

MUT-TP53 2.0: A Novel Versatile Matrix for Statistical Analysis of TP53 Mutations in Human Cancer

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ABSTRACT: Analysis of the literature reporting p53 mutations shows that 8% of report display typographical mistakes with a notable increase in recent years. These errors are sometimes isolated, but in some cases, they concern several or even all mutations described in a single article. Furthermore, some works report unusual profile of p53 mutations whose accuracy is difficult to assess. To handle these problems we have developed MUT-TP53 2.0, an accurate and powerful tool that will automatically handle p53 mutations and generate tables ready for publication that will lower the risk of typographical errors. Furthermore, using functional and statistical information issued from the UMD p53 database, it allows to assess the biological activity and the likelihood of every p53 mutant.

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KEY WORDS: TP53; database; curation; cancer; tumor

Introduction

Identification of novel genes associated with tumor development will provide new insight into cancer biology, and should also identify whether some of these mutated genes could be effective targets for anticancer drug development. For this purpose, partial and whole cancer genome sequencing has been initiated, but has led to the discovery of an unexpected landscape of in vivo somatic mutations with 10 to 20,000 base substitutions per genome [Stratton et al., 2009; Strausberg and Simpson, 2010]. The majority of these variations are somatic passenger mutations (or hitchhiking mutations) that have no active role in cancer progression and are coselected by the driver mutations, which are the true driving force for cell transformation [Chanock and Thomas, 2007]. Passenger mutations can be found in coding or noncoding regions of any genes, and the distinction of these mutations from driving mutations is a difficult but necessary task

to obtain an accurate picture of the cancer genome. Several statistical approaches have been developed to resolve this problem such as comparing the observed to expected ratios of synonymous:nonsynonymous variants. Alternatively, various bioinformatic methods are used to provide an indication of whether an amino acid substitution is likely to damage protein function on the basis of conservation through species or whether or not the amino acid change is conservative [Ng and Henikoff, 2001].

Reporting, storing, classifying, and analyzing these mutations constitute a major challenge [Horaitis and Cotton, 2004]. For a long time, locus-specific databases have been developed for this purpose. Although each LSDB has been developed for a single gene, they are highly accurate and provide information that can be exploited for large-scale analysis. They often include structural, functional, or evolutionary data that allow easy distinction between passenger and driving mutations. TP53 mutation (TP53; MIM# 191170) databases are a paradigm, as they constitute the largest collection of somatic mutations (30,000 mutations from 29,000 patients) for a single gene.

A review of the literature reporting TP53 mutations shows that 8% of reports comprise typographical errors, with a marked increase over recent years (T. Soussi, unpublished observations). These errors are sometimes isolated, but in some cases, they concern several or even all mutations described in a single article. Furthermore, some articles report an unusual profile of p53 mutations. In 2006, we published a meta-analysis of 2,000 reports describing TP53 mutations and revealed that these dubious reports were associated with methodological bias for p53 analysis [Soussi et al., 2006]. To resolve these problems, we have developed, MUT-TP53, an accurate and powerful tool that automatically processes p53 mutations and generate tables ready for publication that will decrease the risk of typographical errors [Soussi et al., 2006]. Furthermore, using functional and statistical information derived from the UMD p53 database, this matrix could be used to assess the biological activity and likelihood of each TP53 mutant. Although this tool is used by numerous laboratories, reports of unusual patterns of p53 mutations are still published, leading to controversial discussions [Campbell et al., 2008; Roukos, 2008; Soussi-Zander and Soussi, 2008; Zalcman et al., 2008]. To identify these problems before publication, we present MUT-TP53 2.0, an extended version of our previous program comprising novel tools that allow authors, reviewers, editors, and curators to: (1) manage p53 mutation sequences based on the genetic code and wild-type (wt) p53 sequences, (2)

Additional Supporting Information may be found in the online version of this article.

