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Clinical implications of *p53* mutations

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Abstract. The ultimate goal of basic cancer research is to provide a theoretical foundation for rational approaches to improve cancer therapy. Our extensive insight into the biology of the p53 tumour suppressor and the clinical behaviour of tumours harbouring *p53* mutations indicates that information concerning p53 will be useful in diagnosis and prognosis, and may ultimately produce new therapeutic strategies. At the same time, efforts to understand the clinical implications of *p53* mutations have revealed conceptual and technical limitations in translating basic biology to the clinic. The lessons learned from p53 may lay the groundwork for future efforts to synthesize cancer gene function, cancer genetics and cancer therapy.

Key words. Apoptosis; chemotherapy; prognosis; tumor suppressor; cancer genetics; clinical outcome.

Introduction

Despite extraordinary advances in our understanding of cancer, basic cancer research has yet to make a substantial impact on the treatment of human malignancy. Most cancer patients continue to receive highly toxic drugs derived from empirical screens, and the best therapy remains complete surgical resection of the tumour. Still, underlying the massive effort to identify the molecular defects in cancer cells is the premise that this information will eventually produce better diagnostic and prognostic tools, and ultimately suggest rational strategies for the development of more effective therapies.

The high frequency of *p53* mutations in diverse human cancers implies that loss of p53 function is central to tumour development. Consequently, much effort has been devoted to understanding p53 biology, from its precise three-dimensional structure to its evolutionary significance [1, 2]. Similarly, a large number of clinical studies have asked whether information concerning the basic biology of p53 can be used for diagnostic or prognostic benefit, or ultimately to suggest strategies to improve cancer therapy. These studies provide valuable insight into the clinical significance of *p53* mutations, but at the same time have revealed fundamental limitations in extrapolating basic research to patients. These studies may provide lessons to guide future work in exploiting *p53* or other cancer genes for improved cancer treatment.

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Biological activities of p53 and tumour development

Research over the last several years has revealed that p53 has a remarkable number of biological activities, including cell-cycle checkpoints, apoptosis (programmed cell death), senescence, maintenance of genomic integrity and control of angiogenesis (reviewed in refs 3–9). One can envision that disruption of any one of these processes might promote tumour progression. For example, loss of a DNA damage-inducible G1 checkpoint might promote tumour growth by increasing the frequency with which genetic damage is fixed as mutation. By contrast, inactivation of hypoxia or oncogene-induced apoptosis might provide a selective advantage to cells acquiring *p53* mutations, allowing them to more readily emerge [10–13]. Still, it is not known which of these p53 activities is most critical during the course of tumour development – that is, what provides the selective pressure to mutate *p53*?

p53 mutation as a prognostic indicator

Irrespective of which p53 function(s) account for its tumour suppressor activity, the nature of p53 biology and the consequences of p53 loss predict that tumours with *p53* mutations should be inherently more aggressive than tumours bearing wild-type *p53* genes. For example, loss of a G1 checkpoint and genomic integrity could increase the mutation rate, allowing *p53* mutant tumours to evolve more rapidly. Loss of apoptosis or enhancement of angiogenesis would accelerate tumour expansion, and perhaps promote metastasis by allowing tumour cells to better survive foreign environments. Finally, inactivation of senescence programmes might more readily allow developing tumours to bypass a molecular clock that would otherwise provide a brake to tumour growth by limiting cell division. All of these factors, alone or in combination, predict an ominous outcome for patients harbouring *p53* mutations.

Owing to the fundamental role of p53 in tumour development, clinical studies have asked whether p53 can be a prognostic indicator in various tumour types. These studies typically ask whether *p53* mutations affect the disease-free interval or, ultimately, patient survival. In general, *p53* mutations are associated with more aggressive cancers of higher tumour grade, and in many instances, *p53* mutations correlate with reduced patient survival independently of tumour grade or stage. Among the tumour types for which substantial data are available, *p53* mutations are associated with poor prognosis in certain lymphomas [14] and leukaemias [15–17], carcinomas of the breast [18–24], liver [25], colon [26], endometrium [27], and lung [28, 29], soft-tissue sarcomas [30], Wilms' tumour [31], transitional cell carcinoma of the bladder [32], head and neck squamous cell carcinoma [33], and glioma [34].

Perhaps the most well-studied situation relating *p53* status to patient prognosis is breast carcinoma, a tumour type where there is a clear need for better prognostic indicators. Early diagnosis and surgical resection of node-negative (i.e., nonmetastatic) breast cancer can be curative without requiring adjuvant chemotherapy. However, a percentage (20–30%) of these patients relapse; hence, good markers of relapse (a poor prognosis) might identify those patients in need of adjuvant therapy while sparing those patients who have a low risk of recurrence [35]. Early studies using p53 immunohistochemistry as a surrogate marker of *p53* mutations were ambiguous; however, more recent studies using DNA sequencing now clearly indicate a striking association between *p53* mutations and poor prognosis. Indeed, *p53* may be the most clear-cut indicator of tumour recurrence in breast cancer identified to date [21].

Although the biological explanation underlying the association between *p53* mutations and poor prognosis is not known, *p53* mutant tumours possess characteristics consistent with functional studies using model systems. For example, tumours with *p53* mutations can display reduced apoptosis and a high degree of genomic instability [36, 37], or increased angiogenesis in some cancer types [38, 39]. Also, some studies suggest that *p53* mutant tumours more readily immortalize in cell culture, suggesting that they have lost growth controls that appear as increased proliferative capacity in cell culture [40].

Of course, the ultimate goal of identifying prognostic indicators is that they might eventually guide oncologists in the design of appropriate treatment regimens. While the clinical utility of p53 as a prognostic indicator remains uncertain, it seems likely that this information will be useful in conjunction with other indicators. Despite its promise, technology used to determine *p53* status is nonuniform and suffers from other serious limitations (see below). Clearly, for p53 to be used routinely to predict prognosis, it will be necessary to standardize the methodology.

p53 and the cytotoxicity of anticancer agents

p53 can be activated by a large number of cellular stresses, including ribonucleotide depletion, hypoxia, oxidative stress and certain mitogenic oncogenes [11, 41–43]. Perhaps the best-studied activator of p53 is DNA damage, which can promote p53-dependent arrest or apoptosis depending on the genetic background of the cell or its tissue of origin [44–46]. Since most commonly used cytotoxic drugs either directly or indirectly damage DNA, one can easily imagine that *p53* status might affect the outcome of cancer therapy. The precise nature of this impact, however, is not intuitively obvious.

p53 and the G1 checkpoint

p53 is involved in a DNA damage-inducible G1 checkpoint and, albeit indirectly, a mitotic spindle checkpoint. The role of p53 in the radiation-induced G1 checkpoint suggests that p53 promotes cell-cycle arrest to facilitate accurate DNA repair; consequently, loss of p53 might allow the persistence of unrepaired damage, leading to enhanced radiation toxicity. However, studies using isogenic fibroblasts from normal and p53 knockout mice found no difference in radiation-induced loss of clonogenic survival, suggesting that this was not the case [47].

Wild-type p53 is also implicated in the mitotic checkpoint based on the observation that p53-deficient fibroblasts do not arrest following treatment with microtubule-disrupting agents, but instead reenter S phase [48–50]. p53 expression is not required for mitotic arrest [51], but it is required to prevent endoreduplication of 4N cells. Following microtubule disruption, mouse embryo fibroblast (MEFs) null for p21 display a similar phenotype to p53 null MEFs, suggesting that adapted 4N cells may be similar to G1 cells; thus wild-type p53 and p21 are needed to prevent improper entry into S phase that would result in polyploidy [52]. This predicts that cells lacking p53 might be more sensitive to microtubule-disrupting agents.

p53 and apoptosis in mouse systems

In addition to promoting cell-cycle arrest, p53 can promote apoptosis following radiation treatment. This was first demonstrated in a series of studies comparing knockout mice to normal mice. For example, radiation induces apoptosis in normal but not p53-deficient thymocytes both in vitro [44, 45] and in vivo [53]. Second, p53 is required for radiation-induced apoptosis in stem cells of the intestinal crypt [54] as well as bone marrow-derived cells [55]. Of note, cell death can also be p53-independent in these systems, since glucocorticoids induce p53-independent apoptosis in thymocytes [44, 45], and radiation induces a more delayed p53-independent form of apoptosis in the intestine [54, 56]. Nevertheless, the indication that ionizing radiation could induce cell death in a p53-dependent manner raised the possibility that cells lacking p53 might more readily survive certain forms of cancer therapy.

