluted, giving rise to false negatives.

As far as immunohistochemical analysis is concerned, there are even more biases. So far there is no consensus as to the threshold of positivity, which has ranged from 1 to 20% of labeled tumor cell nuclei in different studies. The starting material also influences the results. The degree of immunohistochemical labeling shows large variations, depending on whether the material is frozen or paraffin embedded, and according to the method of fixation.[79,80] A recent study showed that antigens can degrade if the slides are stored for too long, giving rise to false negatives.[81] The choice of antibody can also affect the results.[39] Several monoclonal antibodies are commercially available, but they do not all have the same affinity for p53 and do not all recognize the same p53 epitopes. The monoclonal antibodies against the amino terminus of p53, such as DO1 or DO7, appear to give the most reproducible results. Methods such as microwaves to demask antigenic epitopes are also highly controversial,[82–85] because it appears that they enhance sensitivity at the price of specificity.

Moreover, there are highly contradictory data suggesting that some monoclonal antibodies are specific for wild-type or mutant p53. This information has been used in a highly ambiguous manner by the companies that distribute these products. In fact, the truth lies elsewhere. These antibodies recognize only certain p53 mutants, and only when the p53 protein is in native conformation. In immunohistochemical analysis, the p53 protein in a fixed tissue section is usually denatured (*a fortiori* if epitope demasking is used) and will be recognized by all antibodies. Similarly, the wild type–specific monoclonal antibody Pab1620 should absolutely not be used in

immunohistochemical studies because it has specificity only for the native, nondenatured protein.

## CLINICAL STUDIES

It has been reported that p53 mutations are associated with poor prognosis in colorectal cancer.[86–89] However, conflicting results have also been claimed.[90] In some studies, p53 protein accumulation has been shown to correlate with patient survival,[91–94] a finding that has not been observed in other studies.[89,95–99] In a recent work, Kressner *et al*. compared a complementary DNA-based sequencing method and an immunohistochemical (IHC) method for detecting p53 protein accumulation in colorectal cancer. The entire coding region was sequenced in 191 frozen tumors. Seventeen (15%) of the 107 mutations were located outside exon 5–8, which is the region usually routinely screened. Furthermore, 22 genetic changes (20%) were either deletions, insertions, or stop. Such genetic alteration cannot be detected by immunohistochemical analysis. Indeed, in this study the concordance in the results of the cDNA sequencing and the IHC was 74 percent. There was a significant relationship between the p53 mutational status and the cancer-specific survival time, with shorter survival time for patients who had p53 mutations than for those who did not. Multivariate analysis showed that the presence of p53 mutations was an independent prognostic factor.

As stated above, the majority of p53 gene mutations are missense mutations, often within the conserved DNA binding core domain of the protein. The primary selective advantage of such mutation may well be the elimination of cellular wild-type p53 activity. However, at variance with the observations for several other tumor suppressor genes, cells with p53 mutations typically maintain expression of stable full-length mutant p53. It has been suggested that certain mutant forms of p53 may posses a gain of function, whereby they contribute positively to cancer progression. Because p53 mutants can also exert negative dominant effects on wild-type p53, formal evidence for gain of function has been difficult to test. Furthermore, this situation is more complex, because it has been demonstrated that mutant p53 are heterogeneous. Missense point mutations involve either (1) DNA contact residues, (residues 248 and 273) or (2) residues important for conformational structure (residue 175). Extensive analyses of these mutants have led to the discovery that they have heterogeneous behavior concerning their implication in the regulation of cell cycle growth or apoptosis.[100–104] These observations have prompted several authors to check whether such heterogeneity could be linked to clinical parameters.

In colon cancer, Goh *et al*. found that patients with tumors harboring mutated p53 genes showed a significantly poorer prognosis than did those patients with genes without point mutations and, moreover, that patient response to postoperative therapies depended significantly on mutation status in both adjuvant and palliative treatment cohorts.[105] However, not all point mutations were the same functionally; point mutations within the conserved domains of the p53 tumor suppressor gene were inherently more aggressive than tumors with point mutations outside of these domains, and mutations of codon 175 were particularly aggressive. Borresen Dale analyzed a series of 222 colorectal carcinomas.[106] Mutations were found in 102 cases (45.9%). Mutations were found more frequently in rectal tumors versus other locations and in

aneuploid compared to diploid tumors. Presence of a p53 mutation was also significantly associated with absence of microsatellite instability, as well as with loss of heterozygosity at 17p13. The p53 mutations in the left-sided and rectal tumors were more often transversions than transitions, indicating a different etiology in the development of these tumors. The tendency for shorter cancer-related survival among patients with mutations in their tumors was only statistically significant for patients with left-sided tumors. All patients with mutations affecting the L3 domain of the protein involved in zinc binding had significantly shorter cancer-related survival, indicating that mutations affecting this domain have biological relevance in terms of colorectal cancer disease course. Kressner *et al*. found that mutations outside the evolutionarily conserved regions were associated with the worst prognosis.[107] Such a finding is in contrast to those of Goh *et al*.[105] and Borresen Dale *et al*.[106]

All these results suggest that knowledge of a patient's TP53 status, with respect to both the presence and the localization of the mutation, may be important in prognosis evaluation, particularly when selecting patients for more aggressive postoperative therapeutic intervention.