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check the frequency and activity of p53 mutations, (3) generate a p53 mutation table ready for publication that will lower the risk of typographical errors, (4) compare the profile of p53 inactivation with other publications, and (5) perform statistical analysis of p53 loss of function.

Materials and Methods

MUT-TP53 2.0 was developed using Microsoft Excel™. The spreadsheet is available for both Windows and OS X platforms. Two different databases were used for development: First, the latest version of the UMD p53 mutation database (July 2010, 31,000 p53 mutations, <http://p53.free.fr>). It contains data on mutation frequency, mutation identity, and transcriptional activity in yeast that are used to generate the result page in the spreadsheet (Fig. 1). The second database is OPMA, a novel unpublished database that contains mutant p53 activity (T. Soussi et al., manuscript in preparation). Briefly, OPMA has been developed by mining the literature focusing on mutant p53 loss of activity such as loss of transactivation in mammalian cells, dominant negative activity, in vitro and in vivo DNA binding, growth arrest, and apoptosis. This data are used to generate the mutant page in the spreadsheet (Supp. Fig. S1 and Supp. Table S1).

The algorithm used for the development of MUT-TP53 2.0 and the cutoffs used for the various comments are fully described in the Supporting Information available with this manuscript (Supp. Fig. S2 and Supp. Table S2). Mutant p53 activity has been described in detail in a previous report [Kato et al., 2003]. 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 WAF1 promoter sequence of the plasmid after 3 days of growth at 37°C. The activity of the yeast without p53 or with wt p53 was −1.58 and 2.03, respectively. The activity of the majority of p53 mutants was situated between these two values.

An approach similar to that used for meta-analyses comparing clinical trials was used for data analysis and presentation of the statistical study [Soussi et al., 2006]. For each publication, the mean and 95% confidence interval (CI) of p53 activity of each mutant were displayed graphically. The reference value corresponds to the mean and 95% CI of all studies for the specific cancer. Although the mean value of the entire database can be used as the reference value, the use of an individual reference value for each cancer type would more closely reflect the heterogeneous etiology and pattern of p53 mutations in various cancers. This procedure has been fully described in previous publications [Soussi et al., 2006].

Statistical analyses were performed with PRISM software (GraphPad Software Inc., LaJolla, CA) on a Mac OS X platform.

MUT-TP53 2.0 is available for download at <http://p53.free.fr>.

Results and Discussion

The latest version of the UMD p53 database (2010) contains 31,000 mutations from 29,200 patients (several patients have multiple mutations). As the p53 gene has several mutation hot

N°	Pos.	p.Mutant	c.Mutant	Freq.	Activity	Comments 1	Comments 2	Comments 3
1	175	p.R175H	c.524G>A	1233	12,41	This is a hot spot mutant	This mutant is inactive	No Problem
2	132	p.K132E	c.394A>G	28	0,56	This mutant is frequent	This mutant is inactive	No Problem
3	72	p.P72R	c.215C>G	0	x	---	---	This is not a mutation ! This change has been reported as a natural polymorphism of the p53 gene
4	273	p.R273R	---	4	x	---	---	Wt and Mutant codon are identical : this is not a mutation !!
5	125	p.T125T	c.375G>A	14	x	This mutant is not frequent	This mutation does not change the amino acid residue but it is known to lead to aberrant splicing.	No Problem
6	260	p.S260S	c.780C>A	8	x	This mutant is not frequent	This mutation does not change the amino acid. It can change splicing, translation or RNA stability.	This codon is at the vicinity of an exon extremity. Check RNA if possible.
7	337	p.R337H	c.1010G>A	87	x	This mutant is very rare	The stability of this mutant is highly sensitive to pH	Germline alteration R337H is associated with children from southern Brazil with adrenocortical tumours
8	250	p.P250F	c.[748C>T;749C>T]	6	ND	This mutant is not frequent	The remaining activity for this mutant p53 is unknown	Unusual mutational event except in skin cancer: check the trace or the autoradiogram
9	115	p.H115K	c.[343C>A;345T>G]	0	ND	This mutant has never been described so far	The remaining activity for this mutant p53 is unknown	This is an unusual mutational event: check the trace or the autoradiogram
10	230	p.T230W	c.[688A>T;689C>G; 690C>G]	0	ND	This mutant has never been described so far	The remaining activity for this mutant p53 is unknown	This is a very unusual mutational event: check the trace or the autoradiogram

