Chapter 14

PROGNOSTIC AND PREDICTIVE VALUE OF *TP53* MUTATIONS IN HUMAN CANCER

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INTRODUCTION

Finding reliable molecular markers for early diagnosis, prognosis and prediction of response to treatment is a major challenge for cancer management. A marker of prognosis provides information on the risk of relapse and death independently of treatment, whereas a predicitve marker provides information on the potential benefit of a specific treatment (Lonning, 2003). An early diagnostic marker helps to identify lesions at high risk of malignant transformation. Clinical stage, tumor size and morphological grade are the most reliable factors of prognosis. Among numerous molecular markers that have been tested most recently, only a few are used in clinical practice. In breast cancer for example, estrogen and progesterone receptors are used routinely as predictive markers for tumor response to anti-hormone therapy. However, about 30% of patients with positive receptor status (expected to benefit from anti-hormone treatment) will face a therapeutic failure, showing the limitations of these markers.

The tumor suppressor gene *TP53* plays a key role in many cellular pathways controlling cell proliferation, cell survival and genomic integrity (see other Chapters). It acts in response to various forms of cellular stresses to mediate antiproliferative processes. Disrupting its function promotes checkpoints defects, genomic instability and inappropriate survival, leading to the uncontrolled proliferation of damaged cells. The proliferative

P. Hainaut and K.G. Wiman (eds.), 25 Years of p53 Research, 321-338.

advantage given by its inactivation and the fact that it is ubiquituously expressed explain why it is frequently found mutated in almost every type of cancers (Hainaut and Hollstein, 2000). In addition to its tumor suppressor function, *TP53* also contributes to the anti-neoplastic effects of radio- and chemotherapeutic agents. It has been shown, in various experimental *in vitro* systems as well as in mouse models, that cell cycle arrest or apoptosis induced by radiotherapy and various chemotherapeutic drugs depend on an intact *TP53* pathway (Lowe et al., 1994; O'Connor et al., 1997). These results raise the hypothesis that *TP53* could be a key player in defining tumor-sensitivity to a broad range of anti-cancer treatments in cancer patients. Moreover, the presence of a *TP53* mutation could be one of the underlying causes of drug resistance, which is the major cause of treatment failure and cancer death.

TP53 may thus be a potential marker for malignant transformation, tumor aggressiveness and treatment outcome in a broad range of cancers. Many studies have investigated, in various clinical settings, the predictive value of *TP53* mutation status for tumor response to treatment and patient outcome. Despite these efforts, no consensus has been reached and *TP53* mutation analysis is not yet used in clinical practice. In this chapter we review the pro and con of the use of *TP53* as a biomarker and propose which area of oncology could benefit from its use. We will also discuss study design and methodology issues for the development of a biomarker such as *TP53* and its implementation in clinical settings.

TP53 MUTATION STATUS AND CLINICAL OUTCOME

Although more than 500 studies have investigated the value of *TP53* status as a prognostic and/or predictive marker in various types of cancer, results have often been contradictory. Several reasons can explain this apparent confusion, from differences in study design, heterogeneity in the cohorts to methodology used to assess *TP53* status. Studies that have used gene sequencing to assess *TP53* status, have used different pre-screening methods (none, SSCP, DGGE, CDGE, TTGE, TGGE or IHC), that have different sensitivity. Moreover, a number of studies have only analyzed the central part of the protein. Although this region contains 80% of the mutations, this restricted analysis can lead to the mis-classification of 10 to 20% of the cases. However, the main reason certainly resides in the fact that the majority of studies (about 400/500) have used immuno-histochemistry (IHC) to assess *TP53* status. Earlier observations have shown that the majority of *TP53* mutations are missense mutations that accumulate in cancer cells and thus can be detected by IHC. However, it is now admitted

that IHC is not suitable as a screening method for mutations since not all types of mutations are detected. Sequencing of the complete coding sequence of *TP53* shows that 10 to 25% of the mutations are truncating mutations (nonsense, frameshift or splice site mutations) that are not detected by IHC since they do not lead to a stable protein. Moreover, some cases of IHC positive cells do not carry a mutation but may result from the accumulation of the wild-type protein in response cellular stress signals. Finally, IHC studies have used different antibodies, different labeling procedures and different cut-off value for positive cases. Hence, the use of IHC leads to an unacceptable number of mis-classified cases and to a greater inter-study variability.

