Honoring Pierre Hainaut

Lessons in science and humanity

Greenbaum Lab Journal Club
August 12, 2025

Structure of presentation

We will overview Pierre's very full life.

Our discussion will cover qualities salient at different points of his life.

Curiosity

Vision

Bravery

Empathy

Legacy

Curiosity

March 22, 1958

Pierre is born in Belgium.

The state of the world in 1958

Caption Cancer research laboratory. Researchers wearing protective garments and masks while working with laboratory equipment. Photographed in the USA, in the 1950s.

Life as a student (1980 - 1994)

J. gen. Virol. (1983), **64**, 2535–2548. *Printed in Great Britain*

Key words: *MMTV/virus entry/antigens/intestine*

Peroral Infection of Suckling Mice with Milk-borne Mouse Mammary Tumour Virus: Uptake of the Main Viral Antigens by the Gut

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(*Accepted 22 August 1983*)

SUMMARY

Persistence of mouse mammary tumour virus (MMTV) components in the digestive tract of suckling mice was investigated by immunoperoxidase staining of the main viral antigens and micro-immunoenzyme assays of gp52 and p28; these latter assays were also performed after ingestion of milk enriched in viral antigens using Cr_2O_3 as a marker for the alimentary bolus migration. When compared to the ingested antigens, the amounts of gp52 and p28 decreased during transit, p28 being more rapidly digested than gp52. The antigens were, however, destroyed to a much larger extent in the gut of the adult than in that of the newborn mouse. A fraction of the marker remained for a long time in the stomach; a prolonged retention was also observed with gp52 and especially with p28. Significant amounts of viral antigens were detected in the intestinal walls: both p28 and gp52 were found in the duodenum and small intestine. Moreover, the four viral antigens gp52, gp36, p28 and p8 were clearly observed in very large supranuclear vacuoles inside the epithelial cells of the distal part of the gut. Total particles can reach the intestine; the viral material could then be either destroyed or taken up in the epithelial cells by endocytosis, so that the intestinal epithelium might serve as a portal of entry for MMTV in the suckling mouse.

Path through school:

1. MSc in 1980
2. PhD in 1987 (seven year PhD, there is hope!)
3. Postdocs for another seven years (1987 - 1994)

First publication as a student

Table 1. *Distribution of gp52, p28 and Cr_2O_3 in ligatured intestinal loops**

	Incubation medium	Walls	Contents	Total
10-day-old Swiss mice				
gp52	2·0†	2·3	61·0	65
p28	1·6	2·2	57·4	61
Cr_2O_3	BD‡	BD	83	83
6-month-old Swiss mice				
gp52	1·9	1·8	32·4	36
p28	2·0	2·1	31·3	35
Cr_2O_3	BD	BD	89	89

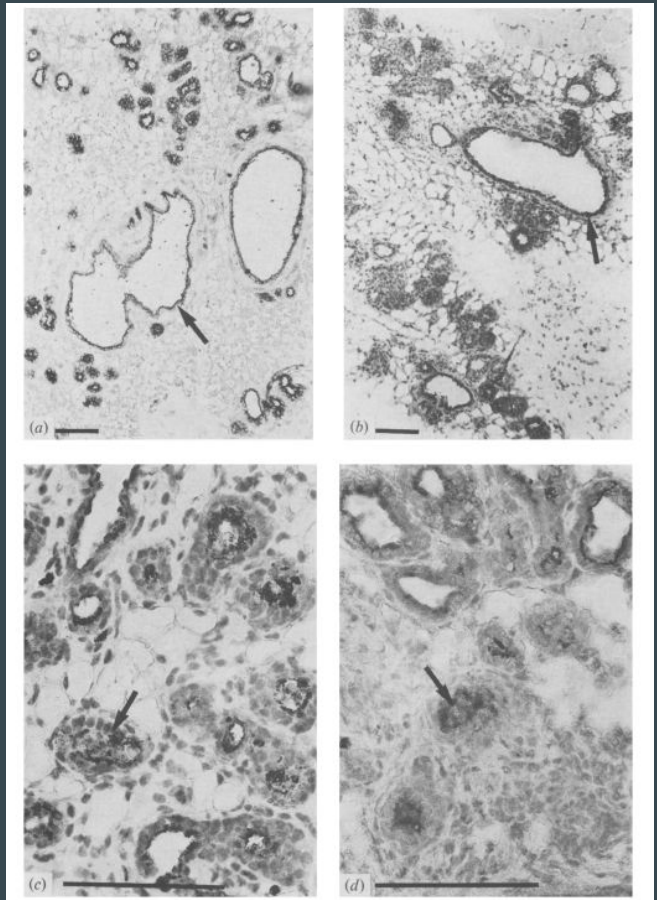
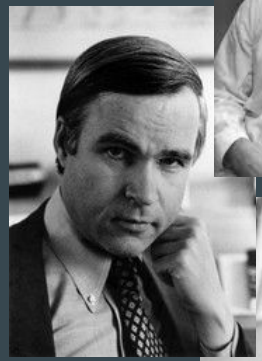
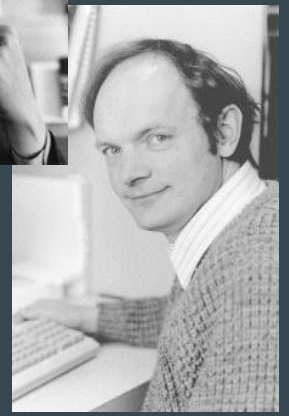