Figure 1. Output example: 10 mutant p53 showing the various features of MUT-TP53 V2.0 were analyzed. Mutants in sample 1 (p.R175H), sample 2 (p.K132E), sample 5 (p.T125T), sample 6 (p.S260S), sample 7 (p.R337H), and sample 8 (p.N200P) have been described in the tumor database. In sample 3, the spreadsheet identifies the exonic SNP in exon 4 at codon 72. In sample 4, the matrix identifies a typographical error, as wt and mutant codon are identical. Mutants in samples 9 and 10 correspond to the substitution of two noncontiguous residues or three residues. These mutational events are very unusual in the p53 gene and are flagged accordingly. See text for more information. Nucleotide numbering reflects cDNA numbering with +1 corresponding to the A of the ATG translation initiation codon in the reference sequence (GenBank X54156.1).

spots, similar mutations are found in different patients and the true number of different p53 mutant is 2,300, including missense and nonsense mutations (whether or not they change the residue) and frameshift mutations. Among these mutations, only 1,439 mutant p53 have a single amino acid change.

These 1,439 mutants have been divided into eight categories according to their frequency in the database (Fig. 2A and B). The residual activity of mutants very frequently found in the *TP53* database (categories 161+, 81–160, 41–80, and 21–40) is usually low, with only a few mutants with higher activity for the 21–40 category (Fig. 2A and B). These categories contain all of the hot spot mutants of the p53 gene and have been shown to be inactive by numerous studies. For categories 4–8, 2–3, or mutants found only once, the scatter is very heterogeneous, ranging from 0 to

160% compared to wt p53. Nonparametric statistical analysis using a Mann–Whitney test did not reveal any statistical difference between the four categories 161+, 81–160, 41–80, and 21–40. However, comparison of each of these categories with each of the low-frequency categories showed a highly significant difference ($P < 0.0001$) (Fig. 2A), as similar distribution and statistical differences was observed using *TP53* data activity on other *TP53* promoters or using different categories to classify *TP53* mutation frequency (Supp. Fig. S3). There is a clearly demonstrated inverse correlation between the frequency of *TP53* mutants and their activity. Fifty-five percent of the rare mutants found only once have an activity greater than 50% compared to wt *TP53*. This picture is similar to our previous analysis performed on 1,100 *TP53* mutants [Soussi et al., 2005]. The presence of a few rare active p53 mutants in a large study is not a problem, as they can be rare passenger mutations. In contrast, several studies have described unusually high numbers of rare mutants that can represent 80% of all mutants. Some of these studies have been shown to be associated with methodologic biases and artefactual data. One of the goals of MUT-*TP53* 2.0 is to detect these rare p53 mutants to warn the user about an unusual mutation pattern.