When only studies that have used gene sequencing to assess *TP53* mutation status are taken into account, *TP53* appears to be of prognostic value in a variety of cancers. A comprehensive list of such studies is provided on the IARC *TP53* database web site (http://www.iarc.fr/p53/). This information is summarized in Table 1, where the number of studies reporting association or lack of association between the presence of a mutation and either poor or good prognosis (patient survival and/or tumor response to treatment) is indicated. This summary table shows that association with poor prognosis has repeatedly been reported for breast, bladder, head and neck and hematological cancers. Results for colorectal, lung and esophageal cancers are more heterogeneous, but a majority of studies found an association with poor prognosis. For ovarian cancer, results remain contradictory. In brain tumors, the majority of studies show a lack of association with prognosis and two studies report an association with good prognosis. Overall, 65/93 studies found that *TP53* is a statistically significant factor of poor prognosis in various cancers. It should be noted that the majority of studies (20/27) reporting no association between *TP53* status and survival have been done on cohorts of less than 100 patients, which give insufficient statistical power to detect moderate differences in survival. Response to adjuvant chemotherapy or radiotherapy is a major determinant of patient outcome. Among 19 studies that have specifically investigated the association between tumor response to treatment and *TP53* mutation status, 14 have found that the presence of a mutation was associated with a poor response to various chemotherapy or radiotherapy regimens in breast, head and neck, hematological, colorectal, ovarian, esophageal cancers and soft tissue sarcomas. These observations are in agreement with experimental data showing a key role for *TP53* in the anti-proliferative response induced by various chemotherapeutic agents. Interestingly, one study on ovarian cancer patients showed that *TP53* status was predictive of response to treatment in patients treated with cyclophosphamide and cisplatin but not in patients treated with a paclitaxel/cisplatin regimen (Smith-Sorensen et al., 1998).

Table 1. TP53 mutation and cancer prognosis

	*Studies reporting that the presence of *TP53* mutation is:		
TUMOR SITE	**Significantly associated with bad prognosis**	**Significantly associated with good prognosis**	**No significant association**
BLADDER	3	-	-
BRAIN	1	2	5
BREAST	17	-	3
COLORECTUM	11	-	5
ESOPHAGUS	3	1	1
HEAD&NECK	7	-	-
HEMATOL.	6	-	-
LIVER	1	-	-
LUNG	8	-	4
LYMPH NODES	1	-	-
OVARY	3	1	4
PANCREAS	1	1	-
PROSTATE	-	-	1
RENAL PELVIS	1	-	-
SINUSES	1	-	-
SOFT TISSUES	1	-	-
Total	65	5	23

*The number of studies is indicated. Data from the IARC *TP53* Database (R9, July 2004), which includes only published studies where *TP53* mutation has been analyzed by gene sequencing. Only studies with cohorts of more than 30 patients have been included in this table. The prognostic parameters investigated are patient survival and/or tumor response to treatment.

In a study on 63 advanced breast cancer patients treated with doxorubicin in a neo-adjuvant setting, a strong correlation between lack of response and presence of *TP53* mutation was observed. The same was seen for 35 breast cancer patients treated with FUMI (5 fluorouracil and Mitomycin C) in a neo-adjuvant setting (Geisler et al., 2003). These observations are in agreement with the fact that DNA-damaging agents have been shown to induce p53-dependent apoptosis whereas paclitaxel (a microtubule stabilizing agent) effects are expected to be independent of p53 function. In 2000, Berns *et al* reported that patients with *TP53* mutation showed the lowest response to tamoxifen on a series of 243 breast cancer patients. This effect was observed in ER positive patients only, suggesting that ER-dependent response to anti-hormone therapy may also depend on an intact *TP53* pathway (Berns et al., 2000).