## p53 IN THE CLINICAL SETTING—WHAT DOES THE FUTURE HOLD?

In addition to our knowledge of the p53 status of the patient and its clinical consequences, all these studies on p53 provide considerable information and material concerning p53 function. It is clear at present that not all of these mutations are equivalent in terms of biological activity. It is now necessary to perform more basic research work to elucidate such p53 mutant activity and its relationship to the transformed phenotype. All these efforts highlight one of the most exciting aspects of p53 studies—that is, the constant exchange between basic research and clinical studies. It is expected that this knowledge will be of future benefit to the patient by enabling earlier, more precise diagnosis and by generating new therapeutic approaches.

## ACKNOWLEDGMENTS

Our work was supported by grants from the Association de Recherche sur le Cancer, Ligue Nationale contre le Cancer (comité de Paris), Ligue Nationale contre le Cancer, the Mutuelle Générale de l'Éducation Nationale (MGEN), the Direction de la Recherche Clinique (DRC) de l'Assistance Publique-Hôpitaux de Paris, and GEFFLUC.

## REFERENCES

1. NIGRO, J.M., S.J. BAKER, A.C. PREISINGER *et al*. 1989. Mutations in the p53 gene occur in diverse human tumour types. Nature **342:** 705–708.
2. TAKAHASHI, T., M.M. NAU, I. CHIBA *et al.* 1989. p53—a frequent target for genetic abnormalities in lung cancer. Science **246:** 491–494.
3. BÉROUD, C. & T. SOUSSI. 1998. p53 gene mutation: software and database. Nucleic Acids Res. **26:** 200–204.