To minimize typographical errors, another problem encountered in many publications, MUT-*TP53* 2.0 automates most of the data entry procedures and keeps the number of manual steps to a minimum. The matrix includes the sequence of the human wt *TP53* cDNA and a genetic code for translation. The user only needs to enter three types of information: (1) case number, (2) position of the mutation (at the codon level), and (3) mutant sequence. The matrix then automatically displays a table comprising all information concerning wt and mutant codon and amino acids (one- and three-letter codes) (Fig. 1). This information is linked to three types of comments and guidelines for the user about the likelihood of each mutation (Fig. 1). Compared to version 1.0, MUT-*TP53* 2.0 contains numerous novel features to cover each single, double or triple nucleotide substitution at each codon of the p53 gene (25, 152 possibilities). Although the majority of p53 mutations are single substitutions, tandem mutations can be common in skin cancer, while triple substitutions are very rare. The nomenclature of the p53 mutants used in the matrix is based on the guideline described by Den Dunnen and Antonarakis [den Dunnen and Antonarakis, 2000]. A typical table generated by MUT-*TP53* 2.0 is shown in Figure 1 with various types of mutant. More examples are available in the Help document that can be downloaded with the program (<http://p53.free.fr>). In sample 1, mutant c.524G>A (p.R175H) is an inactive hot spot mutant described 1,233 times (frequency column) with a residual activity of 12% compared to wt p53 (activity column). Comment 1 concerns the frequency of the mutant in the database and comment 2 concerns the loss of function of this mutant (see Materials and Methods). Comment 3 presents final advice concerning whether or not the user can trust this mutant. This final comment is based on mutant loss of activity and frequency (see Materials and Methods). In sample 2, the mutation is less frequent but associated with loss of p53 activity. In sample 3, c.215C>G (p.P72), comment 3 warns the user that this not a mutation but an exonic polymorphism of the human p53 gene. Although this polymorphism is well known, other polymorphisms, such as p.P36 and p.R213R, are less known and are often described as mutations. All exonic single nucleotide polymorphisms (SNPs) of the p53 gene have been included in MUT-*TP53* 2.0. Although a somatic mutation similar to a natural polymorphism cannot be formally excluded, they are hardly unlikely and difficult to detect if matched normal tissue is not

