## Research Paper

# Analysis of p53 mutation status in human cancer cell lines

## A paradigm for cell line cross-contamination

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**Cancer cell lines are essential tools used in many areas of biomedical research. Using the last release of the UMD_p53 database (2007) (http://p53.free.fr), we analysed the p53 status of 1,211 cell lines published between 1989 and 2007. p53 mutations in cell lines from various types of cancers display a striking similarity in the distribution of mutations and in the pattern of mutational events compared to tumours, indicating that they are representative of the cells from which they were derived. Analysis of the residual transcriptional activity of p53 mutants identified in cell lines that displayed two different p53 mutations show that there is a high frequency of weak mutations that are paired with more potent mutations suggesting a driver/passenger configuration. Strikingly, we found discrepancies in the p53 status for 23% (88/384) of cell lines, for which the p53 status was established independently in two laboratories. Using the NCI-60 cell line panel as a model widely used in the literature, the p53 status could not be ascertained for 13 cell lines due to a lack of homogeneous data in the literature. Our study clearly confirms that misidentified cell lines are still a silent and neglected danger and that extreme care should be taken as a wrong p53 status could lead to disastrous experimental interpretations. The p53 web site has been updated with new sections describing the p53 status in the majority of cell lines and a special section devoted to cell lines with controversial p53 status.**

## Introduction

Continuous cell lines derived from human tumours are widely used in laboratory research. They can be used for drug screening (the NCI-60 panels), for production of various macromolecules, for modelling human tumours or, most frequently, as biological test tubes for a large variety of experiments.[1] To draw valid conclusions from such experiments, it is essential for cell lines to be clearly characterized at the molecular level. For a long time, these genetic characterizations were performed by studies focusing on one gene and the information was scattered in the literature. Recently, the Sanger Institute developed a Catalog Of Somatic Mutations In Cancer (COSMIC) that gathers information on genetic alterations in human tumour cell lines.[2] To date, data in the COSMIC cell line database is a mix of information taken from the literature and in-house sequencing.[2,3]

Cell line cross-contamination (CLCC) is not a novel problem,[4,5] as it was discovered as early as 1974 that one in three cell lines were contaminated, mostly by HeLa cells.[6] Despite the tremendous work conducted by Nelson-Rees et al., this problem is still "a silent and neglected danger", as a recent study indicates a CLCC of 18% at a German cell line repository.[4,7,8] CLCC is not trivial, as the use of wrong cell lines can lead to erroneous conclusions associated with years of wasted time and effort.[9-11]

p53 mutation is the most common genetic abnormality found in human cancer.[12] In cell lines, loss of p53 activity is usually linked with several specific landmarks such as defect in growth arrest or apoptosis after DNA damage and lack of induction of p53-regulated genes.[13,14] The p53 status is also a key factor for the sensitivity to anticancer agents and multiple studies have focused on this subject.[15,16] Although the majority of studies found a correlation between loss of p53 function and p53 alteration, a few publications report opposite results.[15,17,18] This situation is complicated by the observation that some mutant p53 proteins expressed in cell lines have only a partial loss of activity or present a temperature-sensitive transcriptional activity.[19]

For more than 17 years, we have collected and compiled p53 mutations in human tumours and cell lines.[20,21] Although numerous studies on p53 mutations in human tumours have been published, no systematic analysis of the p53 status of cell lines is currently available. In the course of updating the various versions of the UMD p53 database, we have noticed a number of discrepancies in the p53 status of several cell lines. The situation has recently been worsened, as these discrepancies have been randomly published in the literature, a situation that can lead to serious problems of data analysis. Many drug sensitivity studies are based on the p53 status reported in the literature without any new genetic analysis.

In the present study, using the UMD-p53 database as a framework, we performed a precise and thorough analysis of p53 status in 1,211 tumour cell lines. Our analysis shows that p53 mutations in

cell lines from various types of cancers display a striking similarity in the distribution of mutations and in the pattern of mutational events when compared to tumours indicating that they are representative of the cells from which they were derived. Surprisingly, we found discrepancies in the p53 status in 23% of cell lines, some of which are widely used, such as MOLT-4 or CAPAN-1.

## Results and Discussion

**p53 mutations in cell lines versus tumours.** The pattern of p53 mutations can be analysed in two informative ways, either by examining the distribution of p53 mutations in the p53 protein or by scoring the various mutational events that lead to these mutations. Both types of analysis have been very informative when applied to various types of human tumours.[25] These studies demonstrate a link between exposure to various types of carcinogens and the development of specific cancers. The most striking example is that of tandem mutations, specifically induced by ultraviolet radiation, which are only observed in skin cancers.[26] The relationships between G→T transversion and lung cancer in smokers or mutation of codon 249 observed in aflatoxin B1-induced liver cancers are also very demonstrative.[25,27] The distribution of p53 mutations along the p53 protein is similar in tumours and cell lines, indicating that there is no bias in the selection of specific mutant p53 during establishment of a cell culture (Fig. 1A and data not shown). The only exception concerns colorectal cancer cell lines. p.R175H is one of the most frequent p53 mutations in tumours, but is very rare in colorectal cancer cell lines (Suppl. Fig.). This finding is specific for p.R175H and it is not observed for the other two hot spots at codons 248 and 273. The reason for this bias is not known. Comparison of the various mutational events in cell lines and tumours has been performed for all cancers together or for 8 cancer types (Fig. 1A and B and Suppl. Figs.). As previously observed, the pattern of mutations differs between various types of cancers, but there is a striking similarity when comparing tumours and cell lines from the same origin. In colorectal and brain cancer, there is a predominance of GC→AT transition at CpG dinucleotides, whereas in lung cancer or head and neck SCC, the frequency of GC→TA transversion is 30% and 20%, respectively, with only a few transitions at CpG dinucleotides. This high frequency of transversion in these cancers has been shown to be associated with tobacco smoking and will not be discussed in more detail here.[28] This similarity in the pattern of p53 mutations in primary tumours and cell lines is a strong argument suggesting that these p53 mutations did not occur de novo during the establishment of these cell lines. It also supports the small number of studies that have found matched p53 mutations in primary tumours that were used to establish cell lines and confirms that analysis of the spectrum of mutations in oncogenes or tumour suppressor genes in human cell lines accurately reflects the situation observed in primary tumours.