4. GREENBLATT, M.S., W.P. BENNETT, M. HOLLSTEIN & C.C. HARRIS. 1994. Mutations in the p53 tumor suppressor gene: clues to cancer etiology and molecular pathogenesis. Cancer Res. **54:** 4855–4878.
5. SOUSSI, T., K. DEHOUCHE & C. BÉROUD. 2000. Analysis of p53 mutation in human cancer: the link between epidemiology and carcinogenesis. Hum. Mut. In press.
6. LEVINE, A.J. 1997. p53, the cellular gatekeeper for growth and division. Cell **88:** 323–331.
7. MALKIN, D., F.P. LI, L.C. STRONG *et al.* 1990. Germ line p53 mutations in a familial syndrome of breast cancer, sarcomas, and other neoplasms. Science **250:** 1233–1238.
8. KASTAN, M.B., O. ONYEKWERE, D. SIDRANSKY, B. VOGELSTEIN & R.W. CRAIG. 1991. Participation of p53 protein in the cellular response to DNA damage. Cancer Res. **51:** 6304–6311.
9. KUERBITZ, S.J., B.S. PLUNKETT, W.V. WALSH, M.B. KASTAN. 1992. Wild-type p53 is a cell cycle checkpoint determinant following irradiation. Proc. Natl. Acad. Sci. USA **89:** 7491–7495.
10. YONISH-ROUACH, E., D. RESNITZKY, J. LOTEM, L. SACHS, A. KIMCHI & M. OREN. 1991. Wild-type p53 induces apoptosis of myeloid leukaemic cells that is inhibited by interleukin-6. Nature **352:** 345–347.
11. FRITSCHE, M., C. HAESSLER & G. BRANDNER. 1993. Induction of nuclear accumulation of the tumor-suppressor protein p53 by DNA-damaging agents. Oncogene **8:** 307–318.
12. GIACCIA, A.J. & M.B. KASTAN. 1998. The complexity of p53 modulation: emerging patterns from divergent signals. Genes Dev. **12:** 2973–2983.
13. LANE, D. 1992. p53, guardian of the genome. Nature **358:** 15–16.
14. BATES, S., A.C. PHILLIPS, P.A. CLARK *et al.* 1998. p14(ARF) links the tumour suppressors RB and p53. Nature **395:** 124–125.
15. GRAEBER, T.G., C. OSMANIAN, T. JACKS *et al*. 1996. Hypoxia-mediated selection of cells with diminished apoptotic potential in solid tumours. Nature **379:** 88–91.
16. LINKE, S.P., K.C. CLARKIN, A. DILEONARDO, A. TSOU & G.M. WAHL. 1996. A reversible, p53-dependent G(0)/G(1) cell cycle arrest induced by ribonucleotide depletion in the absence of detectable DNA damage. Gene Dev. **10:** 934–947.
17. KAELIN, W.G. 1999. The emerging p53 gene family. J. Nat. Cancer Inst. **91:** 594–598.
18. AGAMI, R., G. BLANDINO, M. OREN & Y. SHAUL. 1999. The tyrosine kinase c-Abl regulates p73 in apoptotic response to cisplatin-induced DNA damage. Nature **399:** 806–809.
19. JOST, C.A., M.C. MARIN & W.G. KAELIN. 1999. p73 is regulated by tyrosine kinase c-Abl in the apoptotic response to DNA damage. Nature **399:** 814–817.
20. YUAN, Z.M., H. SHIOYA, T. ISHIKO *et al.* 1999. Interaction of c-Abl and p73 alpha and their collaboration to induce apoptosis. Nature **399:** 809–813.
21. LOWE, S.W., H.E. RULEY, T. JACKS & D.E. HOUSMAN. 1993. p53-dependent apoptosis modulates the cytotoxicity of anticancer agents. Cell **74:** 957–967.
22. FAN, S.J., W.S. EL-DEIRY, I. BAE *et al*. 1994. p53 gene mutations are associated with decreased sensitivity of human lymphoma cells to DNA damaging agents. Cancer Res. **54:** 5824–5830.
23. ELIOPOULOS, A.G., D.J. KERR, J. HEROD *et al*. 1995. The control of apoptosis and drug resistance in ovarian cancer: influence of p53 and bcl-2. Oncogene **11:** 1217–1228.
24. XIA, F., X. WANG, Y.H. WANG *et al*. 1995. Altered p53 status correlates with differences in sensitivity to radiation-induced mutation and apoptosis in two closely related human lymphoblast lines. Cancer Res. **55:** 12–15.
25. FAN, S.J., M.L. SMITH, D.J. RIVET *et al.* 1995. Disruption of p53 function sensitizes breast cancer MCF-7 cells to cisplatin and pentoxifylline. Cancer Res. **55:** 1649–1654.
26. O'CONNOR, P.M., J. JACKMAN, D. JONDLE, K. BHATIA, I. MAGRATH & K.W. KOHN. 1993. Role of the p53 tumor suppressor gene in cell cycle arrest and radiosensitivity of Burkitts lymphoma cell lines. Cancer Res. **53:** 4776–4780.
27. WU, G.S. & W.S. EL-DEIRY. 1996. Apoptotic death of tumor cells correlates with chemosensitivity, independent of p53 or Bcl-2. Clin. Cancer Res. **2:** 623–633.
28. WU, G.S. & W.S. EL-DEIRY. 1996. p53 and chemosensitivity. Nature Med. **2:** 255–256.
29. MONZO, M., R. ROSELL, J.J. SANCHEZ *et al*. 1999. Paclitaxel resistance in non-small-cell lung cancer associated with beta-tubulin gene mutation. J. Clin. Oncol. **17:** 1786–1793.