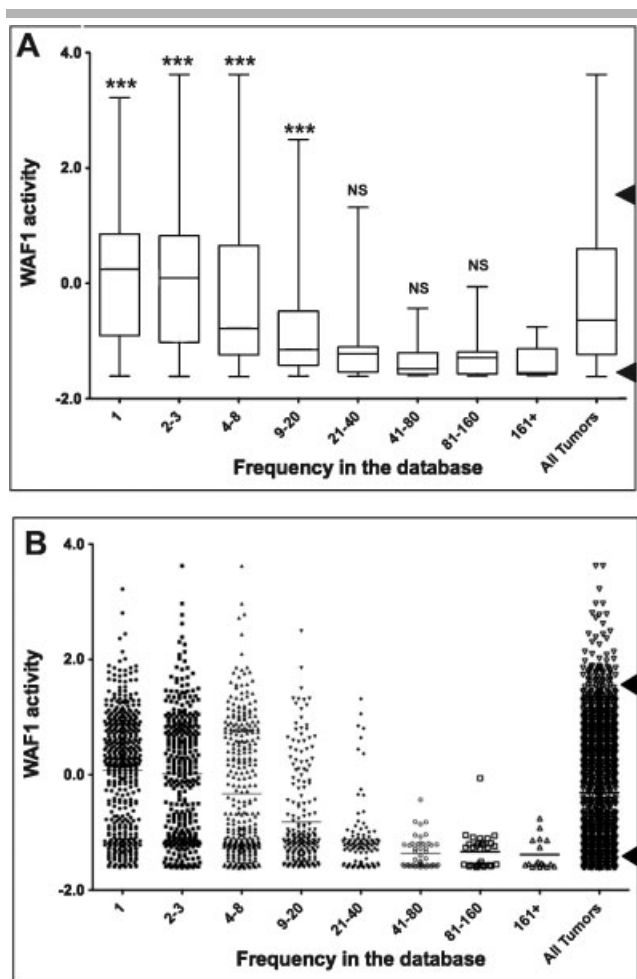


Figure 2. Activity of mutant *TP53* according to their frequency in various subsets of the database. Mutant *TP53* were classified into eight categories according to their frequencies. **A:** Box-and-whisker plots show the upper and lower quartiles and range (box), median value (horizontal line inside the box), and full-range distribution (whisker line); analysis was performed for the 1,439 mutants found in tumors. *P*-Values listed above each bar refer to comparison to the 161+ category. The Mann–Whitney *U*-test was used to evaluate statistical significance. Similar results were observed with the activity of seven other promoters regulated by *TP53* (Supp. Fig. S3). Changing the number of categories leads to similar results (Supp. Fig. S3). **B:** Each dot corresponds to the activity of a single mutant and its *X* position in the column is random. Black lines correspond to the mean value. N.S., not significant; *** $P < 0.0001$. Black triangles corresponds to 0% (bottom) and 100% (top) *TP53* activity.

analyzed. Sample 4 is a simple warning in the case of typographical errors where wt and mutant codon are identical. The mutation in example 5 (c.375G>A, p.T125) is also a good example of a common mistake. For a long time, this mutation was (and still is) described as a neutral somatic mutation, as it does not change the amino acid. Nonetheless, this mutation localized in the last codon of exon 4, has been shown to lead to aberrant splicing and p53 inactivation, a feature now described in comment 2 [Varley et al., 2001]. Exonic mutations leading to aberrant splicing are quite common but difficult to detect if only DNA bases analyses are performed. All codons in the vicinity of an exon (3' and 5') have been documented in MUT-TP53 2.0, and the user is warned accordingly (sample 6, Fig. 2). This feature has allowed the detection of splicing defects in several cell lines. Mutant c.1010G>A (p.R337H) in sample 7 is very unusual and was detected as a germline mutation in Brazilian families with children prone to develop adrenocortical tumors [Ribeiro et al., 2001]. A few tumors with a similar somatic mutation have also been described. Although classical transcriptional assays suggest that this mutant behaves like wt p53, structural studies show that this mutant displays an abnormal conformation at low pH [DiGiammarino et al., 2002]. Whether or not this abnormal conformation is associated with a defect in p53 function in vivo has not been clearly established, but, as shown in Figure 1, this mutant has been flagged. The mutant in sample 8 (c.[748C>T;749C>T], p.P250F), is a tandem mutation only described in skin cancer and which is extremely rare in internal tumors. This double-base substitution found at dipyrimidine sites is associated with UV exposure [Brash, 1997]. As shown in comment 3, a warning is displayed to the user concerning this feature, as a large number of tandem mutations would be very unlikely in internal tumors. Mutations 9 (c.[343C>A;345T>G], p.H115K, two noncontiguous substitutions) and 10 (c.[688A>T;689C>G;690C>G], p.T230W, three substitutions in the same codon) are extremely rare in the p53 mutation database and a warning is displayed for the user. This last feature is not specific to p53 mutations, as a recent release of cancer genome sequences shows that a single nucleotide substitution is the most frequent nucleotide substitution. The table shown in Figure 1 should help the user to pinpoint uncommon p53 mutants and perform verification to validate the mutation.

The second tool included in MUT-TP53 2.0 is entirely new and allows the user to perform statistical comparison of his/her data set with other publications. Since 2003, the UMD p53 mutation database includes functional information about the majority of p53 missense mutants (see also Materials and Methods). This quantitative data has been extremely useful to classify and analyze p53 mutations. The range of p53 loss of function of all p53 mutants for each publication can be displayed by calculating the mean and 95% CI of the residual activity of mutant p53 (Fig. 3). The analysis shows that, for more than 90% of publications, the mean activity was situated between -1 and -1.2 [Kato et al., 2003]. This value corresponds to a residual transcriptional activity of about 10% compared to wt p53. The small range of the 95% CI indicates that the majority of mutant p53 proteins behave in a similar way. Figure 3A shows typical results for lung, colorectal, and breast carcinomas, three types of cancer that have been extensively analyzed for p53 mutations. For each type of cancer, the five publications reporting the highest number of p53 mutants are shown, and one publication situated outside of the range of other studies (Fig. 3) is also shown. The reason why these out-of-range studies display an unusual number of mutant p53 proteins that retain wt activity has already been extensively discussed