**Analysis of p53 mutant activity in cell lines.** Analysis of p53 mutations in human tumours has led to the discovery that at least 5% to 10% of published p53 mutations could be due to PCR or sequencing artefacts.[22] However, these mutations are not randomly distributed among the 2,500 publications reporting p53 mutations. A meta-analysis identified about 30 publications (1,600 p53 mutations) with a high concentration of unusual p53 mutations that shared the following properties: (i) multiple p53 mutations in the same tumour (3 to 14); (ii) a high frequency of synonymous muta-

tions; (iii) a low frequency of mutations at hot spot codons; (iv) most of these mutations retained either partial or total transactivational activity.[29] The vast majority of these studies were associated with the use of nested PCR for amplification and analysis of the p53 gene. Analysis of p53 mutations in cell lines provides several advantages over analysis of tumours to minimize artefactual data: (i) DNA extracted from cell lines is available in large quantities. Analysis requires neither nested PCR nor excessive numbers of PCR cycles and can be easily repeated; (ii) The high quality of the DNA avoids PCR problems associated with DNA extracted from paraffin-embedded tissue; (iii) DNA is not contaminated by normal DNA from stroma or cells or infiltrating lymphocytes.

The UMD p53 mutation database includes functional information about the majority of p53 missense mutants, as originally published by Kato et al.,[23] (see also material and methods). Quantitative data concerning the transcriptional activity of each missense p53 mutation has been extremely useful to classify and analyse p53 mutations in the p53 database.[21,22,29] The mean and 95% confidence interval (CI) of the remaining activity of all mutant p53 proteins found in cell lines or in tumours was calculated by using the activity measured on the p21WAF1 promoter (similar results were obtained with the activity measured on 7 other promoters of transcription, data not shown). The analysis shows that the mean activity was situated between -1 and -1.2. This value corresponds to a residual transcriptional activity of about 10% compared to wild-type p53. The narrower 95% CI in tumours compared to cell lines is due to the greater number of tumours used in the analysis (Fig. 2A). In the majority of cancers, residual p53 activity was lower in cell lines than in tumours, but this difference was only marginally significant in head and neck, breast and SCLC, p = 0.03). On the other hand, residual p53 activity has a wider distribution in tumours compared to cell lines (variance analysis, Fig. 2B). A large number of mutant p53 retain wild-type activity in tumours, but this feature is rarely observed in cell lines. This difference was highly significant for all cancer types (p < 0.0001) except for brain cancers and haematological malignancies. Two non-exclusive explanations can be proposed for this difference between tumours and cell lines. First, it is possible that only tumours with fully inactivated p53 are preferentially selected to establish cell lines. This hypothesis could also explain why the frequency of p53 mutations is always higher in cell lines than in tumours. It is also possible that this profile of p53 inactivation in cell lines is more representative of the true pattern of p53 inactivation and that the tumour p53 database contains passenger mutations and/or artefactual mutations with partial or fully active p53.[21]