30. FUJIWARA, T., E.A. GRIMM, T. MUKHOPADHYAY, W.W. ZHANG, L.B. OWENSCHAUB & J.A. ROTH. 1994. Induction of chemosensitivity in human lung cancer cells *in vivo* by adenovirus-mediated transfer of the wild-type p53 gene. Cancer Res. **54:** 2287–2291.
31. SPITZ, F.R., D. NGUYEN, J.M. SKIBBER, R.E. MEYN, R.J. CRISTIANO & J.A. ROTH. 1996. Adenoviral-mediated wild-type p53 gene expression sensitizes colorectal cancer cells to ionizing radiation. Clin. Cancer Res. **2:** 1665–1671.
32. YANG, B., J.R. ESHLEMAN, N.A. BERGER & S.D. MARKOWITZ. 1996. Wild-type p53 protein potentiates cytotoxicity of therapeutic agents in human colon cancer cells. Clin. Cancer Res. **2:** 1649–1657.
33. BLAGOSKLONNY, M.V. & W.S. EL-DEIRY. 1996. *In vitro* evaluation of a p53-expressing adenovirus as an anti-cancer drug. Int. J. Cancer **67:** 386–392.
34. SOUSSI, T., Y. LEGROS, R. LUBIN, K. ORY & B. SCHLICHTHOLZ. 1994. Multifactorial analysis of p53 alteration in human cancer—a review. Int. J. Cancer **57:** 1–9.
35. AHRENDT, S.A., S. HALACHMI, J.T. CHOW *et al*. 1999. Rapid p53 sequence analysis in primary lung cancer using an oligonucleotide probe array. Proc. Natl. Acad. Sci. USA **96:** 7382–7387.
36. DOWELL, S.P., P.O.G. WILSON, N.W. DERIAS, D.P. LANE & P.A. HALL. 1994. Clinical utility of the immunocytochemical detection of p53 protein in cytological specimens. Cancer Res. **54:** 2914–2918.
37. TOP, B., W.J. MOOI, S.G. KLAVER *et al*. 1995. Comparative analysis of p53 gene mutations and protein accumulation in human non-small-cell lung cancer. Int. J. Cancer **64:** 83–91.
38. CASEY, G., M.E. LOPEZ, J.C. RAMOS *et al.* 1996. DNA sequence analysis of exons 2 through 11 and immunohistochemical staining are required to detect all known p53 alterations in human malignancies. Oncogene **13:** 1971–1981.
39. TENAUD, C., A. NEGOESCU, F. LABATMOLEUR, Y. LEGROS, T. SOUSSI & E. BRAMBILLA. 1994. Methods in pathology—p53 immunolabeling in archival paraffin-embedded tissues: optimal protocol based on microwave heating for eight antibodies on lung carcinomas. Mod. Pathol. **7:** 853–859.
40. LEGROS, Y., C. LAFON & T. SOUSSI. 1994. Linear antigenic sites defined by the B-cell response to human p53 are localized predominantly in the amino and carboxy-termini of the protein. Oncogene **9:** 2071–2076.
41. VOJTESEK, B., J. BARTEK, C.A. MIDGLEY & D.P. LANE. 1992. An immunochemical analysis of the human nuclear phosphoprotein-p53—new monoclonal antibodies and epitope mapping using recombinant-p53. J. Immunol. Methods **151:** 237–244.
42. CRAWFORD, L.V., D.C. PIM & R.D. BULBROOK. 1982. Detection of antibodies against the cellular protein p53 in sera from patients with breast cancer. Int. J. Cancer **30:** 403–408.
43. CARON DE FROMENTEL, C., F. MAY-LEVIN, H. MOURIESSE, J. LEMERLE, K. CHANDRASEKARAN & P. MAY. 1987. Presence of circulating antibodies against cellular protein p53 in a notable proportion of children with B-cell lymphoma. Int. J. Cancer **39:** 185–189.
44. SOUSSI, T. 1996. The humoral response to the tumor-suppressor gene-product p53 in human cancer: implications for diagnosis and therapy. Immunol. Today **17:** 354–356.
45. HOUBIERS, J.G.A., S.H. VANDERBURG, L.M.G. VANDEWATERING *et al*. 1995. Antibodies against p53 are associated with poor prognosis of colorectal cancer. Br. J. Cancer **72:** 637–641.
46. ANGELOPOULOU, K., M. STRATIS & E.P. DIAMANDIS. 1997. Humoral immune response against p53 protein in patients with colorectal carcinoma. Int. J. Cancer **70:** 46–51.
47. HAMMEL, P., B. BOISSIER, M.T. CHAUMETTE *et al*. 1997. Detection and monitoring of serum p53 antibodies in patients with colorectal cancer. Gut **40:** 356–361.
48. SHIBATA, Y., H. KOTANOGI, H. ANDOH, K. KOYAMA, H. ITOH & S. KUDO. 1996. Detection of circulating anti-p53 antibodies in patients with colorectal carcinoma and the antibody's relation to clinical factors. Dis. Colon Rectum **39:** 1269–1274.
49. STHOEGER, Z., E. EVRON, S. GOLAND *et al*. 1997. Anti-p53 antibodies in colon cancer patients. Ann. N.Y. Acad. Sci. **815:** 496–498.
50. POLGE, A., J.F. BOURGAUX, E. BANCEL *et al*. 1998. p53 and follow-up of colorectal adenocarcinomas. Dig. Dis. Sci. **43:** 1434–1442.