[Soussi et al., 2006]. The two out-of-range studies in lung and colorectal cancer have been clearly shown to be artefactual, and they include unusual mutations in other genes as well. The study in breast cancer described an unusual number of clustered mutations at positions never previously described and a large number of mutations that do not change the amino acid sequence. This is not a real problem, as this highly controversial manuscript was published in the *New England Journal of Medicine* and raised the important question of the existence of genetic alterations in stroma cells from breast cancer patients, a highly debated field [Campbell et al., 2008; Roukos, 2008; Soussi-Zander and Soussi, 2008; Zalcman et al., 2008].

This statistical analysis is now available to users of MUT-TP53 2.0. It is performed automatically and displayed in the form of a table and a graphic and can be compared to other publications in the database (Fig. 3B).

It should be stressed that an out-of-range finding should not be considered to be definitive and formal proof of a dubious study, but indicate the need for careful review of the data. If confirmed, this finding should be discussed in the publication, as it may represent a novel finding on p53 loss of activity for a particular set of p53 mutations in a specific type of cancer. For example, the c.1010G>A (p.R337H) mutant discussed above does not display loss of transcriptional activity and could have been missed if structural studies were not performed. Its association with adrenocortical tumor also suggests a specific link of this mutant with a specific cellular environment.

Biochemical analyses have shown that mutant p53 proteins can be heterogeneous in terms of loss of DNA binding activity, transactivation, or other activities [Soussi and Lozano, 2005]. The DNA binding site recognized by p53 is highly degenerated and wt or mutant p53 have variable affinities for the various biological sites [Resnick and Inga, 2003]. Mutant p53 proteins also exhibit a dominant negative effect via inactivation of the function of wt p53. This characteristic increases the significance of a single mutant p53 allele. Although carcinogenesis requires the loss of both alleles of most tumor suppressor genes, mutation of one allele of p53 can result in total loss of function. Although the dominant negative effect clearly occurs in cancer models, the mechanism by which it occurs has not been fully elucidated. Whether or not tumors expressing mutant p53 with this dominant negative characteristic are more aggressive is also still under investigation. MUT-TP53 2.0 includes novel functional data derived from an unpublished database including more than 100,000 entries on mutant p53 in. Residual DNA binding activity (in vitro and in vivo), growth arrest, apoptosis, repressor, and dominant negative activities can now be displayed (Supp. Fig. S1 and Supp. Table S1). Not only will this information be useful to define the degree of loss of activity of a mutant p53, but it should also help the user to define various classes of mutant p53 in order to perform more accurate clinical analysis. MUT-TP53 2.0 with full documentation is available free of charge and can be downloaded from our Website (<http://p53.free.fr>).

Sequencing of tumor DNA for the detection of p53 mutations (as well as other genes) and publication of this information appear to be currently moving in opposite directions. On the one hand, DNA sequencing is now available at very low cost and high throughput, allowing screening of a large number of patients and, on the other hand, the lack of space for publication now means that more than 50% of publications describing p53 mutations no longer provide the mutational data, leading to a decreased number of entries over recent years. An automated spreadsheet such as MUT-TP53 2.0 can be used not only as a verification tool to