During the course of these analyses, we also observed that 82 cell lines displayed two p53 missenses mutations. Preliminary observations suggested that the two mutations may not have the same importance and that only one mutation was the driving force selected during transformation.[22] In order to obtain more information, clustering analysis was performed on cell lines with either single (SM cell lines) or double mutations (DM cell lines). Three clusters were obtained for the two populations, corresponding to mutant p53 with wild-type activity (cluster I), intermediate residual activity (cluster II) or no activity (cluster III) (Table 1). The number of mutants in clusters I and II was significantly higher in DM cell lines than in SM cell lines, whereas mutations with total loss of activity were more frequent in SM cell lines (p < 0.0001, Table 1). Mutations in DM
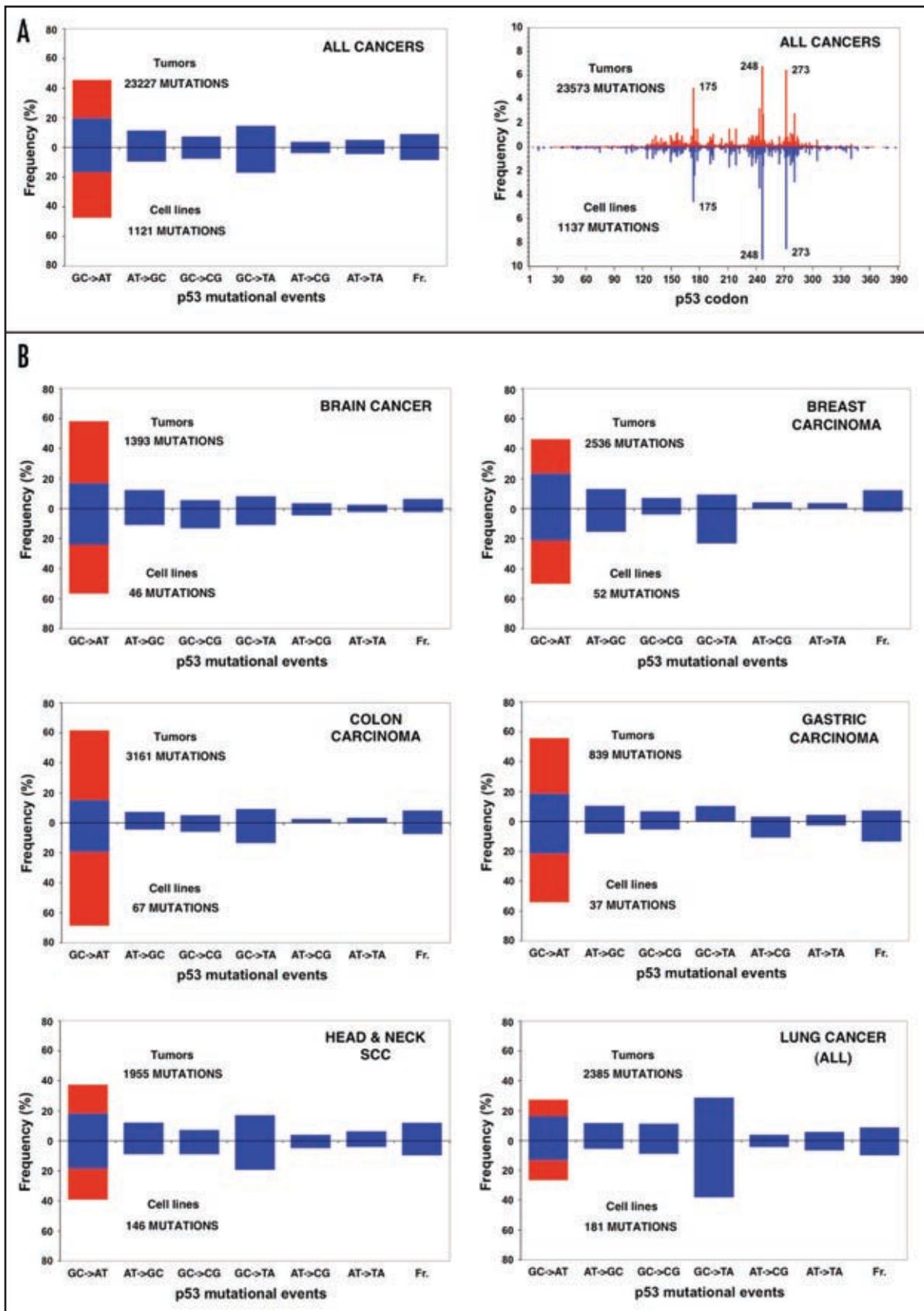
Figure 1. Mutation spectrum in tumours and cell lines: (A) Mutational events (left) and distribution of mutations (right) in all tumours (upper part) and cell lines (lower part). Data were obtained from the UMD p53 database, 2007_R1 release (http://p53/free/fr). (B) mutational events in tumours versus cell lines in various types of cancer. A similar pattern of mutational events is observed for other cancers (melanoma, ovarian carcinoma, oesophageal carcinoma or pancreatic carcinoma, data not shown). Transitions at CpG dinucleotides are shown in red.

cell lines were further analysed to determine how paired mutations (two mutations in a single cell line) were associated (Table 2). Only one of the 41 cell lines presented two mutations in cluster I with wt activity for the two p53 mutant alleles. This choriocarcinoma cell line (NUC-1) displays two unusual p53 mutations at codons 17 and 24 that have never been observed in any other cell lines or tumours. Among the 11 remaining mutations in cluster I, three were paired with mutations in cluster II and 8 were paired with mutations in cluster III. Among the 19 mutations in cluster II, two were paired with a mutation of the same class, 3 with class I mutations and 12 with class III mutations. The majority (30) of the 50 mutations in cluster III were paired with a mutation of the same class and 12 were paired with class II mutations (Table 2).

Double mutations can occur in two configurations, either on the same allele (DMS) or on two different alleles (DMD). Unfortunately, in the majority of cases, this status is unknown (DMU). In the p53 mutation database based on tumours, the majority of DM with a known configuration are DMD (about 90%). No cell lines with two missense mutations in the same allele have been reported and only 10 cell lines with mutations on two different alleles have been reported. All of these cell lines expressed one class III mutation associated with either another class III mutation (6), or class II (3) or class I (1) mutations.

Altogether, our results indicate that: (i) there is a higher frequency of weak mutations in DM than in SM mutations and (ii) the majority of these weak mutations are paired with a more potent mutation. This suggests that the two mutants do not have the same contribution to the transforming process. Whether or not these weak mutations are passenger mutations associated with a driving mutation or true mutations associated with selection of the transforming phenotype is an unresolved question. One of the main problems associated with p53 mutations is the possible dominant negative activity of mutant p53 via hetero-oligomerization making it very difficult to reach any definitive conclusions concerning weak p53 mutations. Weakening of the second allele could possibly accentuate the dominant negative activity of p53.