51. KRESSNER, U., B. GLIMELIUS, R. BERGSTROM, L. PAHLMAN, A. LARSSON & G. LINDMARK. 1998. Increased serum p53 antibody levels indicate poor prognosis in patients with colorectal cancer. Br. J. Cancer **77:** 1848–1851.
52. ZALCMAN, G., B. SCHLICHTHOLZ, J. TRÉDANIEL *et al*. 1998. Monitoring of p53 auto antibodies in lung cancer during therapy: relationship to response to treatment. Clin. Cancer Res. **4:** 1359–1366.
53. LUBIN, R., G. ZALCMAN, L. BOUCHET *et al*. 1995. Serum p53 antibodies as early markers of lung cancer. Nature Med. **1:** 701–702.
54. TRIVERS, G.E., V.M.G. DE BENEDETTI, H.L. CAWLEY *et al*. 1996. Anti-p53 antibodies in sera from patients with chronic obstructive pulmonary disease can predate a diagnosis of cancer. Clin. Cancer Res. **2:** 1767–1775.
55. CAWLEY, H.M., S.J. MELTZER, V.M. DE BENEDETTI *et al*. 1998. Anti-p53 antibodies in patients with Barrett's esophagus or esophageal carcinoma can predate cancer diagnosis. Gastroenterology **115:** 19–27.
56. ISHIOKA, C., T. FREBOURG, Y. YAN *et al*. 1993. Screening patients for heterozygotous p53 mutations using a functional assay in yeast. Nature Genet. **5:** 124–129.
57. FLAMAN, J.M., T. FREBOURG, V. MOREAU *et al*. 1995. A simple p53 functional assay for screening cell lines, blood, and tumors. Proc. Natl. Acad. Sci. USA **92:** 3963–3967.
58. FUJIMOTO, H., J. SASAKI, M. MATSUMOTO *et al*. 1998. Significant correlation of nitric oxide synthase activity and p53 gene mutation in stage I lung adenocarcinoma. Jpn. J. Cancer Res. **89:** 696–702.
59. YUEN, S.T., L.P. CHUNG, S.Y. LEUNG *et al*. 1997. Colorectal carcinoma in Hong Kong: epidemiology and genetic mutations. Br. J. Cancer **76:** 1610–1616.
60. SOUSSI, T. 1996. The p53 tumour suppressor gene: a model for molecular epidemiology of human cancer. Mol. Med. Today **2:** 32–37.
61. SUZUKI, H., T. TAKAHASHI, T. KUROISHI *et al*. 1992. p53 mutations in non-small-cell lung cancer in Japan—association between mutations and smoking. Cancer Res. **52:** 734–736.
62. TAKAGI, Y., H. OSADA, T. KUROISHI *et al*. 1998. p53 mutations in non-small-cell lung cancers occurring in individuals without a past history of active smoking. Br. J. Cancer **77:** 1568–1572.
63. DENISSENKO, M.F., A. PAO, M.S. TANG & G.P. PFEIFER. 1996. Preferential formation of benzo[a]pyrene adducts at lung cancer mutational hotspots in P53. Science **274:** 430–432.
64. CARON DE FROMENTEL, C. & T. SOUSSI. 1992. TP53 tumor suppressor gene: a model for investigating human mutagenesis. Genes Chrom. Cancer **4:** 1–15.
65. HSU, I.C., R.A. METCALF, T. SUN, J.A. WELSH, N.J. WANG & C.C. HARRIS. 1991. Mutational hotspot in the p53 gene in human hepatocellular carcinomas. Nature **350:** 427–428.
66. BRESSAC, B., M. KEW, J. WANDS & M. OZTURK. 1991. Selective G-mutation to T-mutation of p53 gene in hepatocellular carcinoma from southern Africa. Nature **350:** 429–431.
67. OZTURK, M. *et al*. 1991. p53 mutation in hepatocellular carcinoma after aflatoxin exposure. Lancet **338:** 1356–1359.
68. AGUILAR, F., C.C. HARRIS, T. SUN, M. HOLLSTEIN & P. CERUTTI. 1994. Geographic variation of p53 mutational profile in nonmalignant human liver. Science **264:** 1317–1319.
69. PUISIEUX, A., S. LIM, J. GROOPMAN & M. OZTURK. 1991. Selective targeting of p53 gene mutational hotspots in human cancers by etiologically defined carcinogens. Cancer Res. **51:** 6185–6189.
70. BRASH, D.E., J.A. RUDOLPH, J.A. SIMON *et al*. 1991. A role for sunlight in skin cancer—UV-induced p53 mutations in squamous cell carcinoma. Proc. Natl. Acad. Sci. USA **88:** 10124–10128.
71. ZIEGLER, A., A.S. JONASON, D.J. LEFFELL *et al*. 1994. Sunburn and p53 in the onset of skin cancer. Nature **372:** 773–776.
72. DUMAZ, N., A. STARY, T. SOUSSI, L. DAYAGROSJEAN & A. SARASIN. 1994. Can we predict solar ultraviolet radiation as the causal event in human tumours by analysing the mutation spectra of the p53 gene? Mutat. Res. **307:** 375–386.