Fig. 3. Immunoperoxidase staining of gp52 (*a*), gp36 (*b*), p28 (*c*) and p8 (*d*) in the mammary glands of MMTV-infected Swiss mice. Bar markers represent 0·1 mm. This control experiment indicates clearly the apical localization of the viral glycoproteins in the epithelial cells of the alveoli (arrows in *a* and *b*) and, in the same cells, a cytoplasmic localization of the viral proteins (arrows in *c* and *d*). Before use, all sera were absorbed on an acetone powder of C57BL mice mammary glands and none of them stained the uninfected mammary glands.

The protective action of the wild-type *p53* gene helps to suppress tumors in humans. However, the *p53* gene is the most commonly mutated gene in human cancer, and these mutations may actively promote tumor growth. The purple dots indicate some of the many tumor types that may carry *p53* mutations, including brain, esophagus, lung, breast, liver, prostate, and colon. See Editorial, page 1953, Molecule of the Year article, page 1958, and Perspective, page 1980. [Illustration: K. Sutliff and C. Faber Smith]





Publishing as a post-doc: entrance into p53

The EMBO Journal vol.11 no.10 pp.3513 - 3520, 1992

Interaction of heat-shock protein 70 with p53 translated *in vitro*: evidence for interaction with dimeric p53 and for a role in the regulation of p53 conformation

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Communicated by H.Pelham

In intact cells, hsp70 proteins selectively complex with mutant p53. We report here that rabbit reticulocyte lysate contains hsp70 which selectively complexes with the mutant p53 translated *in vitro*. Hsp70 complexes with dimers and possibly monomers of p53 in a manner that requires the terminal 28 amino acids of p53. Using murine $p53^{Val135}$, which is temperature-sensitive for phenotype, we demonstrate that p53–hsp70 complexes can occur after post-translational switching from wild-type to mutant p53 phenotype. Moreover, the temperature-induced switch of full-length $p53^{Val135}$ from wild-type to mutant phenotype is ATP-independent, whereas the switch from mutant to wild-type form requires ATP hydrolysis and involves hsp70. These results imply that hsp70 is involved in the regulation of p53 conformation.

Key words: cell growth control/heat-shock proteins/hsp70/p53

Introduction

The p53 protein plays a role in the control of cell proliferation and the p53 gene is the most commonly affected gene in human cancer (Hollstein *et al.*, 1991; Caron de Fromentel and Soussi, 1992). The p53 gene is a tumour suppressor gene, and mice homozygous for a null p53 allele introduced by homologous recombination appear to be developmentally normal but prematurely susceptible to a variety of neoplasms (Donehower *et al.*, 1992). Point mutations within conserved domains can inactivate p53 suppressor function and some may also activate p53 as an oncogene and promote cell proliferation (see Marshall, 1991 and Levine *et al.*, 1991 for reviews). Point mutations can affect the tertiary structure of p53 protein, altering its reactivity with specific anti-p53 monoclonal antibodies: the wild-type phenotype is reactive with monoclonal antibodies PAb246 and PAb1620 but not with PAb240. Conversely, the mutant phenotype is reactive with PAb240 but not with PAb246 and PAb1620 (Cook and Milner, 1990; Gannon *et al.*, 1990).

Most transforming p53 mutants form stable complexes with both stress-inducible and constitutively expressed members of the 70 kDa family of heat-shock proteins (Pinhasi-Kimhi, 1986; Hinds *et al.*, 1987; Sturzbecher *et al.*, 1987, 1988; Finlay, 1988). Heat-shock proteins (hsps) belong to a class of proteins broadly defined as 'molecular chaperones', involved in facilitating the transport, folding and assembly of many proteins (for reviews, see Gething and Sambrook, 1992; Rothman, 1989). In the case of p53, interaction with hsps appears to involve remarkably conserved structures, since p53 also interacts with a bacterial hsp, dnaK, when expressed in *Escherichia coli* (Clarke *et al.*, 1988). Moreover, both purified p53–hsp70 and p53–dnaK complexes dissociate *in vitro* when incubated with micromolar levels of ATP, suggesting that the stability of the complex is regulated by the intrinsic ATPase activity of hsps (Clarke *et al.*, 1988). However, more detailed study of p53–hsp70 complexes under defined conditions has been hampered by lack of a suitable *in vitro* association assay.