While the role of p53 in radiation-induced apoptosis is limited to a few normal cell types, oncogenic changes can alter p53 function from growth arrest to apoptosis [11, 12]. Since tumour cells have acquired oncogenic mutations, it is possible that p53 might function in apoptosis in diverse tumour types. For example, oncogenically transformed fibroblasts expressing the *E1A* and *ras* oncogenes undergo apoptosis upon treatment with ionizing radiation and several genotoxic chemotherapeutic drugs; by contrast, normal (nontransformed) counterparts simply arrest following these same treatments [57]. A comparison of oncogenically transformed fibroblasts from wild-type and p53-deficient mice demonstrates that p53 plays a critical role in promoting apoptosis. Consequently, p53-deficient cells display a multi-drug-resistant phenotype – they are resistant to a series of diverse agents.

The studies described above provide strong evidence that p53-dependent apoptosis can contribute to anticancer agent cytotoxicity, raising the possibility that *p53* mutations might reduce the efficacy of cancer therapy in vivo. Subsequent studies using genetically controlled fibrosarcomas confirmed this possibility [53]. Specifically, since *E1A* and *ras* transform MEFs to a tumorigenic state without inactivating p53, *p53*-expressing and -deficient tumours could be generated in immunocompromised mice. When tumour-bearing animals are treated with several anticancer agents, tumour response is dependent on p53 status: tumours derived from $p53^{+/+}$ cells displayed massive apoptosis and typically regressed, whereas *p53*-deficient tumours showed little apoptosis and continued to grow. Acquired *p53* mutations are associated with resistance and relapse of tumours derived from $p53^{+/+}$ cells, confirming the importance of p53 in the response of these tumours to therapy.

While the fibroblast system described above utilized highly comparable cells with precisely known genetic alterations, it necessarily suffers from its artificial nature. Despite this limitation, these experiments demonstrate that genetic control of cell death can have a dramatic impact on the efficacy of cancer therapy, and provide a strong illustration of the emerging relationship between cancer genes and the efficacy of cancer therapy. Furthermore, they clearly show that p53 can have a role in therapeutic treatment of tumours and raise the possibility that *p53* mutations – by reducing apoptosis – may contribute to drug resistance in tumours.

p21, cell-cycle checkpoints and chemosensitivity

p21 is a cyclin-dependent kinase inhibitor that is strongly activated by p53 following DNA damage [58–60]. A number of laboratories used gene-targeting technology to ask whether p21 is essential for the p53-induced G1 checkpoint or other p53 functions. In mice, p21-deficient fibroblasts are at least partially defective in cell-cycle checkpoints but not other aspects of p53 biology and are not prone to malignant tumours [59, 60]. In human cells, p21 has been disrupted in the HCT116 colon carcinoma line and in normal diploid fibroblasts [58, 61]. In both instances, inactivation of p21 dramatically compromises radiation-induced cell-cycle arrest. Of note, since parallel p53-null lines were unavailable, it could not be tested whether p21 inactivation was as potent at disrupting this arrest as p53 deficiency.

Remarkably, p21-deficient HCT116 cells treated with genotoxic agents undergo repeated cycles of DNA replication without dividing, resulting in severe aneuploidy and, ultimately, cell death [62]. Furthermore, when these lines are treated with radiation or chemotherapy as xenographs in nude mice, p21-deficient tumours display dramatic regression, and several

of the mice achieved complete remissions [63]. Hence, the p21-deficient HCT116 tumours are more sensitive to therapy than their p21-expressing counterparts.

Since p21 disruption mimics one aspect of p53 loss, these experiments raise the possibility that *p53* mutations may contribute to an improved response to therapy. However, this conclusion requires that one extrapolate the results of p21 deficiency to cells with *p53* mutations. In this regard, p21 can be regulated by non-p53 targets, and p53 regulates many more genes than p21 [64]. In this regard, a critical experiment will be to disrupt p53 function in HCT116 cells to see whether loss of *p53* also enhances drug cytotoxicity and tumour regression. These results may also be affected by additional (unknown) mutations in the HCT116 cells, since p21-deficient normal diploid fibroblasts show neither aneuploidy nor massive cell death following treatment with cytotoxic agents [61]. Despite these caveats, these experiments demonstrate that tumours with loss of p21 function can respond better to cancer therapy, and raise the possibility that therapeutic strategies which target p21 function might have clinical benefit. Furthermore, they provide provocative data to suggest that p53's checkpoint function may be crucial for mediating radiation and drug cytotoxicity.

In summary, experimental studies on p53 biology make two precisely opposite predictions regarding the role of p53 in influencing radiation and drug cytotoxicity and, ultimately, the outcome of cancer therapy. On one hand, loss of the p53-dependent apoptotic programme might promote drug resistance by making tumours less responsive to therapy. By contrast, loss of damage-induced checkpoints might enhance sensitivity by allowing the damage induced by cytotoxic drugs to more readily debilitate tumour cells. Clearly, the situation is complex, and the precise impact might be related to the mode of drug action, tissue of tumour origin or the precise genetic makeup of individual tumours. Given the high frequency of p53 mutations in human tumours, resolving these issues is of substantial clinical importance.

p53 mutations and drug sensitivity: what is the clinical experience?

Model systems demonstrate the potential for p53 status to affect chemosensitivity. The relevance of p53 to therapy outcome in clinical tumours is more difficult to correlate because additional alterations within tumours may affect the response, making it more difficult to attribute differences in chemosensitivity to a single gene.

p53 mutations and drug resistance

In vitro analysis of human tumour cell lines from some tumour types show a correlation between *p53* mutations and resistance to treatment. Burkitt's lymphoma cell lines with mutant p53 are more resistant to a variety of treatments when compared with those with wild-type *p53* [65]. In 56 human astrocytic cell lines, *p53* mutations are associated with resistance to DNA-damaging agents [66], and a broader analysis of 60 NCI cell lines of various tumour origin found that cell lines with *p53* mutations were generally more resistant to treatment that cell lines with wild-type *p53* [67]. Several studies correlate in vitro drug resistance in human tumour cell lines with mutant *p53* and decreased apoptosis [65, 68].

Consistent with in vitro studies, p53 status is linked to drug resistance in several tumour types. Perhaps the most striking examples occur in lymphoid malignancies, including non-Hodgkin's lymphoma, acute myeloid leukaemia, myelodysplastic syndrome and chronic lymphocytic leukaemia [69, 70]. In these tumour types, *p53* mutations are rare but generally associated with disease progression and poor prognosis. When patients are classified by *p53* status, tumour response (i.e., remission vs. nonresponsive) and survival, patients with *p53* mutations are remarkably resistant to therapy and display very short survival times. In this regard, a particularly informative tumour type is acute lymphoblastic leukaemia. Here, *p53* mutations in primary tumours are exceedingly rare, and most patients typically respond to therapy. However, a subfraction of patients relapse, and approximately 30% of relapsed tumours harbour mutant *p53*. Moreover, patients with *p53* mutant tumours are less likely with enter a second remission compared with patients with relapsed tumours harbouring wild-type *p53* [16, 71]. Enrichment for cells with *p53* mutations is exactly what would be predicted if *p53* mutations conferred a survival advantage to cells during chemotherapy.

p53 mutations are linked with drug resistance in carcinomas as well, most notably in breast cancer. Here, *p53* mutations are a strong predictor of treatment failure, relapse and death [21, 72]. For example, *p53* mutations are associated with resistance to tamoxifen [22, 73], radiotherapy [24, 74] and doxorubicin [23]. While theses correlations are provocative, it should be noted that some studies find no correlation [75, 76]. Mutations in genes thought to act in the p53 pathway also correlate with drug resistance. Bax is a proapoptotic protein whose gene expression is regulated by wild-type p53. Several studies associate low Bax protein expression with drug resistance in breast cancer [77–79] or the degree of radiosensitivity [80]. Moreover, Bax overexpression enhances the chemosensitivity of breast cancer cell lines [81, 82].

While basic studies predict that *p53* loss might lead to drug resistance by reducing drug-induced apoptosis, this is difficult to test in human tumours. However, in some tumour types, it appears *p53* mutations correlate with defects in apoptosis. In Wilms' tumour, *p53* mutations are associated with an anaplastic phenotype which is refractory to therapy [31] and is correlated with reduced apoptosis [36]. A positive correlation between p53, apoptosis and chemoresponse is seen in testicular cancer cell lines, where treatment of testicular cancer with chemotherapy produces increased p53 and Bax activity resulting in apoptosis [83, 84].