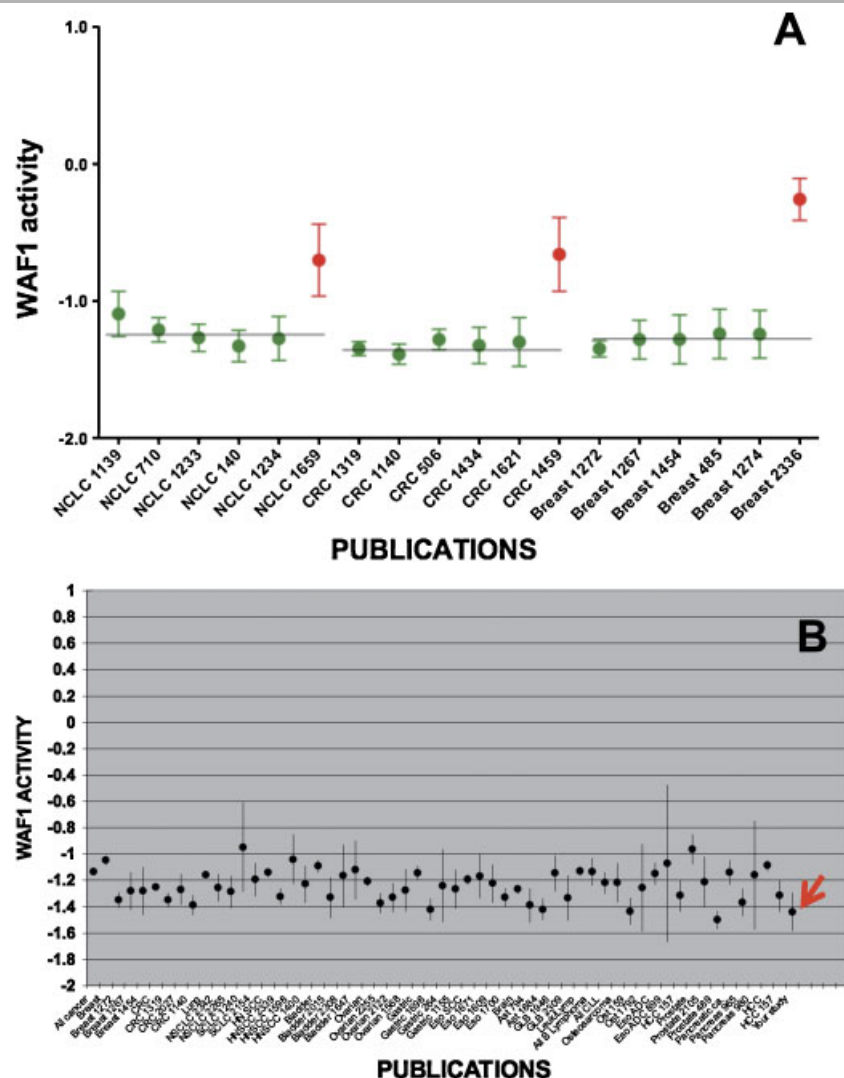


Figure 3. **A:** Meta-analysis of p53 loss of function. Dot and bars: mean and 95% CI of mean p53 activity as measured by transactivation with the WAF1 promoter. The y-axis corresponds to p53 transactivation activity, with a value of -1.58 for the negative control and a value of 2.03 for 100% of wt activity. The publication code is indicated on the x-axis: the first letter corresponds to the cancer type and the second number is an anonymous ID for the publication. Six publications were analyzed for lung (NSCLC), colorectal (CRC), and breast cancer. Five publications displayed a homogenous distribution of p53 loss of activity (green data), whereas one study reported out-of-range results (red data). **B:** Output generated by the spreadsheet. Data from the user are displayed at the far right of the graph (red arrow). Statistical analysis for various cancers is shown in the graph. Publication or cancer type code is indicated on the x-axis: CRC: colorectal cancer; GLB: glioblastoma; HNSCC: head and neck squamous cell carcinoma; HCC: hepatocellular carcinoma; NSCLC: nonsmall cell lung carcinoma; SCLC: small cell lung carcinoma; Eso SCC: esophageal squamous cell carcinoma; Ast: astrocytoma. The first letters correspond to the cancer type and the second number is an anonymous ID for the publication. All cancer: analysis of the entire UMD p53 database.

generate accurate data, but also as a support that can be easily used by the UMD database software to import automatically data and more efficiently update the p53 mutation database.