We now demonstrate the interaction of hsp70 protein present in rabbit reticulocyte lysate with p53 proteins translated in the same lysate. Translation of p53 RNA in reticulocyte lysate is a useful system for studying p53 conformation under defined conditions (Milner *et al.*, 1991; Milner and Medcalf, 1991). For example, a mutant allele of murine p53 ($p53^{Val135}$) which is temperature-sensitive for function (Michalovitz *et al.*, 1990) has been shown to be temperature-sensitive for conformation when expressed *in vitro* (Milner and Medcalf, 1990). $p53^{Val135}$ adopts the wild-type phenotype at 30°C and the mutant phenotype at 37°C. Moreover, a simple post-translational shift of temperature is sufficient to induce the protein to switch from wild-type to mutant phenotype, and vice versa (Milner and Medcalf, 1990). Using this p53 mutant we now show that hsp70 present in reticulocyte lysate complexes with p53 during the translation of mutant, but not wild-type form of p53. Complex formation also occurs when p53 is induced to switch from wild-type to mutant phenotype by a post-translational shift in temperature. This finding allowed us to investigate the role of ATP and of hsp70 binding during the interconversion between wild-type and mutant p53 phenotypes.

Results

Binding of in vitro translated murine p53 to hsp70 present in rabbit reticulocyte lysate

The presence of hsp70 in rabbit reticulocyte lysate was revealed by immunoblotting with a rabbit antiserum against the carboxyl terminus of mammalian hsp70 (data not shown). This antiserum, kindly given by Dr Stephen Ullrich (NCI), recognizes both stress-inducible and constitutively expressed members of the hsp70 family (see Materials and methods). To investigate the association between p53 and lysate hsp70, both wild-type and mutant p53 ($p53^{Val135}$) were translated *in vitro* at 37°C in the presence of [^{35}S]methionine. Immunoprecipitations were carried out with antibodies to p53 and to hsp70, using normal rabbit serum as a negative control. Figure 1A clearly shows that ^{35}S-labelled mutant p53, but not wild-type, co-precipitates with lysate hsp70. Under these conditions the amount of p53 complexed with hsp70 represents ~5–10% of total translated p53.

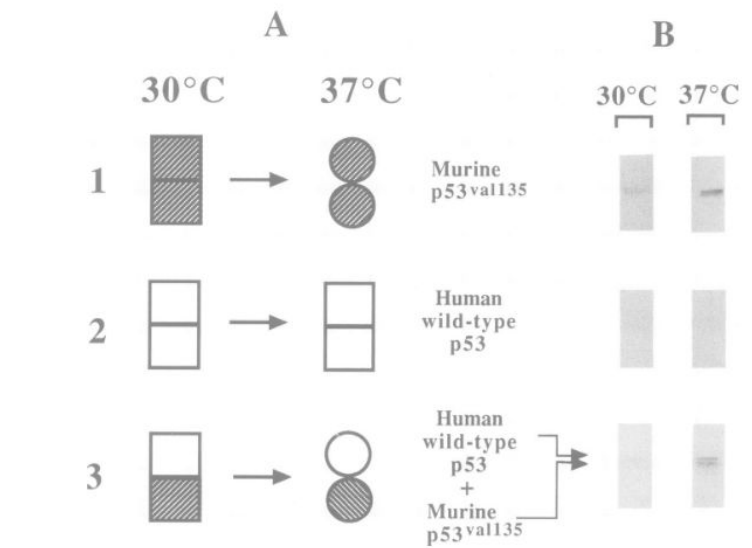

Fig. 4. Association of hsp70 with mixed oligomers formed by co-translation *in vitro* of murine $p53^{Val135}$ and human wild-type p53. **(A)** Schematic representation of the effect of temperature on the conformational phenotype of murine $p53^{Val135}$ and human wild-type p53. Phenotypically wild-type and mutant p53 are represented by a square and a circle, respectively. p53 is represented as a dimer. Murine $p53^{Val135}$ (hatched symbols) is temperature-sensitive for conformation, adopting the wild-type phenotype at 30°C and switching to the mutant phenotype at 37°C. In contrast, human wild-type p53 (white symbols) is phenotypically wild-type at 30°C and at 37°C. Co-translation of murine $p53^{Val135}$ and human wild-type p53 yields mixed oligomers that adopt the wild-type phenotype at 30°C. Upon shifting the temperature to 37°C, mutant murine p53 drives human wild-type to adopt the mutant phenotype (see Milner and Medcalf, 1991). **(B)** Co-immunoprecipitation of p53 with anti-hsp70 serum at 30°C and 37°C. The double arrow in the lower panel indicates human p53 (upper arrow) and murine p53 (lower arrow). Immunoprecipitations were carried out as in the legend to Figure 1.