It is not clear from the available studies whether the prognostic value of *TP53* for the overall survival of patient depends on the administration of adjuvant treatment, or if it has also a value for patients receiving only surgery. In one study of colorectal tumors where patients treated with surgery alone were included, the presence of mutations in specific regions was correlated with a shorter survival (Borresen-Dale et al., 1998). In another study on colorectal cancer, the survival of patients treated with surgery only was compared with patients receiving adjuvant chemotherapy in addition to surgery. It showed that survival was strongly correlated with the presence of *TP53* mutations in the entire cohort. However, when only patients undergoing a radical resection were considered, *TP53* mutation status was no longer of prognostic significance (Tortola et al., 1999). In esophageal cancer, *TP53* alterations (*TP53* mutation plus positive immunostaining) but not *TP53* mutation alone, was found to be significantly associated with shorter overall and disease-free survival in 91 patients treated by surgery only (Casson et al., 2003). Although these results remain to be confirmed and extended, they suggest that *TP53* mutation has a weak prognostic value, if any, in patients treated with surgery only. The capacity of *TP53* to mediate tumor response to chemotherapeutic drugs may thus be the main mechanism explaining its prognostic value.

TP53 MUTATION TYPE AND CLINICAL OUTCOME

There is now much *in vitro* experimental evidence showing that different types of *TP53* mutations have different functional consequences. Unlike other tumor suppressor genes that are inactivated by insertions or deletions leading to an absence of protein expression, most *TP53* mutations are missense mutations that lead to the over-expression of a mutant protein. More than 1800 different missense mutations have been reported in human cancer and functional assays have shown that mutant proteins show a great variability in their functional activities (see *TP53* Function Database, http://www.iarc.fr/P53). WT p53 function relies mainly on the capacity to transactivate target genes through binding to specific response elements. Loss of function (LOF) is the main consequences of missense mutations, however some mutants also exert dominant-negative effects (DNE) or show gain of function (GOF) properties. DNE corresponds to the capacity of the mutant protein to complex with the product of the remaining wild-type allele to inactivate its function. DNE results in the total abrogation of p53 protein function, even if there is still a wild-type protein expressed in the cell (Milner et al., 1991). GOF corresponds to the acquisition of novel properties by mutant p53 that do not depend upon the presence of wild-type p53

(Cadwell and Zambetti, 2001). All hotspot mutations known so far lead to a loss of specific trans-activation capacity, but the degree of LOF vary between mutants. Missense mutations outside the central DNA-binding domain more often retain transcriptional activity on a variety of promoters than mutations within the DNA-binding domain (Kato et al., 2003; Resnick and Inga, 2003). In addition to various degree of LOF, some mutant proteins exert various degrees of DNE (see *TP53* Function Database, http://www.iarc.fr/P53). It has been proposed more than 10 years ago that mutant p53 may exert pro-oncogenic effects, and that mutation was turning p53 into some kind of oncogene (Lane and Benchimol, 1990; Oren, 1992). There is now good evidence that mutant p53 can promote cancer through a GOF mechanism, such as promotion of gene amplification, or resistance to drug-induced apoptosis (reviewed in (Sigal and Rotter, 2000)). Several mutants have been shown to transactivate or potentiate the transactivation of genes such as *MDR1*, (Dittmer et al., 1993), *EGFR*, *c-MYC*, *PCNA, IGF-II* or *VEGF* (see *TP53* Function Database, http://www.iarc.fr/P53). These genes are not transactivated by the wild-type p53 protein and do not necessary possess a p53 binding-site. Mutant p53 proteins can also interact with a network of proteins that differ from wt p53. For example, some mutant p53 can form stable complexes with the products of other members of the *TP53* gene family, p63 and p73, blocking their transactivation capacity (see other chapters).