**p53 status in human tumour cell lines.** The NCI-60 panel is a good example of a series of cell lines that are widely used for both basic research and drug discovery.[1] This panel originally contained 60 cell lines from 9 histological origins (Table 3). Several observations unrelated to p53 status revealed that some cell lines were either mixed up or were derived from the same donor (Table 3).[5] At least 100 studies have analysed the p53 status of a subset of the panel and in 1997, O'Connor et al., reported the p53 status of the entire NCI-60 panel.[16] This paper has been used as a reference for 10 years despite discrepancies with other data in the literature. A second analysis of the entire NCI-60 panel was performed in 2006 and the results are fairly heterogeneous compared to the 1997 study (Table 3). Inspection of the two studies leads to the detection of 19 apparent differences (Table 3). Three differences were due to typographical errors in the 1997 report (RPMI-8226, SK-MEL-28 and Hs-578-T). A more careful examination of four other discrepancies reveals that they are due to a problem of nomenclature associated with a different mutation screening strategy. In the 1997 paper, p53 mutations were analysed by cDNA sequencing, while the 2007 analysis was performed using genomic DNA as starting material. One of the disadvantages of RNA-based analysis is that it is impossible to infer
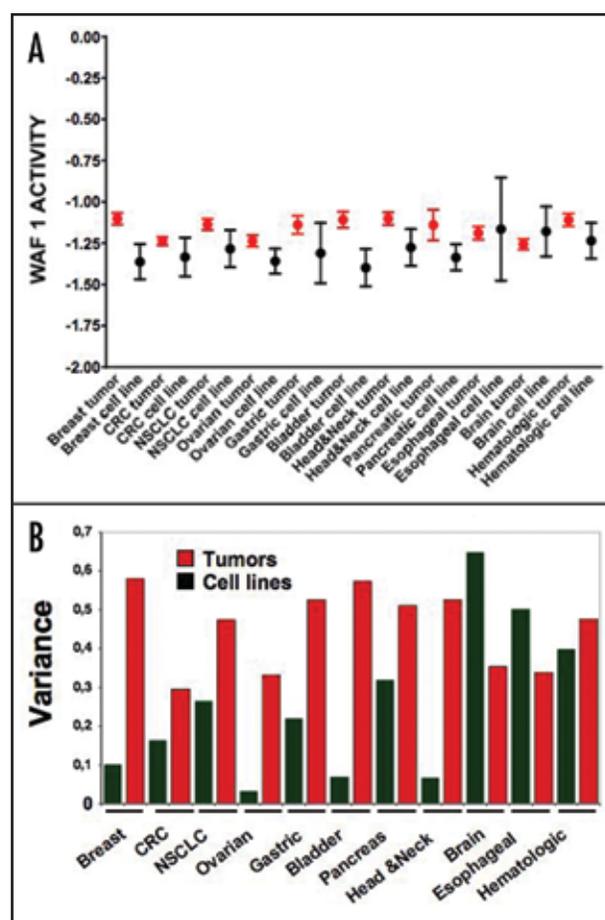


Figure 2. Analysis of the residual p53 activity of mutant p53 in tumours and cell lines. (A) Points, mean p53 activity as measured by transactivation with the p21WAF1 promoter; bars, 95% CI. A similar distribution was observed with other p53 response genes (data not shown). The y-axis corresponds to p53 transactivation activity, with a value of -1.5 for the negative control and 2.5 for wild-type p53. (B) Variance of the p21WAF1 promoter activity in tumors and cell lines. CRC, colorectal carcinoma; NSCLC, non-small cell lung cancer. Data from cell lines and tumours are displayed in black and red respectively.

whether deletions found in the cDNA are due to splicing mutations or intragenic deletions in the gene. On the other hand, it is always difficult to predict the consequence of mutations found in intron or splice junctions after genomic sequencing. Both methods are complementary and may be necessary to ensure an accurate genetic status.

In HOP-62, RNA-based analysis detected an insertion between codon 212–225 but no information about the insertion sequence was available. Codon 225 is at the boundary of exon 6 and intron 6 suggesting a splicing defect, as analysis at the genomic level confirms the presence of a splice mutation in the acceptor signal of exon 6 (Table 3).

In OVCAR-8, the 126–132 deletion detected by the RNA-based assay concerns the first six residues of exon 5. Genomic analysis described a mutation in the acceptor site of exon 5 and a splicing defect leading to a shift of the normal donor site of exon 5 that skips 18 nucleotides (6 aa residues) in exon 5. Examination of the DNA sequence at codon 132 reveals an AG dinucleotide sequence preceded by a pyrimidine tract similar to those found in the splice

Table 1   **Cluster analysis of p53 mutation activity**

|  | SM | DM |
|---|---|---|
| Cluster I (wt activity) | 27 (3.4%) | 13 (15.9%) |
| Cluster II (low acitivity) | 73 (9.3%) | 19 (23.2%) |
| Cluster III (no activity) | 687 (87.5%) | 50 (61.0%) |
| Total | 787 (100%) | 82 (100%) |

The table entries are the number (and %) of mutants classified into the three clusters based on k-means clustering of the promoter activities of p53 target genes. There are significantly more cluster-I and cluster-II mutations among the double mutations (DM) than among the single mutations (SM) (p= 2e - 10 using the chi-square test).

Table 2   **Discordance table of class assignment of the 82 DM mutations (from 41 pairs)**

|  | Cluster I | Cluster II | Cluster III |
|---|---|---|---|
| Cluster I (wt activity) | 1 | 3 | 8 |
| Cluster II (low acitivity) | 0 | 2 | 9 |
| Cluster III (no activity) | 2 | 3 | 15 |

Majority of the weak mutations (cluster I and cluster II) are paired with strong mutations (cluster III).

donor sequence. The same situation is observed for NCI/ADR-RES that has been recently shown to be an ovarian carcinoma cell line originating from the same patient as OVCAR-8.