In addition to apoptosis, at least two other aspects of p53 biology might contribute to the observed relationship between *p53* mutations and drug resistance. First, p53 dysfunction can upregulate expression of P-glycoprotein (Pgp), a membrane pump implicated in multidrug resistance by virtue of its ability to prevent intracellular accumulation of certain drugs. Consequently, *p53* mutations might indirectly promote drug resistance by enhancing Pgp levels [85]. Nevertheless, *p53* mutations do not correlate with elevated Pgp expression in human tumours [17, 86, 87], and both radiation and non-Pgp substrate drugs can display p53-dependent toxicity (e.g., refs 88 and 89). Second, *p53* mutant tumours are more genetically unstable, and it is conceivable that this instability might allow *p53* mutant tumours to more rapidly evolve towards drug resistance. However, it seems unlikely that this property can explain the drug resistance occurring in p53 mutant tumours, since reintroduction of wild-type *p53* has been shown to enhance chemosensitivity (e.g., refs 90 and 91). This would not be expected if loss of *p53* was simply facilitating the emergence of drug-resistant variants.

p53 mutations and increased chemosensitivity

In some human tumours, *p53* mutations correlate with increased drug sensitivity. This is best illustrated in bladder cancer, where tumours positive for p53 by immunohistochemistry respond better to a variety of treatments than tumours with undetectable p53 [92, 93]. Similarly, in bladder cancer cell lines, cells expressing mutant p53 are more sensitive to radiation than their wild-type counterparts [94]. These observations are consistent with the data from p21-deficient HCT116 colon carcinoma cells associating loss of a p53 cell-cycle checkpoint with increased chemosensitivity. To date, it has not been tested whether increased drug toxicity occurs in conjunction with reduced checkpoint control in human tumours. Better molecular markers and technology may be necessary for such studies.

In summary, clinical data are emerging to support both positive and negative correlations between *p53* mutations and drug sensitivity. Some studies find no correlations at all (e.g., refs 95 and 96). What could explain this apparent paradox? One explanation has to do with the diversity of p53 functions and consequences of *p53* loss. Since defects in damage-induced checkpoints may enhance chemosensitivity, whereas defects in apoptosis promote drug resistance, the clinical impact of *p53* mutation may be determined by which effect predominates. This, in turn, may be influenced by tumour type, chemotherapeutic agent or by other mutations occurring in the tumour cells. In addition, p53 is a member of an emerging protein family [97–99], so other proteins may compensate for p53 loss in some settings. Alternatively, it is possible that at least some of the difficulties in relating *p53* mutations to clinical parameters may be methodological.

Assessing p53 status in the clinic

Since clinical studies are often limited by access to sufficient numbers of suitable specimens, misclassification of even a few tumours can dramatically affect the conclusions, obscuring circumstances where a correlation might otherwise exist, or suggesting weak associations when in fact there are none. Therefore, proper classification of tumours is central to the interpretation and utility of studies relating *p53* status to diagnosis, prognosis or as a predictive factor for therapy response. The methods used to determine *p53* status are limited as to which types of mutations or alteration in p53 they will detect. Even with accurate diagnosis, p53 function can be eliminated by several mechanisms, including mutation, deletion, heteromeric protein interactions or by extragenic mutations in the p53 pathway.

Methodological aspects

Immunohistochemistry (IHC) is an indirect measure of p53 status that is not completely accurate. IHC analysis of p53 is based on the fact that *p53* mutations often stabilize the protein, leading to higher steady-state p53 levels than in wild-type cells [100]. However, not all mutations stabilize p53 [101]; consequently, IHC detection would underestimate mutation frequency in tumours with deletion, frameshift or nonsense *p53* mutations. By contrast, wild-type protein is upregulated in response to DNA damage, hypoxia or activated oncogenes; this might produce an overestimate of *p53* mutation frequency. In studies where a small number of specimens are analysed, misclassification of only a few tumours could dramatically affect the interpretation and conclusions.

IHC analysis also suffers from a lack of technical and classification standards resulting in subjective evaluation of results. There are no standard criteria for defining positive p53 immunostaining, so a tumour that is classified as mutant in one study may be defined as wild-type in another study. In addition, different p53 antibodies do not always give the same results [102], and some antibodies cross react with non-p53 proteins. Because studies use different antibodies and criteria to define *p53* status by IHC, it is difficult to compare results between studies and may account for some of the discrepancies in correlating p53 to prognosis or therapy response.

Direct sequence analysis is perhaps the most precise method for determining *p53* mutation status. Studies that compare IHC with sequence analysis find disparities between the two methods. IHC does not identify all *p53* mutations, and sometimes p53 immunostaining is observed in the absence of sequence mutations [103, 104]. In some instances, direct sequencing has identified correlations between *p53* and therapy outcome when IHC has not [104]. However, sequence analysis of *p53* is expensive, time-consuming and sensitive to normal cell contamination. An alternate technique that utilizes *p53* gene sequence is the yeast functional assay [105]. This assay takes advantage of the fact that functional p53 transactivates reporter genes when expressed in yeast. *p53* complementary DNA (cDNA) can be isolated from tumour cells and expressed in yeast – a colour change indicates whether the tumour-derived *p53* is transcriptionally active and, hence, whether it is mutant or wild-type. This technique is able to distinguish between silent *p53* mutations and those that disrupt p53 transcription. However, it does not identify mutations in modifiers of p53 activity or downstream effectors and requires total RNA.

Clearly the methodology chosen to measure *p53* status will affect tumour classification and interpretations of the results. Studies use different techniques or variations within a method, making comparisons between studies or overall conclusions regarding the role of p53 impossible. Contradictory results may be due to different methodologies for analysing *p53* status. Thus the technology used will bias the conclusions in favour of the mutation type that methodology detects. Indirect indicators of p53 combined with a lack of standards for interpreting data may account for discrepancies regarding the role for p53 in the clinic.

Heterogeneity in *p53* mutations

The most frequent route to p53 inactivation in human tumours is point mutations that produce altered proteins with single amino acid substitutions. *p53* mutations have been identified in more than 100 different codons [106], implying that diverse structural alterations in *p53* promote carcinogenesis. Although these mutants can encode proteins that inactivate wild-type p53 by a dominant negative mechanism, missense mutations are often accompanied by deletion of the normal *p53* allele [107]. This implies that many mutant proteins do not completely abolish the activity of wild-type p53.

The threshold for p53-dependent apoptosis is highly dosage dependent, and deletion of even a single *p53* allele can provide a significant survival advantage following treatment with apoptotic stimuli. For example, thymocytes and bone marrow cells harbouring a single *p53* allele show an intermediate resistance to radiation-induced apoptosis compared with their wild-type and *p53*-deficient counterparts [44, 45, 108]. Similarly, oncogene-expressing cells derived from $p53^{+/-}$ fibroblasts are more resistant to apoptosis compared with isogenic cells expressing two *p53* alleles [11, 57]. This dosage dependence implies that the intrinsic ability of individual p53 mutants to inhibit wild-type p53 function, as well as the status of the remaining allele, may contribute to heterogeneity in cellular response to anticancer agents. Indeed, in some settings only a specific *p53* mutation type correlates with prognosis or response to therapy. For example, in soft-tissue sarcomas, only nonframeshift mutations in *p53* correlate with poor prognosis, whereas frameshift mutations show no correlation [109]. In non-small cell lung cancer, mutations in exon 5 are associated with the worst prognosis [110]. In certain haematological malignancies, where a wild-type *p53* allele is retained, *p53* mutation is not associated with drug resistance, whereas *p53* null malignancies correlate with resistance [111]. The fact that not all *p53* mutations affect p53 function equally further clouds the interpretation of clinical studies.

Defects in the p53 pathway

Our knowledge of p53 biology tells us that p53 integrates cellular stress with the cell-cycle or apoptotic machinery. A major caveat in p53 methodology is the current inability to account for the integrity of the p53 pathway – hence, extragenic mutations may affect p53 function without affecting p53 structure or protein expression.

Proteins that bind and inactivate p53. Both cellular and viral protein bind p53 and modulate its activity. Mdm-2 is a cellular protein overexpressed in 30% of sarcomas which binds and inactivates p53 at least in part by promoting its degradation [112]. Mdm-2 is overexpressed in a subset of other tumour types (breast, brain, bladder, lung and leukaemia) [113–118]. In these tu-

mours, Mdm-2 overexpression and *p53* mutations are mutually exclusive, suggesting that either mechanism inactivate p53.