These observations suggest that different mutations may have different biological consequences *in vivo* and have led several investigators to explore whether tumor progression and tumor response to therapy may depend on the nature and localization of *TP53* mutations. In 1995, a study on colorectal cancer and another on breast cancer, have found that mutations affecting regions involved in zinc binding were of worse prognosis than others in term of survival (Borresen et al., 1995; Goh et al., 1995). Table 2 gives a summary of all studies that have found an association between the presence of specific *TP53* mutations and poor prognosis. It shows that, in various cancers, mutations affecting residues involved in zinc binding and DNA contacts (L2 and L3 loops in the DNA-binding domain) are associated with a worse prognosis than others. In breast cancer for example, it has been shown that mutations disrupting the zinc binding domain were associated with primary resistance to doxorubicin and were predicitive of an early relapse (Aas et al., 1996; Geisler et al., 2001). Similar findings were observed in another small cohort of advanced breast cancer patients treated with FUMI (5-fluorouracil and mitomycin-C) (Geisler et al., 2003). These mutations have also been found to be associated with a poor response to tamoxifen in a cohort of breast cancer patients (Berns et al., 2000), and with a shorter survival in lung and head and neck cancers (see Table 2). Functional

assessments of the most common missense mutations falling within the L2-L3 loops show loss of transactivation activity towards most p53-target genes, resulting in defects in p53-dependent responses such as cell-cycle arrest or apoptosis (Aurelio et al., 2000; Ory et al., 1994). These properties fit well with the observations in cancer patients and are in agreement with a major role for *TP53* in the anti-proliferative response induced by radio- and chemo-therapeutic agents.

Table 2. Specific *TP53* mutations associated with poor prognosis in cancer.

Tumor site	Country	Mutation frequency	Region associated with poor prognosis	References
BREAST	Australia	178/1037 (17%)	Exon 4	(Powell et al., 2000)
BREAST	Austria	42/205 (20%)	L2/L3 loops	(Kucera et al., 1999)
BREAST	Brazil	33/242 (14%)	DNA/Zn binding	(Nagai et al., 2003)
BREAST	Denmark	74/315 (23%)	DNA/Zn binding	(Alsner et al., 2000)
BREAST	Japan	30/76 (40%)	DNA contact	(Takahashi et al., 2000)
BREAST	Norway	?/119 (?%)	L2/L3 loops	(Borresen et al., 1995)
BREAST	Norway	26/90 (29%)	L2/L3 loops	(Aas et al., 1996; Geisler et al., 2001)
BREAST	Norway	18/35 (51%)	L2/L3 loops	(Geisler et al., 2003)
BREAST	Sweden	69/315 (22%)	Conserved regions II and V	(Bergh et al., 1995)
BREAST	Sweden	21/123 (17%)	Zn binding	(Gentile et al., 1999)
BREAST	The Netherlands	53/177 (30%)	DNA contact	(Berns et al., 1998)
COLON	USA	665/1464 (45%)	Codon 245	(Samowitz et al., 2002)
COLORECTUM	Norway	102/222 (46%)	L3 loop	(Borresen-Dale et al., 1998)
COLORECTUM	Singapore	109/192 (57%)	Non-conserved regions, codon 175	(Goh et al., 1995)
COLORECTUM	Sweden	99/189 (52%)	Non-conserved regions	(Kressner et al., 1999)
ESOPHAGUS	Japan	78/138 (56%)	L2/L3 loops	(Kihara et al., 2000)
HEAD AND	France	40/105 (38%)	DNA contact	(Temam et

Tumor site	Country	Mutation frequency	Region associated with poor prognosis	References
NECK				al., 2000)
HEAD AND NECK	Germany	39/86 (45%)	DNA contact	(Erber et al., 1998)
HEAD AND NECK	Italy	40/70 (57%)	Missense mutations on codon72 Arg allele	(Bergamaschi et al., 2003)
HEAD AND NECK	Japan	51/121 (42%)	Conserved regions and DNA/Zn binding	(Yamazaki et al., 2003)
LUNG	Europe	34/151 (22%)	Null mutations	(de Anta et al., 1997)
LUNG	Japan	75/204 (37%)	Exon 8	(Huang et al., 1998)
LUNG	Japan	65/144 (45%)	Null mutations	(Hashimoto et al., 1999)
LUNG	Japan	49/103 (47%)	Missense mutations	(Tomizawa et al., 1999)
LUNG	Norway	83/148 (56%)	L2/L3 loops	(Skaug et al., 2000)
LUNG	USA	107/188 (57%)	truncating/structural/ DNA contact mutants	(Ahrendt et al., 2003)
OVARY	Germany/ USA	99/178 (57%)	Conserved domains	(Reles et al., 2001)
OVARY	Norway		Missense on Arg ??	(Wang et al., 2004)
OVARY	USA	125/267 (47%)	Null mutations	(Rose et al., 2003)
SOFT TISSUE	Germany	15/145 (10%)	Non-frameshift mutations	(Taubert et al., 1996)

Data from the IARC *TP53* Database (R9, July 2004).