In EKVX, the deletion of codon 187 to 224 detected on RNA-based analysis corresponds exactly to the deletion of the entire exon 6, a strong argument for a splicing defect. Genomic analysis did not reveal a splicing defect but a tandem mutation at codons 203 and 204 in exon 6 (Table 3). If the two cell lines analysed were really EKVX, this result suggests that a mutation at either codon 203 and/or 204 could affect p53 gene splicing. This observation is not surprising, as it is now well known that exons contain exonic splicing enhancers (ESE) that regulate either alternative splicing or normal splicing.[30] These ESE are recognized by the SR proteins that regulate the various splicing events. Mutations in ESE have been identified in numerous genes including APC or NF1.[31,32] Exonic mutations that can change p53 splicing have also been described.[33,34] Taken together, the contradictions noted in the p53 status of the four cell lines, HOP-62, OVCAR-8, NCI/ADR-RES and certainly EKVX are only due to the different strategies used for their analysis and a lack of homogeneity in the nomenclature used to report p53 mutations. The problem of the nomenclature of p53 mutations as well as other gene defects is a recurrent problem in publications.[35] Despite numerous recommendations, the description of p53 mutations in the literature is highly heterogeneous and can reach a high degree of fantasy with tables that are either totally non-informative or with so many typographical errors that they cannot be interpreted. In a recent survey, the editors of 80 journals with frequent publications of p53 mutations were contacted in order to stress this problem and define certain guidelines for the publication of p53 mutations (Soussi T, Unpublished). Unfortunately, this survey was a complete failure with less than 10% of replies and no change in the trends of reporting accurate p53 mutations. In fact, the number of typographical errors or incomprehensible mutations has increased over the last five years (Soussi T, unpublished observations).

After eliminating typographical errors and possible splice muta-

tions, the p53 status of 15 cell lines was different between the two studies. Using the UMD p53 database and the literature, we checked for other publications that have analysed the p53 status of these cell lines. For two cell lines, CCRF-CEM and HL-60, sufficiently concordant publications are available to define a consensus concerning the p53 status (Table 3). For 13 cell lines, analysis of the literature revealed a very heterogeneous situation and no consensus could be reached (Table 3, Inconclusive). Cell lines such as MOLT-4 or NCI-H226 represent an extreme situation, as multiple publications do not show any common p53 mutations. For other cell lines such as DU-145, which have been shown to display two different p53 mutations in two different alleles (p.V274F and p.P223L), the ambiguity concerns the fact that several authors have detected only one of the two mutations, either p.V274F or p.P223L. It is therefore possible that during long-term cell culture, one of the two mutant p53 alleles is lost, as no selection pressure is exerted on cell growth.

A similar situation is observed for other cell lines that do not belong to the NCI-60 panel, but with many discrepancies (Table 4 and Suppl. Table S1, see also p53 website). In many cell lines, the p53 status has been analysed in only one or two reports and the information is subsequently reproduced in the literature. This is a very dangerous situation as it could lead to erroneous phenotype-genotype correlations in various types of studies. The pancreatic carcinoma cell line CAPAN-2 is a good example of the problems raised by erroneous phenotype. This cell line has been described as either wt, mutated (p.R273H) or p53 null (Table 4 and reference within). A Pubmed literature search indicates that all three phenotypes are used in various studies.

The "p53-null" status is used in different ways in the literature. The two most common meanings are a cell line with a documented p53 gene deletion (both alleles) or a cell line with a p53 mutation. We have also observed more "unusual" situations in which this status is only based on p53 expression (RNA or protein). Unfortunately, this type of information diffuses rapidly in the literature without any verification of the original publication. The p53 status of the two cell lines SK-OV-3 (Ovarian cancer) and FRO (anaplastic thyroid carcinoma cell line) are a good example of this ambiguity. In the majority of publications, the p53 status of these two cell line is stated as "p53 null". In fact, close examination of the original manuscript shows that the p53 gene in SK-OV-3 is not deleted and did not sustain any gross rearrangement but neither p53 RNA or protein are found. In these publications, no p53 mutations were found but the recent analysis performed at the Sanger Institute detected a deletion of a single nucleotide at position 267 (codon 90).[3] It is therefore possible that nonsense-mediated mRNA decay (NMD) eliminates p53 aberrant mRNA. NMD has been observed in the human leukaemia cell line K562 where p53 is also inactivated via a 1 base pair insertion at nucleotide 136. For the FRO cell line, the original reference for the analysis of the p53 gene status is always correctly quoted, but a closer look at this original paper demonstrates a marked decrease of p53 RNA in the cell but no mutation was detected by sequencing exons 5 to 8. Either a mutation is situated outside this region leading to a decrease of RNA expression (frameshift mutation associated with Nonsense-Mediated mRNA Decay) or the altered p53 expression is due to another mechanism. Because the whole p53 gene is present, it is incorrect to define SK-OV-3 or FRO cell lines as "p53 null", as in the case of H1299 or Saos-2 cell lines in which the p53 gene is