Many viral oncogenes disrupt p53 during virus infection. High-risk papilloma viruses are involved in the pathogenesis of 84% of cervical carcinomas [119]. These viruses encode the E6 protein, which binds p53 and degrades it via ubiquitin-mediated proteolysis. In cervical cancer, HPV expression and *p53* mutations are exclusive: HPV-positive tumours rarely have *p53* mutations, whereas HPV-negative tumours typically do have p53 mutations. Thus, by IHC or sequence analysis, the majority of cervical carcinomas and a subset of sarcomas would be misclassified as having wild-type *p53*.

Proteins that modulate p53. Normal p53 function may also require cofactors or modifiers. Inhibitor of growth factor (*ING1*) is necessary for normal p53 activity. Cells lacking *ING1* are defective in p53 mediated transcription and growth inhibition [120]. Thus loss or mutation of *ING1* could alter p53 activity. ARF also functions in the p53 pathway [121]. $p19^{ARF}$ degrades Mdm-2, thus blocking Mdm-2-mediated p53 degradation, leading to increased transactivation of p53 target genes. Interestingly, ARF is not required for p53 activation following DNA damage, but activated p53 in response to mitogenic oncogenes [122]. However, the synergistic activation of p53 by ARF and DNA damage potentiated radiation and drug-induced death in oncogene-expressing cells [123]. In addition, p53 is activated by phosphorylation. Mutations in the kinase(s) that phosphorylate p53 could also disrupt p53 function, although it is not clear which kinases are responsible for this activity. Since *ING* and *ARF* are potential or bona fide tumour suppressors, respectively, it may ultimately become necessary to consider their mutational status as well as p53.

Mutations in the p53 pathway. In principle, mutations in downstream p53 effectors could lead to a p53 'null' phenotype even though *p53* sequence is wild-type. This may be the case in HNPCC, a cancer where *p53* mutations are rare, but *bax* mutations are common [124, 125]. Even in the presence of wild-type *p53*, Bax inactivation correlates with reduced apoptosis and chemosensitivity [126]; thus in these tumours *Bax* mutations may reduce the selective pressure to mutate *p53*.

The failure to account for the p53 pathway brings into question the interpretation of all clinical studies to date. While it may be possible to ask whether a particular method for assessing p53 is a good prognostic or predictive tool, these limitations preclude translating these results to the underlying biology. Tumours classified with wild-type *p53* may be null for p53 function due to mutations in upstream mediators or downstream effectors. Consequently, a complete analysis of p53 in the clinic requires analysis of the p53 pathway. One way to do this is with DNA 'array' technology, where the expression of p53 and p53-responsive genes could be measured to determine p53 functionality.

Strategies to exploit p53 for cancer diagnosis or therapy

The clinical utility of p53 may extend beyond its usefulness in predicting tumour behaviour. Indeed, a number of groups have begun to investigate strategies to exploit p53 for improved therapy.

Diagnostic strategies

Understanding the role of p53 in tumour development and in response to treatment would be useful in the clinic on several fronts. Because *p53* alterations are so frequent, the detection of *p53* mutations could be a useful diagnostic tool. Since successful treatment of cancer relies on early detection, molecular methods that identify tumours at their early stages would be invaluable.

Using polymerase chain reaction (PCR)-based techniques, mutant *p53* has been detected in exfoliated cells in bladder cancer and lung cancer [127, 128]. *p53* mutations were identified prior to clinical diagnosis of cancer in some cases, thus demonstrating the potential use of *p53* as a diagnostic tool, particularly for high-risk individuals. However, since only a small percentage of cells contain *p53* mutations, they may be missed due to insufficient sensitivity of the assay. A subset of cancer patients (5–40%) mount an immune response to mutant p53 [129]. Another potential diagnostic tool is screening for anti-p53 antibodies in patients. Using enzyme linked immunosorbent assay (ELISA) on serum samples, anti-p53 antibodies were identified in two smokers prior (5–15 months) to their clinical diagnosis with lung cancer [130]. In addition, antibody levels drop following therapy and thus may provide a marker for treatment success. While this tool would only apply to a portion of patients, a major benefit to the approach is that the analysis requires only a blood sample. These diagnostic strategies are low-risk, noninvasive screening tools that if used in conjunction with current screening procedures may provide valuable diagnostic information.

Therapeutic strategies

A hope in understanding the biology of p53 is that the information can be used to develop new therapeutic strategies for treating cancer. Most cancer agents used currently were discovered through empirical screens, and their action on tumour cells is not well understood. Since *p53* is frequently mutated in human cancer, it is an obvious candidate for rational drug or therapy development.

Restoring p53 function. Irrespective of the biological impact of p53 on tumour development or response, it is reasonable to suggest that restoring p53 functions to *p53* mutant tumours would have a positive impact on cancer therapy. One approach is through *p53* gene therapy, which can directly induce apoptosis and enhance drug cytotoxicity in several tumour cell lines [90, 131, 132] and has shown promise in vivo [90, 91]. While gene therapy strategies alone may be insufficient, Roth and colleagues have documented synergy between *p53* gene therapy and chemotherapy, suggesting that *p53* may prove a useful adjuvant to current therapies.

Non-gene therapy strategies to restore p53 function are also possible. In some cells lines mutant p53 can be reactivated by the addition of a small C-terminal peptide of wild-type p53 or antibodies to the C-terminus of p53 [133, 134]. Conceivably, drugs which mimic p53 itself or modify its effectors may also functionally replace p53.

Induction of p53-independent cell death. Many chemotherapeutic agents work, at least in part, by damaging DNA and inducing apoptosis, and wild-type p53 is necessary for this response in some settings. Development of drugs that target p53-independent apoptotic pathways may improve therapy of tumours with *p53* mutations. As an example, microtubule inhibitors can kill tumour cells irrespective of *p53* status [66, 67, 135]. Drugs such as taxol or UCN-01, which disrupt the G2 checkpoint, sensitize p53 null tumours to DNA damage [136]. While a better understanding of factors affecting drug cytotoxicity may be necessary for this approach, these examples illustrate its potential.

p53 immunotherapy. Since *p53* mutations typically produce altered proteins, several groups have attempted to exploit the immune system to target tumour cells. Immunotherapy targeting of mutant p53 would provide a nontoxic, tumour-specific treatment for tumours. The potential for this type of therapy has been illustrated in mice. Treatment with dendritic cells specific for mutant p53 were able to slow tumour growth of preexisting tumours [137]. In addition, dendritic cells are able to act as a vaccine, protecting mice against a lethal injection of sarcoma cells and promoting tumour rejection [138].

Selective killing of *p53* mutant tumours. Cancer therapy could also take advantage of a tumour's p53 null status in treatment specificity. An example of this is the ONYX-015 adenovirus, which is unable to replicate in normal cells but selectively divides in cells lacking p53 [139, 140]. In the absence of p53, viral replication occurs followed by cell lysis; consequently, tumour cells are specifically killed by the virus, whereas normal cells are unaffected. The ability of the virus to replicate and spread overcomes some of the limitations of standard gene therapy strategies, although it remains to be determined whether such a therapy would be effective at treating metastatic disease.

Summary

Since factors that influence the efficacy of cancer therapy remain poorly understood, a molecular understanding of neoplasia will certainly improve cancer diagnosis and treatment. However, attempts to translate basic biology to clinically useful information have uncovered many pitfalls. While p53 clearly has an important role in tumourigenesis and therapy outcome, it does not function in isolation. To date, most studies attempting to correlate phenotypes to *p53* status have failed to account for other members of the p53 pathway, and hence the interpretations of current studies are incomplete. Better methods to assess both *p53* status and the p53 pathway will be required before we can understand the clinical behaviour of *p53* mutant tumours in the context of our knowledge of p53 biology.

In any case, functional studies on p53 suggest rational strategies for designing better therapies. Since p53 has such diverse functions, all of which may contribute to tumourigenesis, there are many avenues to pursue. Tumours harbouring *p53* mutations are inherently more aggressive and, in many instances, more resistant to traditional therapies. Hence, they represent a tumour subpopulation desperately in need of new approaches. The preliminary success of approaches that specifically address *p53* status in tumours underscores the notion that a molecular understanding of cancer will have a positive impact on treatment.