73. KINZLER, K.W. & B. VOGELSTEIN. 1996. Lessons from hereditary colorectal cancer. Cell **87:** 159–170.
74. SPARKS, A.B., P.J. MORIN, B. VOGELSTEIN & K.W. KINZLER. 1998. Mutational analysis of the APC/beta-catenin/Tcf pathway in colorectal cancer. Cancer Res. **58:** 1130–1134.
75. POTTER, J.D. 1999. Colorectal cancer: molecules and populations. J. Natl. Cancer Inst. **91:** 916–932.
76. OLSCHWANG, S., R. HAMELIN, P. LAURENT-PUIG *et al*. 1997. Alternative genetic pathways in colorectal carcinogenesis. Proc. Natl. Acad. Sci. USA **94:** 12122–12127.
77. NORDGREN, H., A. LINDGREN & J. BERGH. 1996. The p53 gene in breast cancer: prognostic value of complementary DNA sequencing versus immunohistochemistry—response. J. Natl. Cancer Inst. **88:** 1500.
78. TOMIZAWA, Y., T. KOHNO, T. FUJITA *et al*. 1999. Correlation between the status of the p53 gene and survival in patients with stage I non-small cell lung carcinoma. Oncogene **18**: 1007–1014.
79. FISHER, C.J., C.E. GILLETT, B. VOJTESEK, D.M. BARNES & R.R. MILLIS. 1994. Problems with p53 immunohistochemical staining—the effect of fixation and variation in the methods of evaluation. Br. J. Cancer **69:** 26–31.
80. VOJTESEK, B., C.J. FISHER, D.M. BARNES & D.P. LANE. 1993. Comparison between p53 staining in tissue sections and p53 proteins levels measured by an ELISA technique. Br. J. Cancer **67:** 1254–1258.
81. JACOBS, T.W., J.E. PRIOLEAU, I.E. STILLMAN & S.J. SCHNITT. 1996. Loss of tumor marker-immunostaining intensity on stored paraffin slides of breast cancer. J. Natl. Cancer Inst. **88:** 1054–1059.
82. WYNFORD-THOMAS, D. 1992. p53 in tumour pathology—can we trust immunocytochemistry. J. Pathol. **166:** 329–330.
83. WYNFORD-THOMAS, D. 1995. Assessment of p53 overexpression by non-immunohistochemical methods—reply. J. Pathol. **175:** 94–95.
84. HALL, P.A. & D.P. LANE. 1994. P53 in tumour pathology—can we trust immunohistochemistry—revisited. J. Pathol. **172:** 1–4.
85. DIAMANDIS, E.P. & M.A. LEVESQUE. 1995. Assessment of p53 overexpression by non-immunohistochemical methods. J. Pathol. **175:** 93–94.
86. HAMELIN, R., P. LAURENT-PUIG, S. Olschwang *et al*. 1994. Association of p53 mutations with short survival in colorectal cancer. Gastroenterology **106:** 42–48.
87. GOH, H.S., C.S. CHAN, K. KHINE & D.R. SMITH. 1994. p53 and behaviour of colorectal cancer. Lancet **344:** 233–234.
88. SMITH, D.R., C.Y. JI & H.S. GOH. 1996. Prognostic significance of p53 overexpression and mutation in colorectal adenocarcinomas. Br. J. Cancer **74:** 216–223.
89. PRICOLO, V.E., S.D. FINKELSTEIN, T.T. WU *et al*. 1996. Prognostic value of TP53 and k-ras-2 mutational analysis in stage III carcinoma of the colon. Am. J. Surg. **171:** 41–46.
90. DIX, B.R., P. ROBBINS, R. SOONG *et al*. 1994. The common molecular genetic alterations in dukes' b and c colorectal carcinomas are not short-term prognostic indicators of survival. Int. J. Cancer **59:** 747–751.
91. REMVIKOS, Y., O. TOMINAGA, P. HAMMEL *et al*. 1992. Increased p53 protein content of colorectal tumours correlates with poor survival. Br. J. Cancer **66:** 758–764.
92. SUN, X.F., J.M. CARSTENSEN, H. ZHANG *et al*. 1992. Prognostic significance of cytoplasmic-p53 oncoprotein in colorectal adenocarcinoma. Lancet **340:** 1369–1373.
93. AUVINEN, A., J. ISOLA, T. VISAKORPI, T. KOIVULA, S. VIRTANEN & M. HAKAMA. 1994. Overexpression of p53 and long-term survival in colon carcinoma. Br. J. Cancer **70:** 293–296.
94. BOSARI, S., G. VIALE, P. BOSSI *et al*. 1994. Cytoplasmic accumulation of p53 protein—an independent prognostic indicator in colorectal adenocarcinomas. J. Natl. Cancer Inst. **86:** 681–687.
95. BELL, S.M., N. SCOTT, D. CROSS *et al*. 1993. Prognostic value of p53 overexpression and c-Ki-ras gene mutations in colorectal cancer. Gastroenterology **104:** 57–64.
96. MORRIN, M., M. KELLY, N. BARRETT & P. DELANEY. 1994. Mutations of ki-ras and p53 genes in colorectal cancer and their prognostic significance. Gut **35:** 1627–1631.
97. MULDER, J.W.R., I.O. BAAS, M.M. POLAK, S.N. GOODMAN & G.J.A. OFFERHAUS. 1995. Evaluation of p53 protein expression as a marker for long-term prognosis in colorectal carcinoma. Br. J. Cancer **71:** 1257–1262.