**Table 3A  p53 status in the NCI-60 panel cell lines**

| Cell line | ATCC number | Cancer | Mutation in UMD[1] | | Mutation in COSMIC[2] | | Consensus[3] | Comments |
|---|---|---|---|---|---|---|---|---|
| | | | Mutation | Reference | Protein | DNA | | |
| CCRF-CEM | CCL-119 | Leukemia | R248Q | 16 | p.R175H, p.R248Q | c.524G>A, c.743G>A | p.[R175H] + p.[R248Q] | Two mutations in separate alleles. Derivative CCRF-CEM-VLB100 has a third mutation |
| | | | R175H+ R248Q | 394 | | | | |
| HL-60[5] | CCL-240 | | R248L | 16 | p.M1_*394del | c.1_1182del1182 | p.M1_*394del | |
| | | | p53 Null | 40 [4] | | | | |
| K-562 | CCL-243 | | ND | 16 | p.Q136fs*13 | c.406_407insC | p.Q136fs*13 | |
| | | | 136ins1 | 41 [4] | | | | |
| MOLT-4[6] | CRL-1582 | | wt | 16,39 | p.R306X | c.916C>T | Inconclusive | |
| | | | L111V | 42 | | | | |
| | | | R248Q | 43 | | | | |
| RPMI-8226 | CCL-155 | | E285L[7] | 16 | p.E285K | c.853G>A | p.E285K | This mutant is temperature-sensitive |
| | | | E285K | 44 | | | | |
| SR | CRL-2262 | | wt | 16 | wt | wt | wt | |
| A549 | CCL-185 | Non-Small Cell Lung | wt | 16,45 | wt | wt | wt | |
| EKVX | | | del187-224 | 16 | p.V203_E204>V* | c.609_610GG>TT | Inconclusive | Possible splicing defect See text for discussion |
| HOP-62 | | | Ins212-225 | 16 | p.? | c.673-2A>G | Splicing defect | See text for discussion |
| HOP-92 | | | R175L | 16 | p.R175L | c.524G>T | p.R175L | |
| NCI-H226 | CRL-5826 | | P309A | 16 | wt | wt | Inconclusive | |
| | | | R158L | 46 | | | | |
| NCI-H23 | CRL-5800 | | M246I | 16,47 | p.M246I | c.738G>C | p.M246I | |
| NCI-H322M | | | R248L | 16,46 | p.R248L | c.743G>T | p.R248L | |
| NCI-H460 | HTB-177 | | wt | 16 | wt | wt | wt | |
| NCI-H522 | CRL-5810 | | 191delG | 16, 46 | p.P191fs*56 | c.572_572delC | p.P191fs*56 | |
| COLO-205 | CCL-222 | Colon | G266E | 16 | p.Y103_L111>L | c.308_333>TA | Inconclusive | |
| | | | 103del27 | 48 | | | | |
| HCC-2998 | | | R213X | 16 | p.R213x | c.637C>T | p.R213x | |
| HCT-116 | CCL-247 | | wt | 16 | wt | wt | wt | |
| HCT-15 | CCL-225 | | P153A | 16 | p.S241F, p.? | c.722C>T, c.1101-2A>C | Inconclusive | HCT-15 and DLD1 are derived from the same individual. DLD1 display a p.S241F mutation |
| | | | S241F | 49 | | | | |
| HT-29 | HTB-38 | | R273H | 16, 48 | p.R273H | c.818G>A | p.R273H | |
| KM12 | | | H179R | 16 | p.R72fs*51 | c.215delG | Inconclusive | |
| SW620 | CCL-227 | | R273H | 16, 50 | p.R273H, p.P309S | c.818G>A, c.925C>T | p.[R273H] (+) p.[P309S] | SW480 and SW620 are derived from the same individual with a similar p53 alteration. The p.P309S mutation is not always reported |
| | | | R273H, P309S | 51 | | | | |
| SF268 | | CNS | R273H | 16,52 | p.R273H | c.818G>A | p.R273H | |
| SF295 | | | R248Q | 16 | p.R248Q | c.743G>A | p.R248Q | |
| SF539 | | | wt | 16 | p.R342fs*3 | c.1024delC | Inconclusive | |
| SNB75 | | | E258K | 16 | p.E258K | c.772G>A | p.E258K | |
| U251/SNB19 | | | R273H | 16,53 | p.R273H | c.818G>A | p.R273H | SNB19 and U251 cell lines are derived from the same individual and are similar |

entirely deleted. These cell lines are commonly used as recipients to reintroduce either wild-type of mutant p53. Whether the presence of an endogenous p53 gene which is still transcriptionally active in the SK-OV-3 or FRO cell could interfere with this reconstitution experiment is not known, but should be carefully considered before conducting this type of experiment. The recent finding of p53 isoforms that could be expressed by alternative splicing may also increase the complexity of this problem, as the various delta133 isoforms could be theoretically expressed in this cell line.

Another reason why "p53-null" should be used cautiously to describe cell lines that express mutant p53 is the observation that p53 mutations are fairly heterogeneous in terms of loss of function and several cell lines display a normal or partial p53 response. Finally, there is now ample evidence that some mutant p53 behave as dominant oncogenes with a gain of function activity. We therefore believe that the "p53 null" status should be used only for cell lines that are totally devoid of p53 gene. Any other situation should be referred to as "mutant p53".

The UMD_p53 database (2007_R1 release) includes p53 mutations in 1,211 cell lines: 827 of these mutations have only been described once, preventing any verification. A discrepancy was detected in 88 of the remaining 384 cell lines (23%), in line with the study by Macleod et al., who showed that 18% of cell lines in the DSMZ-German Collection of Microorganisms and cell Cultures were cross-contaminated.[8] The p53 status in various cell lines is a