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1 Cho Y., Gorina S., Jeffrey P. D. and Pavletich N. P. (1994) Crystal structure of a p53 tumor suppressor-DNA complex: understanding tumorigenic mutations. Science **265:** 346–355
2 Hall P. A. and Lane D. P. (1997) Tumor suppressors: a developing role for p53? Curr. Biol. **7:** R144–147
3 Bellamy C. O. (1997) p53 and apoptosis. Br. Med. Bull. **53:** 522–538
4 Dameron K. M., Volpert O. V., Tainsky M. A. and Bouck N. (1994) The p53 tumor suppressor gene inhibits angiogenesis by stimulating the production of thrombospondin. Cold Spring Harb. Symp. Quant. Biol. **59:** 483–489
5 El-Deiry W. (1998) p21/p53, cellular growth control and genomic integrity. Curr. Top. Microbiol. Immunol. **227:** 121–137
6 Levine A. J. (1997) p53, the cellular gatekeeper for growth and division. Cell **88:** 323–331
7 Moll U. M. and Schramm L. M. (1998) p53 – an acrobat in tumorigenesis. Crit. Rev. Oral. Biol. Med. **9:** 23–37
8 O'Connor P. M. (1997) Mammalian G1 and G2 phase checkpoints. Cancer Surv. **29:** 151–182
9 Wynford T. D. (1996) p**53:** guardian of cellular senescence. J. Pathol. **180:** 118–121

10 Symonds H., Krall L., Remington L., Saenz-Robles M., Lowe S., Jacks T. et al. (1994) p53-dependent apoptosis suppresses tumor growth and progression in vivo. Cell **78:** 703–711
11 Lowe S. W., Jacks T., Housman D. E. and Ruley H. E. (1994) Abrogation of oncogene-associated apoptosis allows transformation of p53-deficient cells. Proc. Natl. Acad. Sci. USA **91:** 2026–2030
12 Hermeking H. and Eick D. (1994) Mediation of c-Myc-induced apoptosis by p53. Science **265:** 2091–2093
13 Graeber T. G., Osmanian C., Jacks T., Housman D. E., Koch C. J., Lowe S. W. et al. (1996) Hypoxia-mediated selection of cells with diminished apoptotic potential in solid tumours. Nature **379:** 88–91
14 Rodriguez M. A., Ford R. J., Goodacre A., Selvanayagam P., Cabanillas F. and Deisseroth A. B. (1991) Chromosome 17p and p53 changes in lymphoma. Br. J. Haematol. **79:** 575–582
15 Nakai H., Misawa S., Taniwaki M., Horiike S., Takashima T., Seriu T. et al. (1994) Prognostic significance of loss of a chromosome 17p and p53 gene mutations in blast crisis of chronic myelogenous leukaemia. Br. J. Haematol. **87:** 425–427
16 Hsiao M. H., Yu A. L., Yeargin J., Ku D. and Haas M. (1994) Nonhereditary p53 mutations in T-cell acute lymphoblastic leukemia are associated with the relapse phase. Blood **83:** 2922–2930
17 El Rouby S., Thomas A., Costin D., Rosenberg C. R., Potmesil M., Silber R. et al. (1993) p53 gene mutation in B-cell chronic lymphocytic leukemia is associated with drug resistance and is independent of MDR1/MDR3 gene expression. Blood **82:** 3452–3459
18 Thompson A. M., Anderson T. J., Condie A., Prosser J., Chetty U., Carter D. C. et al. (1992) p53 allele losses, mutations and expression in breast cancer and their relationship to clinico-pathological parameters. Int. J. Cancer **50:** 528–532
19 Thorlacius S., Borresen A. L. and Eyfjord J. E. (1993) Somatic p53 mutations in human breast carcinomas in an Icelandic population: a prognostic factor. Cancer Res. **53:** 1637–1641
20 Andersen T. I., Holm R., Nesland J. M., Heimdal K. R., Ottestad L. and Borresen A. L. (1993) Prognostic significance of TP53 alterations in breast carcinoma. Br. J. Cancer **68:** 540–548
21 Kovach J. S., Hartmann A., Blaszyk H., Cunningham J., Schaid D. and Sommer S. S. (1996) Mutation detection by highly sensitive methods indicates that p53 gene mutations in breast cancer can have important prognostic value. Proc. Natl. Acad. Sci. USA **93:** 1093–1096
22 Bergh J., Norberg T., Sjogren S., Lindgren A. and Holmberg L. (1995) Complete sequencing of the p53 gene provides prognostic information in breast cancer patients, particularly in relation to adjuvant systemic therapy and radiotherapy. Nature Med. **1:** 1029–1034
23 Aas T., Borresen A. L., Geisler S., Smith-Sorensen B., Johnsen H., Varhaug J. E. et al. (1996) Specific P53 mutations are associated with de novo resistance to doxorubicin in breast cancer patients. Nature Med. **2:** 811–814
24 Formenti S. C., Dunnington G., Uzieli B., Lenz H., Keren-Rosenberg S., Silberman H. et al. (1997) Original p53 status predicts for pathological response in locally advanced breast cancer patients treated preoperatively with continuous infusion 5-fluorouracil and radiation therapy. Int. J. Radiat. Oncol. Biol. Phys. **39:** 1059–1068
25 Honda K., Sbisa E., Tullo A., Papeo P. A., Saccone C., Poole S. et al. (1998) p53 mutation is a poor prognostic indicator for survival in patients with hepatocellular carcinoma undergoing surgical tumour ablation. Br. J. Cancer **77:** 776–782
26 Pricolo V. E., Finkelstein S. D., Hansen K., Cole B. F. and Bland K. I. (1997) Mutated p53 gene is an independent adverse predictor of survival in colon carcinoma. Arch. Surg. **132:** 371–374; discussion 374–375
27 Backe J., Gassel A. M., Hauber K., Krebs S., Bartek J., Caffier H. et al. (1997) p53 protein in endometrial cancer is related to proliferative activity and prognosis but not to expression of p21 protein. Int. J. Gynecol. Pathol. **16:** 361–368
28 Mitsudomi T., Oyama T., Kusano T., Osaki T., Nakanishi R. and Shirakusa T. (1993) Mutations of the p53 gene as a predictor of poor prognosis in patients with non-small-cell lung cancer. J. Natl. Cancer Inst. **85:** 2018–2023
29 Huang C. L., Taki T., Adachi M., Konishi T., Higashiyama M., Kinoshita M. et al. (1998) Mutations of p53 and K-ras genes as prognostic factors for non-small cell lung cancer. Int. J. Oncol. **12:** 553–563
30 Cordon-Cardo C., Latres E., Drobnjak M., Oliva M. R., Pollack D., Woodruff J. M. et al. (1994) Molecular abnormalities of mdm2 and p53 genes in adult soft tissue sarcomas. Cancer Res. **54:** 794–799
31 Bardeesy N., Falkoff D., Petruzzi M. J., Nowak N., Zabel B., Adam M. et al. (1994) Anaplastic Wilms' tumour, a subtype displaying poor prognosis, harbours p53 gene mutations. Nature Genet. **7:** 91–97
32 Esrig D., Elmajian D., Groshen S., Freeman J. A., Stein J. P., Chen S. C. et al. (1994) Accumulation of nuclear p53 and tumor progression in bladder cancer. N. Engl. J. Med. **331:** 1259–1264
33 Koch W. M., Brennan J. A., Zahurak M., Goodman S. N., Westra W. H., Schwab D. et al. (1996) p53 mutation and locoregional treatment failure in head and neck squamous cell carcinoma. J. Natl. Cancer Inst. **88:** 1580–1586
34 Pollack I. F., Hamilton R. L., Finkelstein S. D., Campbell J. W., Martinez A. J., Sherwin R. N. et al. (1997) The relationship between TP53 mutations and overexpression of p53 and prognosis in malignant gliomas of childhood. Cancer Res. **57:** 304–309
35 Falette N., Paperin M. P., Treilleux I., Gratadour A. C., Peloux N., Mignotte H. et al. (1998) Prognostic value of P53 gene mutations in a large series of node-negative breast cancer patients. Cancer Res. **58:** 1451–1455
36 Bardeesy N., Beckwith J. B., and Pelletier J. (1995) Clonal expansion and attenuated apoptosis in Wilms' tumors are associated with p53 gene mutations. Cancer Res. **55:** 215–219
37 Moll U. M., Ostermeyer A. G., Ahomadegbe J. C., Mathieu M. C. and Riou G. (1995) p53 mediated tumour cell response to chemotherapeutic DNA damage: a preliminary study in matched pairs of breast cancer biopsies. Hum. Pathol. **26:** 1293–1301
38 Fontanini G., Vignati S., Lucchi M., Mussi A., Calcinai A., Boldrini L. et al. (1997) Neoangiogenesis and p53 protein in lung cancer: their prognostic role and their relation with vascular endothelial growth factor (VEGF) expression. Br. J. Cancer **75:** 1295–1301
39 Takahashi Y., Bucana C. D., Cleary K. R. and Ellis L. M. (1998) p53, vessel count, and vascular endothelial growth factor expression in human colon cancer. Int. J. Cancer **79:** 34–38
40 Tada M., Iggo R. D., Ishii N., Shinohe Y., Sakuma S., Estreicher A. et al. (1996) Clonality and stability of the p53 gene in human astrocytic tumor cells: quantitative analysis of p53 gene mutations by yeast functional assay. Int. J. Cancer **67:** 447–450
41 Linke S. P., Clarkin K. C., Di Leonardo A., Tsou A. and Wahl G. M. (1996) A reversible, p53-dependent G0/G1 cell cycle arrest induced by ribonucleotide depletion in the absence of detectable DNA damage. Genes Dev. **10:** 934–947
42 Lotem J., Peled-Kamar M., Groner Y. and Sachs L. (1996) Cellular oxidative stress and the control of apoptosis by wild-type p53, cytotoxic compounds and cytokines. Proc. Natl. Acad. Sci. USA **93:** 9166–9171
43 Serrano M., Lin A. W., McCurrach M. E., Beach D. and Lowe S. W. (1997) Oncogenic ras provokes premature cell