98. KRESSNER, U., G. LINDMARK, B. GERDIN, L. PAHLMAN & B. GLIMELIUS. 1996. Immunohistochemical p53 staining is of limited value in the staging and prognostic prediction of colorectal cancer. Anticancer Res. **16:** 951–957.
99. POLLER, D.N., K.J. BAXTER & N.A. SHEPHERD. 1997. p53 and Rb1 protein expression: are they prognostically useful in colorectal cancer? Br. J. Cancer **75:** 87–93.
100. FRIEDLANDER, P., Y. HAUPT, C. PRIVES, M. OREN. 1996. A mutant p53 that discriminates between p53-responsive genes cannot induce apoptosis. Mol. Cell. Biol. **16:** 4961–4971.
101. FRIEDLANDER, P., Y. LEGROS, T. SOUSSI & C. PRIVES. 1996. Regulation of mutant p53 temperature-sensitive DNA binding. J. Biol. Chem. **271:** 25468–25478.
102. FORRESTER, K., S.E. LUPOLD, V.L. OTT *et al*. 1995. Effects of p53 mutants on wild-type p53-mediated transactivation are cell type dependent. Oncogene **10:** 2103–2111.
103. LUDWIG, R.L., S. BATES & K.H. VOUSDEN. 1996. Differential activation of target cellular promoters by p53 mutants with impaired apoptotic function. Mol. Cell. Biol. **16:** 4952–4960.
104. RYAN, K.M. & K.H. VOUSDEN. 1998. Characterization of structural p53 mutants which show selective defects in apoptosis but not cell cycle arrest. Mol. Cell. Biol. **18:** 3692–3698.
105. GOH, H.S., J. YAO & D.R. SMITH. 1995. p53 point mutation and survival in colorectal cancer patients. Cancer Res. **55:** 5217–5221.
106. BORRESEN DALE, A.L., R.A. LOTHE, G.I. MELING, P. HAINAUT, T.O. ROGNUM & E. SKOVLUND. 1998. Tp53 and long-term prognosis in colorectal cancer—mutations in the l3 zinc-binding domain predict poor survival. Clin. Cancer Res. **4:** 203–210.
107. KRESSNER, U., M. INGANAS, S. BYDING *et al*. 1999. Prognostic value of p53 genetic changes in colorectal cancer. J. Clin. Oncol. **17:** 593–599.

## DISCUSSION

D. HERLYN (*The Wistar Institute, Philadelphia, PA*): You said that the antibodies were present in those individuals who had p53 mutations. However, to my knowledge, the antibodies are directed not to mutated epitopes but to normal epitopes. On the other hand, there are data in the literature showing that one can vaccinate against normal p53, and the tumors (with mutated p53) disappear. So what are your comments on those findings in view of the specificity of the antibodies in your patients?

SOUSSI: It is true, and it has been demonstrated that the antibodies are not specific for the mutant p53. When we performed an epitope mapping of the p53 protein to map the region that is recognized, we found that both wild-type and mutant p53—and, in fact, p53 antibodies—are directed toward immunodominant epitopes that are localized in the nonmutated amino terminus of the p53 protein. These immunodominant epitopes are similar in the mouse: when one immunizes mice with human p53, most of the antibodies are also directed against this region. Then, mutation per se is not important for the induction of humoral response. By contrast, accumulation of p53 protein is very important, and it is the main trigger of humoral response. There are some animal models in which p53 is stabilized through SV 40 antigen. There is no p53 mutation, but there are anti-p53 antibodies because wild-type p53 is stabilized through its interaction with T antigen. These antibodies recognize both wild-type and mutant p53. For use of p53 protein as a vaccine for immunotherapy, mutation is not really an important feature.

HERLYN: So you wouldn't say that your antibodies have a beneficial effect?

SOUSSI**:** I did not say so.

HERLYN: Why not? According to the some studies, they could have beneficial effect for the patient and improve prognosis.