## Table 3B   p53 status in the NCI-60 panel cell lines

| Cell line | ATCC | Tissue | Mutation[1] | Ref | [2] | cDNA | Consensus[3] | Notes |
|---|---|---|---|---|---|---|---|---|
| LOXIMV1 | | Melanoma | wt | 16 | wt | wt | wt | |
| Malme-3M | HTB-64 | | wt | 16 | wt | wt | wt | |
| M14 | | | G266E | 16 | p.G266E | c.797G>A | p.G266E | |
| SK-MEL-2 | HTB-68 | | G245S | 16 | p.G245S | c.733G>A | p.G245S | |
| SK-MEL-28 | HTB-72 | | C145V[7] | 16 | p.L145R | c.434_435TG>GT | p.L145R | |
| | | | L145R | 54 | | | | |
| SK-MEL-5 | HTB-70 | | wt | 16 | wt | wt | wt | |
| UACC-257 | | | wt | 16 | wt | wt | wt | |
| UACC-62 | | | wt | 16 | wt | wt | wt | |
| MDA-MB-435 | | | G266E | 16, 55 | p.G266E | c.797G>A | p.G266E | This cell line was originally reported as a breast carcinoma cell line but recent SNP analysis indicates that it is similar to the M14 melanoma cell line |
| MDA-N | | | G266E | 16 | ND | ND | p.G266E | This cell line is a derivative of MDA-MB-435 transfected with a plasmid expressing erbB2 |
| IGROV1 | | Ovarian | wt | 16, 56 | p.Y126C | c.377A>G | Inconclusive | |
| OVCAR-3 | HTB-161 | | R248Q | 16, 57 | p.R248Q | c.743G>A | p.R248Q | |
| OVCAR-4 | | | wt | 16 | p.L130V | c.388C>G | Inconclusive | |
| OVCAR-5 | | | ins224 | 16 | wt | wt | Inconclusive | |
| | | | 224ins3[8] | 58 | | | | |
| OVCAR-8 | | | del 126-132 | 16 | p.? | c.376-1G>A | Splicing defect | Same as NCI/ADR-RES |
| NCI/ADR-RES | | | del 126-132 | 16 | p.? | c.376-1G>A | Splicing defect | Originally labelled as MCF-7/Adr but was later found to be different from MCF-7. SNP analysis indicates that it is similar to OVCAR-8 |
| | | | 126del21 | 59 | | | | |
| SK-OV-3[9] | HTB-77 | | H179R | 16 | p.S90fs*33 | c.267delC | Inconclusive | |
| 786-0 | CRL-1932 | Renal | P278A | 16 | p.P278A, p.? | c.832C>G, c.560-2A>G | Inconclusive | |
| A498 | HTB-44 | | wt | 16 | wt | wt | wt | |
| ACHN | CRL-1611 | | wt | 16 | wt | wt | wt | |
| CAKI-1 | HTB-46 | | wt | 16 | wt | wt | wt | |
| RXF393 | | | R175H | 16 | p.R175H | c.524G>A | p.R175H | |
| SN12C | | | E336X | 16 | p.E336X | c.1006G>T | p.E336X | |
| TK10 | | | L264R | 16 | p.L264R | c.791T>G | p.L264R | |
| U031 | | | wt | 16 | wt | wt | wt | |
| DU-145 | HTB-81 | Prostate | P223L | 16 | p.V274F | c.820G>T | Inconclusive | See text for more information |
| | | | P223L, V274F | 60,61 | | | | |
| PC-3 | CRL-1435 | | 138del | 16 | p.K139fs*31 | c.414delC | p.K139fs*31 | |
| | | | 138del1 | 60 | | | | |
| | | | R282W | 62 | | | | |
| BT-549 | HTB-122 | Breast | ND | 16 | p.R249S | c.747G>C | p.R249S | |
| | | | R249S | 63 | | | | |
| Hs 578T | HTB-126 | | D157E[7] | 16 | p.V157F | c.469G>T | p.V157F | |
| | | | V157F | 64, 4 | | | | |
| MCF7 | HTB-22 | | wt | 16 | wt | wt | wt | |
| MDA-MB-231 | HTB-26 | | R280K | 16, 63 | p.R280K | c.839G>A | p.R280K | |
| T47D | HTB-133 | | L194F | 16,51 | p.L194F | c.580C>T | p.L194F | |

[1]Mutations as reported in the 2007_R1 of the UMD p53 mutation database. The description of the mutations have been left as originally published by the authors; [2]Mutations described by Ikediobi et al.,[3]; [3]A mutation consensus was defined for cell lines using the following rules: (i) at least two independent studies reporting sequencing and identifying the same mutation without any contradictory reports; (ii) at least three independent studies reporting sequencing and identifying the same mutation and one fourth contradictory report. All other possibilities were not considered to be consensual and have been assigned as uncertain. The nomenclature for TP53 mutation uses either the cDNA (RefSeqNM_000546.2) or the protein (RefSeq NP 000537) as reference: For numbering, +1 is A of the ATG initiation codon in the correct RefSeq (NM_000546.2). Mutations are described using the international nomenclature[65] and http://www.hgvs.org/mutnomen/; [4]Mutation found independently by multiple authors. Only the first publication is shown; [5]HL 60(TB) was used for the analysis, but it is reported to have a p53 deletion similar to HL60; [6]The status of MOLT-4 is highly heterogeneous in the literature. The report of a wt status could be due to the fact that only exons 5 to 8 (residues 126–306) were screened in several publications; [7]Typographical error in the publication; [8]it is not clear whether these authors checked the p53 status of the cell line or report the mutation described by O' Connor et al; [9]This cell line has been reported to be null for p53 RNA or protein. Whether this is due to a small DNA rearrangement or RNA-mediated decay associated with a frameshift mutation is unknown.

paradigm for CLCC. (i) p53 mutation is sufficiently diverse to allow comparison of various cell lines. Statuses of other genes with fewer mutation hot spots (Ha-ras) or a lower frequency of mutations are not as useful. (ii) Due to its importance in cell phenotype, p53 status has been analysed in more than 1,200 cell lines. Although p53 mutation analysis cannot replace DNA fingerprinting, our finding is a strong