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References

Brash DE. 1997. Sunlight and the onset of skin cancer. *Trends Genet* 13:410–414.
 Campbell IG, Qiu W, Polyak K, Haviv I. 2008. Breast-cancer stromal cells with TP53 mutations. *N Engl J Med* 358:1634–1635; author reply 1636.
 Chanock SJ, Thomas G. 2007. The devil is in the DNA. *Nat Genet* 39:283–284.

den Dunnen JT, Antonarakis SE. 2000. Mutation nomenclature extensions and suggestions to describe complex mutations: a discussion. *Hum Mutat* 15:7–12.
 DiGiammarino EL, Lee AS, Cadwell C, Zhang W, Bothner B, Ribeiro RC, Zambetti G, Kriwacki RW. 2002. A novel mechanism of tumorigenesis involving pH-dependent destabilization of a mutant p53 tetramer. *Nat Struct Biol* 9:12–16.
 Horaitis O, Cotton RG. 2004. The challenge of documenting mutation across the genome: the human genome variation society approach. *Hum Mutat* 23:447–452.
 Kato S, Han SY, Liu W, Otsuka K, Shibata H, Kanamaru R, Ishioka C. 2003. Understanding the function–structure and function–mutation relationships of p53 tumor suppressor protein by high-resolution missense mutation analysis. *Proc Natl Acad Sci USA* 100:8424–8429.
 Ng PC, Henikoff S. 2001. Predicting deleterious amino acid substitutions. *Genome Res* 11:863–874.
 Resnick MA, Inga A. 2003. Functional mutants of the sequence-specific transcription factor p53 and implications for master genes of diversity. *Proc Natl Acad Sci USA* 100:9934–9939.
 Ribeiro RC, Sandrini F, Figueiredo B, Zambetti GP, Michalkiewicz E, Lafferty AR, DeLacerda L, Rabin M, Cadwell C, Sampaio G, Cat I, Stratakis CA, Sandrini R.

2001. An inherited p53 mutation that contributes in a tissue-specific manner to pediatric adrenal cortical carcinoma. *Proc Natl Acad Sci USA* 98:9330–9335.
- Roukos DH. 2008. Breast-cancer stromal cells with TP53 mutations. *N Engl J Med* 358:1636; author reply 1636.
- Soussi T, Asselain B, Hamroun D, Kato S, Ishioka C, Claustres M, Beroud C. 2006. Meta-analysis of the p53 mutation database for mutant p53 biological activity reveals a methodologic bias in mutation detection. *Clin Cancer Res* 12: 62–69.
- Soussi T, Kato S, Levy PP, Ishioka C. 2005. Reassessment of the TP53 mutation database in human disease by data mining with a library of TP53 missense mutations. *Hum Mutat* 25:6–17.
- Soussi T, Lozano G. 2005. p53 mutation heterogeneity in cancer. *Biochem Biophys Res Commun* 331:834–842.
- Soussi T, Rubio-Nevado JM, Ishioka C. 2006. MUT-TP53: a versatile matrix for TP53 mutation verification and publication. *Hum Mutat* 27:1151–1154.
- Soussi-Zander C, Soussi T. 2008. Breast-cancer stromal cells with TP53 mutations. *N Engl J Med* 358:1635; author reply 1636.
- Stratton MR, Campbell PJ, Futreal PA. 2009. The cancer genome. *Nature* 458:719–724.
- Strausberg RL, Simpson AJ. 2010. Whole-genome cancer analysis as an approach to deeper understanding of tumour biology. *Br J Cancer* 102:243–248.
- Varley JM, Attwooll C, White G, McGown G, Thorncroft M, Kelsey AM, Greaves M, Boyle J, Birch JM. 2001. Characterization of germline TP53 splicing mutations and their genetic and functional analysis. *Oncogene* 20:2647–2654.
- Zalcman G, Bergot E, Hainaut P. 2008. Breast-cancer stromal cells with TP53 mutations. *N Engl J Med* 358:1635–1636; author reply 1636.