A question that remains to be fully elucidated is how the GOF activities observed for certain mutant proteins specifically affect tumor response to treatment, tumor aggressiveness and patient outcome. Among the various GOF described for mutant p53 proteins, the capacity to interact with the *TP53* family members, p63 and p73, provides interesting clues. There are now several examples showing that TAp73 is induced by various chemotherapeutic drugs, and that this activation results in the selective activation of apoptosis-related target genes (reviewed in (Gasco and Crook, 2003)). Cell assays have shown that some tumor-derived p53 mutant are able to bind to and inhibit TAp73 transactivation function (Di Como et al., 1999;

Monti et al., 2003; Strano et al., 2000). Such mutant p53 proteins are thus expected to confer a drug-resistant phenotype to tumors, due to the combined loss of p53 anti-proliferative activities and inhibition of p73 pro-apoptotic function. This hypothesis is substanciated by a recent study on advanced head and neck cancer, which showed that the efficiency with which p53 mutants inhibit TAp73-dependent apoptosis was related to efficacy of cisplatin-based chemo-radio-therapy (Bergamaschi et al., 2003).

A *TP53* polymorphism at codon 72, encoding an Arg or a Pro, has been shown to affect some *TP53* activities *in vitro*. The Arg72 variant is more susceptible to degradation by HPV E6 protein (Storey et al., 1998) and is more potent in inducing apoptosis (Dumont et al., 2003). Inhibition of TAp73 by some p53 mutants is enhanced if they expressed the arginine rather than the proline allele (Marin et al., 2000). In the study on advanced head and neck cancer by Bergamaschi *et al*, tumors carrying a p53 mutant capable of TAp73 inhibition and expressing the arginine allele had lower response rates than those expressing the same mutant with a proline allele (Bergamaschi et al., 2003). These results suggest that the two polymorphic variants of p53 are functionally distinct and that *TP53* codon72 polymorphism may influence individual responsiveness to cancer therapy.

TP53 MUTATION FOR EARLY DETECTION AND FOLLOW-UP

Although *TP53* mutations are found in almost any types of cancers, the timing of occurrence of the mutation during cancer progression is extremely variable from one cancer to another. In the classical model of stepwise progression of colorectal cancers, Fearon and Vogelstein have identified that *TP53* mutation and loss of alleles preferentially occur at the transition between late adenoma and carcinoma *in situ*, that is, at a relatively late stage in the histopathological development of these lesions (Fearon and Vogelstein, 1990). Similar findings have been reported in many common cancers, including breast and prostate cancers, although *TP53* mutations have been seen in atypical hyperplasia and DSCIS of the breast (Chitemerere et al., 1996).

In contrast, *TP53* mutation has been reported to occur at an early stage in many types of cancer that are directly caused by exogenous carcinogens. It is the case for lung cancers of smokers, non-melanoma skin cancers after exposure to UV irradiation, head and neck squamous cell carcinoma and esophageal cancers. In these cancers, *TP53* mutations are often detectable in hyperplastic and dysplastic lesions, as well as in non-involved, apparently normal tissues surrounding the tumor (Hussain et al., 2001; Mandard et al.,