senescence associated with accumulation of p53 and p16INK4a. Cell **88:** 593–602
44 Lowe S. W., Schmitt E. M., Smith S. W., Osborne B. A. and Jacks T. (1993) p53 is required for radiation-induced apoptosis in mouse thymocytes. Nature **362:** 847–849
45 Clarke A. R., Purdie C. A., Harrison D. J., Morris R. G., Bird C. C., Hooper M. L. et al. (1993) Thymocyte apoptosis induced by p53-dependent and independent pathways. Nature **362:** 849–852
46 Kastan M. B., Zhan Q., El Deiry W., Carrier F., Jacks T., Walsh W. V. et al. (1992) A mammalian cell cycle checkpoint pathway utilizing p53 and GADD45 is defective in ataxia-telangiectasia. Cell **71:** 587–597
47 Slichenmyer W. J., Nelson W. G., Slebos R. J. and Kastan M. B. (1993) Loss of a p53-associated G1 checkpoint does not decrease cell survival following DNA damage. Cancer Res. **53:** 4164–4168
48 Guillouf C., Rosselli F., Krishnaraju K., Moustacchi E., Hoffman B. and Liebermann D. A. (1995) p53 involvement in control of G2 exit of the cell cycle: role in DNA damage-induced apoptosis. Oncogene **10:** 2263–2270
49 Di Leonardo A., Khan S. H., Linke S. P., Greco V., Seidita G. and Wahl G. M. (1997) DNA rereplication in the presence of mitotic spindle inhibitors in human and mouse fibroblasts lacking either p53 or pRb function. Cancer Res. **57:** 1013–1019
50 Cross S. M., Sanchez C. A., Morgan C. A., Schimke M. K., Ramel S., Idzerda R. L. et al. (1995) A p53-dependent mouse spindle checkpoint. Science **267:** 1353–1356
51 Minn A. J., Boise L. H. and Thompson C. B. (1996) Expression of Bcl-xL and loss of p53 can cooperate to overcome a cell cycle checkpoint induced by mitotic spindle damage. Genes Dev. **10:** 2621–2631
52 Lanni J. S. and Jacks T. (1998) Characterization of the p53-dependent postmitotic checkpoint following spindle disruption. Mol. Cell. Biol. **18:** 1055–1064
53 Lowe S. W., Bodis S., McClatchey A., Remington L., Ruley H. E., Fisher D. E. et al. (1994) p53 status and the efficacy of cancer therapy in vivo. Science **266:** 807–810
54 Merritt A. J., Potten C. S., Kemp C. J., Hickman J. A., Balmain A., Lane D. P. et al. (1994) The role of p53 in spontaneous and radiation-induced apoptosis in the gastrointestinal tract of normal and p53-deficient mice. Cancer Res. **54:** 614–617
55 Sachs L. and Lotem J. (1993) Control of programmed cell death in normal and leukemic cells: new implications for therapy. Blood **82:** 15–21
56 Clarke A. R., Gledhill S., Hooper M. L., Bird C. C. and Wyllie A. H. (1994) p53 dependence of early apoptotic and proliferative responses within the mouse intestinal epithelium following gamma-irradiation. Oncogene **9:** 1767–1773
57 Lowe S. W., Ruley H. E., Jacks T. and Housman D. E. (1993) p53-dependent apoptosis modulates the cytotoxicity of anticancer agents. Cell **74:** 957–967
58 Waldman T., Kinzler K. W. and Vogelstein B. (1995) p21 is necessary for the p53-mediated G1 arrest in human cancer cells. Cancer Res. **55:** 5187–5190
59 Deng C., Zhang P., Harper J. W., Elledge S. J. and Leder P. (1995) Mice lacking p21CIP1/WAF1 undergo normal development, but are defective in G1 checkpoint control. Cell **82:** 675–684
60 Brugarolas J., Chandrasekaran C., Gordon J. I., Beach D., Jacks T. and Hannon G. J. (1995) Radiation-induced cell cycle arrest compromised by p21 deficiency. Nature **377:** 552–557
61 Brown J. P., Wei W., and Sedivy J. M. (1997) Bypass of senescence after disruption of p21CIP1/WAF1 gene in normal diploid human fibroblasts. Science **277:** 831–834
62 Waldman T., Lengauer C., Kinzler K. W. and Vogelstein B. (1996) Uncoupling of S phase and mitosis induced by anticancer agents in cells lacking p21. Nature **381:** 713–716
63 Waldman T., Zhang Y., Dillehay L., Yu J., Kinzler K., Vogelstein B. et al. (1997) Cell-cycle arrest versus cell death in cancer therapy. Nature Med. **3:** 1034–1036
64 Polyak K., Xia Y., Zweier J. L., Kinzler K. W. and Vogelstein B. (1997) A model for p53-induced apoptosis. Nature **389:** 300–305
65 Fan S., el-Deiry W. S., Bae I., Freeman J., Jondle D., Bhatia K. et al. (1994) p53 gene mutations are associated with decreased sensitivity of human lymphoma cells to DNA damaging agents. Cancer Res. **54:** 5824–5830
66 Iwadate Y., Tagawa M., Fujimoto S., Hirose M., Namba H., Sueyoshi K. et al. (1998) Mutation of the p53 gene in human astrocytic tumours correlates with increased resistance to DNA-damaging agents but not to anti-microtubule anti-cancer agents. Br. J. Cancer **77:** 547–551
67 Weinstein J. N., Myers T. G., O'Connor P. M., Friend S. H., Fornace A. J. Jr., Kohn K. W. et al. (1997) An information-intensive approach to the molecular pharmacology of cancer. Science **275:** 343–349
68 Xia F., Wang X., Wang Y. H., Tsang N. M., Yandell D. W., Kelsey K. T. 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
69 Wattel E., Preudhomme C., Hecquet B., Vanrumbeke M., Quesnel B., Dervite I. et al. (1994) p53 mutations are associated with resistance to chemotherapy and short survival in hematologic malignancies. Blood **84:** 3148–3157
70 Wilson W. H., Teruya-Feldstein J., Fest T., Harris C., Steinberg S. M., Jaffe E. S. et al. (1997) Relationship of p53, bcl-2 and tumour proliferation to clinical drug resistance in non-Hodgkin's lymphomas. Blood **89:** 601–609
71 Diccianni M. B., Yu J., Hsiao M., Mukherjee S., Shao L. E. and Yu A. L. (1994) Clinical significance of p53 mutations in relapsed T-cell acute lymphoblastic leukemia. Blood **84:** 3105–3112
72 Clahsen P. C., van de Velde C. J., Duval C., Pallud C., Mandard A. M., Delobelle D. A. et al. (1998) p53 protein accumulation and response to adjuvant chemotherapy in premenopausal women with node-negative early breast cancer. J. Clin. Oncol. **16:** 470–479
73 Berns E. M., Klijn J. G., van Putten W. L., de Witte H. H., Look M. P., Meijer-van Gelder M. E. et al. (1998) p53 protein accumulation predicts poor response to tamoxifen therapy of patients with recurrent breast cancer. J. Clin. Oncol. **16:** 121–127
74 Degeorges A., de Roquancourt A., Extra J. M., Espie M., Bourstyn E., de Cremoux P. et al. (1998) Is p53 a protein that predicts the response to chemotherapy in node negative breast cancer? Breast Cancer Res. Treat. **47:** 47–55
75 Rozan S., Vincent-Salomon A., Zafrani B., Validire P., De Cremoux P., Bernoux A. et al. (1998) No significant predictive value of c-erbB-2 or p53 expression regarding sensitivity to primary chemotherapy or radiotherapy in breast cancer. Int. J. Cancer **79:** 27–33
76 Veronese S., Mauri F. A., Caffo O., Scaioli M., Aldovini D., Perrone G. et al. (1998) Bax immunohistochemical expression in breast carcinoma: a study with long term follow-up. Int. J. Cancer **79:** 13–18
77 Krajewski S., Blomqvist C., Franssila K., Krajewska M., Wasenius V. M., Niskanen E. et al. (1995) Reduced expression of proapoptotic gene BAX is associated with poor response rates to combination chemotherapy and shorter survival in women with metastatic breast adenocarcinoma. Cancer Res. **55:** 4471–4478
78 Kapranos N., Karaiosifidi H., Valavanis C., Kouri E. and Vasilaros S. (1997) Prognostic significance of apoptosis related proteins Bcl-2 and Bax in node-negative breast cancer patients. Anticancer Res. **17:** 2499–2505
79 Bargou R. C., Daniel P. T., Mapara M. Y., Bommert K., Wagener C., Kallinich B. et al. (1995) Expression of the bcl-2 gene family in normal and malignant breast tissue: low bax-alpha expression in tumor cells correlates with resistance towards apoptosis. Int. J. Cancer **60:** 854–859