**Table 4  Cell lines with controversial p53 mutations**

| Cell line | ATCC number | Cancer | p53 Mutation UMD | |
|---|---|---|---|---|
| | | | AA Change | Reference* |
| HT-1197 | CRL-1473 | *Bladder carcinoma* | p.His365Arg | 66 |
| | | | wt | 2 |
| EJ | | *Bladder carcinoma* | p.Tyr126X | 67 |
| | | | p.Lys164Glu | 68 |
| | | | wt | 69 |
| RT-112 | | *Bladder carcinoma* | p.Ser183X, p.Arg248Gln | 2 |
| | | | p.Arg248Gln | 70 |
| SD | | *Bladder carcinoma* | p.Arg110Leu | 71 |
| | | | p.Ser116Cys | 67 |
| T-24 | HTB-4 | *Bladder carcinoma* | p.Tyr126X | 67 |
| | | | p.Tyr126delTAC | 66 |
| VM-CUB-1 | | *Bladder carcinoma* | p.Tyr126X, p.Arg175His | 2 |
| | | | p.Arg175His | 67 |
| VM-CUB-2 | | *Bladder carcinoma* | p.Arg158Leu, p.Tyr163Cys | 67 |
| | | | p.Arg158Leu | 71 |
| MDA-MB-436 | HTB-130 | *Breast carcinoma* | p.Glu204delinsAspfsX6 | 55 |
| | | | p.Arg273His | 72 |
| DAUDI | CCL-213 | *Burkitt lymphoma* | p.Arg213X | 73 |
| | | | p.Gly266Glu | 2 |
| A-172 | CRL-1620 | *Glioblastoma* | p.Cys242Phe | 74 |
| | | | wt | 75 |
| U-118-MG | HTB-15 | *Glioblastoma* | p.Arg213Gln | 76 |
| | | | wt | 2 |
| SK-LMS-1 | | *Leiomyosarcoma* | p.Met237Lys, p.Gly245Ser | 77 |
| | | | p.Gly245Ser | 78 |
| SK-UT-1 | HTB-114 | *Leiomyosarcoma* | p.Arg175His, p.Arg248Gln | 2 |
| | | | p.Arg175His | 78 |
| NCI-H1048 | CRL-5853 | *Lung (SCLC)* | p.Ser46fsX76, p.Arg273Cys | 2 |
| | | | p.Arg273Cys | 79 |
| MeWo | HTB-65 | *Melanoma* | p.Glu258Lys, p.Gln317X | 2 |
| | | | p.Glu258Lys | 80 |
| PA-1 | CRL-1572 | *Ovarian carcinoma* | p.Asn239Asp | 81 |
| | | | p.Pro316Pro | 57 |
| | | | wt | 2 |
| SW626 | HTB-78 | *Ovarian carcinoma* | p.Gly262Val | 58 |
| | | | p.Arg273His | 82 |
| Capan-2 | HTB-80 | *Pancreatic cancer* | p.Arg273His | 83 |
| | | | wt | 2 |

*References correspond to studies in which the p53 gene status was analysed experimentally and not deduced from other reports in the literature.

argument to suggest that CLCC should not be ignored. We are also very concerned by the observation that the p53 status based on a cell line (either correct or false) can be reproduced from a single publication in the literature without any subsequent confirmation. Finally, we have also noticed a marked heterogeneity in the labelling of cell lines, a problem that can also lead to confusion between mislabelled cell lines with similar names.[36] CLCC includes several situations: (i) cross-contamination between two cell lines (the best example being HeLa cells); (ii) cell lines with an incorrect origin (such as the KB cell line often wrongly described as an oral cancer when it is actually a cervical cancer); and (iii) cell lines that have been contaminated during manipulation. We believe that the problem identified in the present analysis is predominantly related to confusion or incorrect labelling of cell lines. Although, the material and methods sections of published articles usually state that cell lines were derived from cell banks such as ATCC or DMSZ, it is well known that many cell lines have been exchanged between research groups, a situation that increases the probability of CLCC. These problems have already been extensively discussed over the past year, but seem to be ignored by the scientific community. We strongly encourage all scientists to comply with the various recently published guidelines for correct handling of cell lines.[37,38]

The p53 status in cell lines is now available at the p53 web site (http://p53.free.fr/Database/Cancer_cell_lines/p53_cell_lines.html).

A specific section is devoted to cell lines with a controversial p53 status. We invite all scientists to update these tables with their own findings so that a consensus concerning the p53 status of each cell line can be reached. Finally, we strongly encourage those involved in studies dealing with p53 (or other p53 family members) to regularly check the p53 status of their cell lines.

## Material and Methods

**Analysis of the biological activity of mutant p53 proteins.** <u>Data analysis.</u> The p53 database used for this study contains 21,717 mutations derived from 1,992 publications (UMD p53 database (htpp://p53.free.fr), 2007_R1 release released in January 2007).[22] This release contains functional data for the majority of missense p53 mutants. Mutant p53 activity has been described previously.[22,23] Briefly, 2,314 haploid yeast transformants containing p53 mutations and a GFP-reporter plasmid have been constructed. Mutant p53 activity was tested by measuring the fluorescent intensity of GFP that is controlled by the p21WAF1 promoter sequence of the plasmid after 3 days of growth at 37°C. For functional analysis, frameshift and nonsense mutations were also excluded, as their biological significance has not been clearly established (see text for more information). The mean and 95% Confidence Interval (CI) of the biological activity of all mutants was calculated by using the transactivational activity measured on the p21WAF1 promoter. Similar results were obtained with the activity measured on 7 other promoters of transcription (data not shown).

<u>Statistical analysis.</u> To identify the distinct levels of p53 residual activities among the mutants we used the k-means clustering,[24] whose aim is to partition the data into 3 groups such that the sum of squares from each mutant to the assigned cluster centres is minimized. Three clusters were chosen to represent mutants with no, low and wild-type activity levels. The analysis was based on the measurements of promoter activities of 8 p53 target genes, including p21WAF1, MDM2, BAX, v14-3-3-σ, AIP, GADD45, NOXA and p53R2.

### Note

Supplementary materials can be found at:
www.landesbioscience.com/supplement/BerglindCBT7-5-Sup.pdf

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