2000). Moreover, the position of *TP53* mutation in the temporal sequence of events leading to cancer is not always constant for similar types of cancer. For example, in hepatocellular carcinoma (HCC), *TP53* mutations are late events in most cancers occurring in the Western population, but are very early events in most cases from West Africa and South-east Asia (Montesano et al., 1997). In these regions, HCC occurs as a consequence of exposure to aflatoxins (hepatocarcinogen contaminant of diet) and HBV. In this context, *TP53* mutations are detectable in cirrhotic liver before the onset of cancer (Livni et al., 1995). Another example is colon cancer. Apart from the well-characterised "late" involvement of *TP53* in polypoid carcinomas, there is evidence that *TP53* mutation can occur at an early stage in serrated carcinoma (Hawkins et al., 2000). *TP53* mutations have also been found in non-tumorous colonic tissue from inflamed regions in patients with ulcerative colitis (UC) (Hussain et al., 2000). In this case, they may result from an endogenous carcinogenic stress (reactive oxygen species and nitrogen species produced by the inflammatory micro-environment due to UC). It should be emphasized that the timing of occurrence of *TP53* mutations is not well established for a majority of cancers. The terms "early" or "late" event are based on the frequency of mutations observed at different tumor stages. It can't be excluded that a late event may correspond to a mutation acquired at an early stage in a tumor detected at an advanced pathological stage, the mutation providing a growth advantage and leading to the rapid development of the tumor. Therefore, screening for *TP53* mutations in dysplastic and early lesions may help identify those lesions that are at a high risk for rapid malignant evolution.

TP53 mutations have been detected in circulating free DNA in the plasma of cancer patients, in feces of patients with colorectal cancer, in the saliva of oral cancer patients, in urine of bladder cancer patients, in sputum of lung cancer patients and in other body fluids. In many instances, these mutations were identical to the ones found in the primary tumour tissue of the patient, thus clearly establishing their tumoral origin. *TP53* mutation screening in these surrogate materials may thus be of potential use in the early detection of malignant lesions in individual at risk for these cancers, and may also be used to detect early relapse during post-treatment follow-up of patients with defined mutations in the primary lesion. In a recent study, we have analysed *TP53* and *KRAS* mutations in free DNA extracted from the plasma of healthy subjects who later developed lung, bladder, larynx, pharynx, oral cancers or leukemias. *TP53* mutations were detected in 9/374 (2.4%) and *KRAS* mutations in 11/1025 (1.0%) subjects. Six *TP53* positive (OR: 3.3; 95% CI: 0.8-13.4) and 3 *KRAS*-positive (OR: 1.0; 95% CI: 0.3-3.4) subjects developed cancer. Thus, *TP53* mutations in plasma of healthy subjects may be associated with subsequent occurrence of some types of

cancers. However, further work is needed to evaluate the reliability and sensitivity of these approaches.

Circulating p53 auto-antibodies have been found in 1 to 50% of cancer patients with solid tumors (see (Soussi, 2000) for review) (Caron et al., 1987; Crawford et al., 1982). They are directed against the N- and C-terminal domains of the protein although the mutations are mainly located in the DNA-binding domain. It has been shown that they are due to a self-immunization process linked to the strong immunogenicity of the p53 mutant protein and that they correlate with the presence of missense mutations and the accumulation of p53 mutant protein in the tumor. They have been associated with high-grade tumors and poor survival in several cancers such as breast, colon, stomach and head and neck. In rare instances, these antibodies have been found in blood samples collected months to years before cancer diagnosis. Monitoring these antibodies could thus serve as early detection markers as well as markers of relapse. However, large inter-individual differences are observed suggesting that the capacity to elicit this humoral response is dependent on the biological and genetic background of the patients. Further studies are thus required to assess the significance of these antibodies in term of specificity and sensitivity for their use in clinical practice.

PERSPECTIVES: BRINGING P53 INTO CLINICAL PRACTICE

The examples discussed above show that *TP53* is a potential useful biomarker for early detection and follow-up of cancer, prediction of patient outcome and response to treatment in several types of cancer. However, its use in clinical practice still requires validation studies to precisely define in which conditions it presents a real advantage over currently available markers. In many studies, the presence of a *TP53* mutation has been found to be associated with classical clinico-pathological predictors of poor survival, ie large tumor size, positive node status, high histological grade, and low hormone receptor contents. It was also found to be associated with markers of increased cell proliferation such as high mitotic frequency and high expression of Ki-67, which are also of prognostic value. Although, in multivariate analysis, *TP53* mutation was often found to be an independent factor of prognosis in various cancer types (http://www.iarc.fr/p53/), the clinical parameters included in the multivariate models vary between studies, rendering results difficult to compare. Further studies should be carried out to specifically address this issue. These studies should be conducted on a large scale and designed with the same standards as drug trials, with well-

characterized cancer cases and well-documented treatment regimens and clinical information.