80 Sakakura C., Sweeney E. A., Shirahama T., Igarashi Y., Hakomori S., Tsujimoto H. et al. (1997) Overexpression of bax enhances the radiation sensitivity in human breast cancer cells. Surg. Today **27:** 90–93
81 Sakakura C., Sweeney E. A., Shirahama T., Igarashi Y., Hakomori S., Nakatani H. et al. (1996) Overexpression of bax sensitizes human breast cancer MCF-7 cells to radiation-induced apoptosis. Int. J. Cancer **67:** 101–105
82 Wagener C., Bargou R. C., Daniel P. T., Bommert K., Mapara M. Y., Royer H. D. et al. (1996) Induction of the death-promoting gene bax-alpha sensitizes cultured breast-cancer cells to drug-induced apoptosis. Int. J. Cancer **67:** 138–141
83 Chresta C. M. and Hickman J. A. (1996) Oddball p53 in testicular tumors. Nature Med. **2:** 745–746
84 Chresta C. M., Masters J. R. and Hickman J. A. (1996) Hypersensitivity of human testicular tumors to etoposide-induced apoptosis is associated with functional p53 and a high Bax:Bcl-2 ratio. Cancer Res. **56:** 1834–1841
85 Chin K. V., Ueda K., Pastan I. and Gottesman M. M. (1992) Modulation of activity of the promoter of the human MDR1 gene by Ras and p53. Science **255:** 459–462
86 Preudhomme C., Lepelley P., Vachee A., Soenen V., Quesnel B., Cosson A. et al. (1993) Relationship between p53 gene mutations and multidrug resistance (mdr1) gene expression in myelodysplastic syndromes. Leukemia **7:** 1888–1890
87 Schneider J., Rubio M. P., Barbazan M. J., Rodriguez-Escudero F. J., Seizinger B. R. and Castresana J. S. (1994) P-glycoprotein, HER-2/neu, and mutant p53 expression in human gynecologic tumors. J. Natl. Cancer Inst. **86:** 850–855
88 Nabeya Y., Loganzo F. Jr., Maslak P., Lai L., de Oliveira A. R., Schwartz G. K. et al. (1995) The mutational status of p53 protein in gastric and esophageal adenocarcinoma cell lines predicts sensitivity to chemotherapeutic agents. Int. J. Cancer **64:** 37–46
89 O'Connor P. M., Jackman J., Jondle D., Bhatia K., Magrath I. and Kohn K. W. (1993) Role of the p53 tumor suppressor gene in cell cycle arrest and radiosensitivity of Burkitt's lymphoma cell lines. Cancer Res. **53:** 4776–4780
90 Liu T. J., Zhang W. W., Taylor D. L., Roth J. A., Goepfert H. and Clayman G. L. (1994) Growth suppression of human head and neck cancer cells by the introduction of a wild-type p53 gene via a recombinant adenovirus. Cancer Res. **54:** 3662–3667
91 Roth J. A., Nguyen D., Lawrence D. D., Kemp B. L., Carrasco C. H., Ferson D. Z. et al. (1996) Retrovirus-mediated wild-type p53 gene transfer to tumors of patients with lung cancer. Nature Med. **2:** 985–991
92 Sengelov L., Horn T. and Steven K. (1997) p53 nuclear immunoreactivity as a predictor of response and outcome following chemotherapy for metastatic bladder cancer. J. Cancer Res. Clin. Oncol. **123:** 565–570
93 Cote R. J., Esrig D., Groshen S., Jones P. A. and Skinner D. G. (1997) p53 and treatment of bladder cancer. Nature **385:** 123–125
94 Ribeiro J. C., Barnetson A. R., Fisher R. J., Mameghan H. and Russell P. J. (1997) Relationship between radiation response and p53 status in human bladder cancer cells. Int. J. Radiat. Biol. **72:** 11–20
95 Hanzal E., Gitsch G., Kohlberger P., Dadak C., Miechowiecka N. and Breitenecker G. (1992) Immunohistochemical detection of mutant p53-suppressor gene product in patients with breast cancer: influence on metastasis-free survival. Anticancer Res. **12:** 2325–2329
96 Ostrowski J. L., Sawan A., Henry L., Wright C., Henry J. A., Hennessy C. et al. (1991) p53 expression in human breast cancer related to survival and prognostic factors: an immunohistochemical study. J. Pathol. **164:** 75–81
97 Jost C. A., Marin M. C. and Kaelin W. J. (1997) p73 is a human p53-related protein that can induce apoptosis. Nature **389:** 191–194
98 Kaghad M., Bonnet H., Yang A., Creancier L., Biscan J. C., Valent A. et al. (1997) Monoallelically expressed gene related to p53 at 1p36, a region frequently deleted in neuroblastoma and other human cancers. Cell **90:** 809–819
99 Bian J. and Sun Y. (1997) p53CP, a putative p53 competing protein that specifically binds to the consensus p53 DNA binding sites: a third member of the p53 family? Proc. Natl. Acad. Sci. USA **94:** 14753–14758
100 Davidoff A. M., Humphrey P. A., Iglehart J. D. and Marks J. R. (1991) Genetic basis for p53 overexpression in human breast cancer. Proc. Natl. Acad. Sci. USA **88:** 5006–5010
101 Casey G., Lopez M. E., Ramos J. C., Plummer S. J., Arboleda M. J., Shaughnessy M. 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
102 Jacquemier J., Moles J. P., Penault-Llorca F., Adelaide J., Torrente M., Viens P. et al. (1994) p53 immunohistochemical analysis in breast cancer with four monoclonal antibodies: comparison of staining and PCR-SSCP results. Br. J. Cancer **69:** 846–852
103 Barnes D. M., Hanby A. M., Gillett C. E., Mohammed S., Hodgson S., Bobrow L. G. et al. (1992) Abnormal expression of wild type p53 protein in normal cells of a cancer family patient. Lancet **340:** 259–263
104 Sjogren S., Inganas M., Norberg T., Lindgren A., Nordgren H., Holmberg L. et al. (1996) The p53 gene in breast cancer: prognostic value of complementary DNA sequencing versus immunohistochemistry. J. Natl. Cancer Inst. **88:** 173–182
105 Flaman J. M., Frebourg T., Moreau V., Charbonnier F., Martin C., Chappuis P. et al. (1995) A simple p53 functional assay for screening cell lines, blood and tumors. Proc. Natl. Acad. Sci. USA **92:** 3963–3967
106 Greenblatt M. S., Bennett W. P., Hollstein M. and Harris C. C. (1994) Mutations in the p53 tumour suppressor gene: clues to cancer etiology and molecular pathogenesis. Cancer Res. **54:** 4855–4878
107 Fearon E. R. and Vogelstein B. (1990) A genetic model for colorectal tumorigenesis. Cell **61:** 759–767
108 Lotem J. and Sachs L. (1993) Hematopoietic cells from mice deficient in wild-type p53 are more resistant to induction of apoptosis by some agents. Blood **82:** 1092–1096
109 Taubert H., Meye A. and Wurl P. (1996) Prognosis is correlated with p53 mutation type for soft tissue sarcoma patients. Cancer Res. **56:** 4134–4136
110 Vega F. J., Iniesta P., Caldes T., Sanchez A., Lopez J. A., de Juan C. et al. (1997) p53 exon 5 mutations as a prognostic indicator of shortened survival in non-small-cell lung cancer. Br. J. Cancer **76:** 44–51
111 Preudhomme C., Dervite I., Wattel E., Vanrumbeke M., Flactif M., Lai J. L. et al. (1995) Clinical significance of p53 mutations in newly diagnosed Burkitt's lymphoma and acute lymphoblastic leukemia: a report of 48 cases. J. Clin. Oncol. **13:** 812–820
112 Leach F. S., Tokino T., Meltzer P., Burrell M., Oliner J. D., Smith S. et al. (1993) p53 Mutation and MDM2 amplification in human soft tissue sarcomas. Cancer Res. **53:** 2231–2234
113 Bueso R. C., Manshouri T., Haidar M. A., Yang Y., McCown P., Ordonez N. et al. (1996) Abnormal expression of MDM-2 in breast carcinomas. Breast Cancer Res. Treat. **37:** 179–188
114 Barbareschi M., Girlando S., Fellin G., Graffer U., Luciani L. and Dalla P. P. (1995) Expression of mdm-2 and p53 protein in transitional cell carcinoma. Urol. Res. **22:** 349–352
115 Ehrmann J. J., Kolar Z., Vojtesek B., Kala M., Komenda S. and Oulton A. (1997) Prognostic factors in astrocytomas: relationship of p53, MDM-2, BCL-2 and PCNA immunohistochemical expression to tumor grade and overall patient survival. Neoplasma **44:** 299–304
116 Jiang M., Shao Z. M., Wu J., Lu J. S., Yu L. M., Yuan J. D. et al. (1997) p21/waf1/cip1 and mdm-2 expression in breast carcinoma patients as related to prognosis. Int. J. Cancer **74:** 529–534