SOUSSI: It is totally different. These antibodies are only, I would say, neutral to the patient. My feeling is that these antibodies have no effect on the development or progression of the tumor. They are a result of a self-immunization process that occurs because there is a huge accumulation of the p53 protein. The fact that the patients with antibodies have a very bad prognosis is due not to the presence of antibodies, but just to the fact that there is a mutation in the tumor that inactivates p53.

C.R. BOLAND (*University of California San Diego, La Jolla, CA*): What has long puzzled me is the fact that in the multistep carcinogenesis of colon cancer, in the very earliest adenomas in which, even if you use histochemistry, you do not see any stabilized p53. After transition to the carcinoma one sees loss of the wild-type allele. The question is: Why in larger adenomas are there expanded clones of cells with p53 mutations that still have a wild-type allele? Is there any growth advantage for an epithelial cell having one mutant and one wild-type allele?

SOUSSI: I have no explanation for this, but clearly the presence of one wild-type and one mutant allele is detrimental to the cells. The fact that you have just one p53 allele can lead to genetic instability. Mice that have been knocked out for p53 and have only one p53 allele will develop cancer. As p53 protein is a tetramer, it is possible to have a dominant negative effect of mutant p53 toward wild-type p53.

J. O'CONNELL (*University Hospital, Cork, Ireland*): Just a point about the p53 serving as a target for antigen recognition in the tumor cells: I think it is fair to say that a lot of peptides derived from intracellular proteins are presented on the cell surface in complex with MHC molecules so that these peptides can be seen on the surface by the immune system. Why is there humoral response to p53 at all?

SOUSSI: We do not know how this B cell–mediated antitumoral response is triggered. We have no indication of the anti-p53 T cell response in patients, either. It is more difficult to study.

R. OFFRINGA (*University Hospital Leiden, Leiden, The Netherlands*): I don't think antibodies against p53 can do anything against tumor, because p53 is simply a nuclear antigen and is quite different from the antigen that Dr. Herlyn is working on (EpCAM or GA 733), which is a surface antigen. So the only way the immune system can attack tumors through p53 as a target is by T cells that recognize processed p53 peptides. The antibodies are just an indication of the tumor burden, and that's all.

W. BALLHAUSEN (*Biozentrum-Halle, Halle, Germany*): Have you checked colorectal cancer patients also for antibodies against the APC gene product, or altered ß-catenin? Would it make sense?

SOUSSI: Never. For APC, I don't know; for ß-catenin, yes, it would be interesting.

E.A. SAUSVILLE (*National Cancer Institute, Bethesda, MD*): You alluded to the mdm-2 expression and consequently p53 expression in sarcoma. Is there evidence that there may be broader applicability of that mechanism in other cases of wild-type p53-positive tumors?

SOUSSI: Mdm2 accumulation leads to p53 degradation. There are several mechanisms that lead to such accumulation, such as gene amplification or mRNA overexpression.

QUESTION: I wanted to ask you about the anti-p53 antibodies in lupus patients. They have very high titers. What are they directed against?

SOUSSI: With our assay we have never been able to find anti-p53 antibodies in patients with autoimmune disease. I do know of reports from other labs indicating that they could detect such anti-p53 antibodies. The only noncancer disease in which we could find anti-p53 antibodies is rheumatoid arthritis. Interestingly enough, p53 mutations have also been discovered in this disease, but we don't exactly understand the significance of it.

BOLAND**:** Are there any small compounds that can interfere with the interaction between the HPV protein and p53 interaction that would allow the p53 to function normally in those tumor cells?

SOUSSI: I know that private companies are working on this. For now they have failed. It is very difficult. We don't work on this ourselves, but in my lab we do work on small compounds that could dissociate mdm2 and p53 protein. We have some small molecules, but it is very difficult to see if it will really be useful *in vivo* or not. I think it is a very important way to develop new therapeutic means for such types of cancer.

C. HANSKI (*Universitätsklinikum Benjamin Franklin, Berlin*): Dr. Soussi, when you treat patients, p53 expression is increasing. Why don't you find a humoral response to p53 after prolonged chemotherapy or radiotherapy?

SOUSSI: You speak about patients who have wild-type p53. The increase of p53 protein expression in these patients is really transient, and there is not enough time to have immunization. The titer depends on the size of the tumor and the tumor burden. We have some patients with colon cancer with a high titer of anti-p53 antibodies. You can dilute these antibodies 10,000 times and in Western blot still get a very good signal. But when you remove the colon cancer by surgery, in one month you observe a decrease of the titer. Therefore, I think that p53 needs to be present all the time in order to maintain the high level of p53 antibodies. As soon as the tumor is removed and p53 protein is not present, there is a decrease in the titer of these antibodies.