One of the difficulties of conducting large validation studies is the absence of validated, high-throughput screening technology. So far, mutation analysis still relies on the sequencing of portions of the *TP53* gene to determine the exact nature and position of the mutation. Tumor DNA always contains a proportion of wild-type material due to the presence of wild-type alleles in cancer cells, or of non-cancer cells in the original tissue specimens. Therefore, DNA sequencing needs to be highly sensitive in order to detect mutant DNA against a background of wild-type material. Such sensitivity is not always achieved by standard, automated direct sequencing methods. Thus, *TP53* mutation analysis remains an expensive and labor-intensive work. The most common techniques used for *TP53* mutation analysis include PCR-based assays like single strand conformational polymorphism (SSCP), denaturing gradient gel electrophoresis (DGGE) and its variations, and DNA sequencing. Another method based on yeast functional assays was developed to detect *TP53* mutations (Ishioka et al., 1995; Moshinsky and Wogan, 1997; Scharer and Iggo, 1992a; Scharer and Iggo, 1992b). In this assay, the loss of DNA binding and transcriptional-transactivation function in mutant p53 is detected by the colony color of yeast. A very elegant and reliable assay, SOMA (short oligonucleotide mass assay), has also been developed that involves PCR and mass spectrometry (Laken et al., 1998). This assay is very reliable and enables simultaneous analysis of both strands of the gene. However, it can only detect one specific mutation at a time, which is not suitable for screening the entire TP53 gene. More recently, microarray-based methods have been described. The microarray developed by Affymetrix (Santa Clara, CA) is based on direct hybridization of *TP53* DNA fragments on immobilized oligonucleotides. This array shows good specificity but its sensitivity is still limited and its application in large-scale studies needs to be further evaluated (Ahrendt et al., 1999; Wikman et al., 2000a; Wikman et al., 2000b). Another, commercial array is currently developed by Asper technologies (Tartu, Estonia), based on APEX (Arrayed Primer Extension). APEX uses solid-phase primer extension to incorporate fluorescent terminators into fixed oligos, thus requiring less oligos than methods based on specific hybridization. This method is extremely specific and sensitive and is currently being evaluated for scaling-up (Tonisson et al., 2002). These new array technologies should allow the scaling-up of *TP53* mutation screening in a close future.

The diversity of the type and functional consequences of *TP53* mutations is another issue that needs to be taken into account when analyzing the predictive value of *TP53* mutations. This is a difficult point since a clear knowledge of the biological impact of each specific mutation is still lacking

for the majority of reported mutations. The impact of *TP53* polymorphisms (in particular R/P at codon 72) on the activity of mutant proteins need also to be further explored. It is of note that available studies have used various way of classifying mutation, none of which fully reflecting the biological reality. For example, mutations have often been grouped according to their codon position, despite experimental evidence showing that different amino-acid substitutions at a given codon have different functional impact (Ryan and Vousden, 1998). Gene expression profiling is another area that should help decifering the functional impact of p53 mutants. A recent study on breast cancer has identified subclasses of breast tumors based on gene expression profiles (Sorlie et al., 2001). These tumor subcalsses had different prognosis and had different frequency of *TP53* mutation. Studies on a larger scale are needed to determine if specific types of mutants are associated with specific gene expression profiles.

Future studies should address all these issues to determine to which extent the identification of a *TP53* mutation may help clinicians in the diagnosis of cancer and in the selection of the appropriate therapeutic approach. The availibility of public databases that integrate mutation data with clinical and pathological annotations as well as with functional annotations on mutant proteins will be necessary to estimate the clinical impact of specific *TP53* mutations. In the early nineties, the rapid accumulation of data on the occurrence of *TP53* mutations raised high expectations for clinical exploitation. However, over 10 years later, these expectations are still waiting to materialize into clinical practice. Recent findings show us that, in this area as in many others, the clinical reality is more complex than initially suspected and that interpretation of *TP53* mutations cannot be restricted to a "yes/no" answer. However, with the accumulation of knowledge on the specific properties of mutant proteins, we are closing down to the stage when mutation analysis will become standard practice in molecular pathology.

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