117 Gorgoulis V. G., Rassidakis G. Z., Karameris A. M., Papastamatiou H., Trigidou R., Veslemes M. et al. (1996) Immunohistochemical and molecular evaluation of the mdm-2 gene product in bronchogenic carcinoma. Mod. Pathol. **9:** 544–554
118 Zhou M., Yeager A. M., Smith S. D. and Findley H. W. (1995) Overexpression of the MDM2 gene by childhood acute lymphoblastic leukemia cells expressing the wild-type p53 gene. Blood **85:** 1608–1614
119 Howley P. M. (1991) Role of the human papillomaviruses in human cancer. Cancer Res. **51:** 5019s–5022s
120 Garkavtsev I., Grigorian I. A., Ossovskaya V. S., Chernov M. V., Chumakov P. M. and Gudkov A. V. (1998) The candidate tumour suppressor p33ING1 cooperates with p53 in cell growth control. Nature **391:** 295–298
121 Kamijo T., Zindy F., Roussel M. F., Quelle D. E., Downing J. R., Ashmun R. A. et al. (1997) Tumor suppression at the mouse INK4a locus mediated by the alternative reading frame product p19ARF. Cell **91:** 649–659
122 Sherr C. (1998) Tumor surveillance via the ARG-p53 pathway. Genes Dev. **12:** 2984–2991
123 de Stanchina E., McCurrach M. E., Zindy F., Shieh S. Y., Ferbeyre G., Samuelson A. V. et al. (1998) E1A signaling to p53 involves the p19(ARF) tumor suppressor. Genes Dev. **12:** 2434–2442
124 Rampino N., Yamamoto H., Ionov Y., Li Y., Sawai H., Reed J. C. et al. (1997) Somatic frameshift mutations in the BAX gene in colon cancers of the microsatellite mutator phenotype. Science **275:** 967–969
125 Yamamoto H., Sawai H., Weber T. K., Rodriguez B. M. and Perucho M. (1998) Somatic frameshift mutations in DNA mismatch repair and proapoptosis genes in hereditary nonpolyposis colorectal cancer. Cancer Res. **58:** 997–1003
126 McCurrach M. E., Connor T. M., Knudson C. M., Korsmeyer S. J. and Lowe S. W. (1997) bax-deficiency promotes drug resistance and oncogenic transformation by attenuating p53-dependent apoptosis. Proc. Natl. Acad. Sci. USA **94:** 2345–2349
127 Mao L., Hruban R. H., Boyle J. O., Tockman M. and Sidransky D. (1994) Detection of oncogene mutations in sputum precedes diagnosis of lung cancer. Cancer Res. **54:** 1634–1637
128 Sidransky D., Von Eschenbach A., Tsai Y. C., Jones P., Summerhayes I., Marshall F. et al. (1991) Identification of p53 gene mutations in bladder cancers and urine samples. Science **252:** 706–709
129 Angelopoulou K., Diamandis E. P., Sutherland D. J., Kellen J. A. and Bunting P. S. (1994) Prevalence of serum antibodies against the p53 tumor suppressor gene protein in various cancers. Int. J. Cancer **58:** 480–487
130 Lubin R., Zalcman G., Bouchet L., Tredanel J., Legros Y., Cazals D. et al. (1995) Serum p53 antibodies as early markers of lung cancer. Nature Med. **1:** 701–702
131 Shaw P., Bovey R., Tardy S., Sahli R., Sordat B. and Costa J. (1992) Induction of apoptosis by wild-type p53 in a human colon tumor-derived cell line. Proc. Natl. Acad. Sci. USA **89:** 4495–4499
132 Fujiwara T., Cai D. W., Georges R. N., Mukhopadhyay T., Grimm E. A. and Roth J. A. (1994) Therapeutic effect of a retroviral wild-type p53 expression vector in an orthotopic lung cancer model. J. Natl. Cancer Inst. **86:** 1458–1462
133 Hupp T. R. and Lane D. P. (1994) Allosteric activation of latent p53 tetramers. Curr. Biol. **4:** 865–875
134 Selivanova G., Iotsova V., Okan I., Fritsche M., Strom M., Groner B. et al. (1997) Restoration of the growth suppression function of mutant p53 by a synthetic peptide derived from the p53 C-terminal domain. Nature Med. **3:** 632–638
135 O'Connor P. M., Jackman J., Bae I., Myers T. G., Fan S., Mutoh M. et al. (1997) Characterization of the p53 tumor suppressor pathway in cell lines of the National Cancer Institute anticancer drug screen and correlations with the growth-inhibitory potency of 123 anticancer agents. Cancer Res. **57:** 4285–4300
136 Wang Q., Fan S., Eastman A., Worland P. J., Sausville E. A. and O'Connor P. M. (1996) UCN-01: a potent abrogator of G2 checkpoint function in cancer cells with disrupted p53. J. Natl. Cancer Inst. **88:** 956–965
137 Gabrilovich D. I., Cunningham H. T. and Carbone D. P. (1996) IL-12 and mutant P53 peptide-pulsed dendritic cells for the specific immunotherapy of cancer. J. Immunother. Emphasis Tumor Immunol. **19:** 414–418
138 Mayordomo J. I., Loftus D. J., Sakamoto H., De Cesare C. M., Appasamy P. M., Lotze M. T. et al. (1996) Therapy of murine tumors with p53 wild-type and mutant sequence peptide-based vaccines. J. Exp. Med. **183:** 1357–1365
139 Lowe S. W. (1997) Progress of the smart bomb cancer virus. Nature Med. **3:** 606–608
140 Heise C., Sampson-Johannes A., Williams A., McCormick F., Von Hoff D. D. and Kirn D. H. (1997) ONYX-015, an E1B gene-attenuated adenovirus, causes tumor-specific cytolysis and antitumoral efficacy that can be augmented by standard chemotherapeutic agents. Nature Med. **3:** 639–645