Publishing as a post-doc: entrance into p53

[CANCER RESEARCH 53, 1739–1742, April 15, 1993]

Advances in Brief

A Structural Role for Metal Ions in the "Wild-type" Conformation of the Tumor Suppressor Protein p53[1]

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Abstract

In human tumors, many different point mutations of the *p53* gene knock out suppressor function and induce the p53 polypeptide to adopt an immunologically distinct, "mutant" conformation. Here we show that exposure to the metal chelator 1,10-phenanthroline induces wild-type *p53* to adopt the mutant conformation and that this process is reversible. Conversion to mutant phenotype also occurs after exposure to (*a*) an organic mercurial reagent targeting cysteinyl residues and (*b*) low concentrations of mercury or cadmium. We propose that binding of metal ions, most probably zinc, to conserved cysteinyl residues stabilizes the tertiary structure of wild-type *p53*.

Introduction

The gene encoding the tumor suppressor protein p53 is the most frequently affected gene in human cancer, where loss of one *p53* allele is often coupled with a point mutation in the remaining allele (1). The wild-type p53 protein acts as a suppressor of abnormal cell proliferation. However, missense mutations within conserved regions can not only inactivate this suppressor function but in some cases also activate p53 as an oncogene and promote cell proliferation (2). The effect of these mutations on p53 function presumably reflects alterations of the tertiary structure of the protein (3). Indeed, many oncogenic mutant p53 proteins differ from wild-type by their reactivity with conformation-specific monoclonal antibodies. Wild-type p53 is characteristi-

linearized with *Stu*I. Translations were carried in rabbit reticulocyte lysate (Promega) for 1 h at 37°C unless otherwise stated, in the presence of 0.75 μM of added [^{35}S]methionine (40.5 TBq/mmol; Amersham) (4). Translations were stopped by addition of anisomycin (2 μg/μl). Given that a typical translation yielded 0.8–1.3 × 10^5 cpm of trichloroacetic acid-precipitable material per μl of lysate, the estimated concentration of p53 synthesized is 100 pmol/ml.

Treatment with Defined Reagents and Immunoprecipitations. OP[3] and CMPS (Sigma) were kept as, respectively, 10 mM and 25 mM solutions in 10 mM Tris-HCl, pH 7.6. Unless otherwise stated, aliquots of translated lysate were incubated for 20 min at 37°C in the presence of OP, CMPS, or metal ions dissolved in 10 mM Tris-HCl, pH 7.6. Using OP, time course experiments revealed that the effect on wild-type p53 was rapid ($t_{1/2}$ 7 min) and reached a maximum after 20 min (data not shown). After incubation, p53 was analyzed by immunoprecipitation as described previously (4), using the following antibodies: PAb 240 (5); PAb246 and PAb248 (10); and rabbit serum to hsp70 (9). Immunoprecipitates were analyzed on 15% SDS-PAGE as described elsewhere (8).

Results and Discussion

In order to investigate factors involved in the tertiary folding of p53, we have first studied the effect of metal chelators on the conformation of wild-type p53, since metal ions play a crucial role in stabilizing conformational structures in many proteins (11). The effect of the chelating agent 1,10-phenanthroline on wild-type p53 translated *in vitro* is shown in Fig. 1. Wild-type p53 reacted with PAb246 but

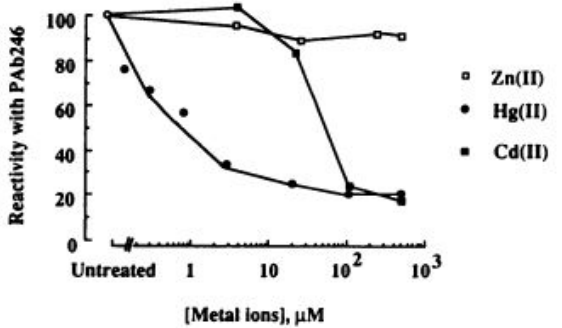


Fig. 4. Effect of metal ions on the wild-type conformation of p53. Wild-type p53 was exposed to varying concentrations of Zn(II), Cd(II), or Hg(II) for 20 min at 37°C. Metal ions were added as sulfate (zinc, cadmium) or chloride (mercury) salts in water (26). Immunoprecipitations with PAb246 and quantification by scintillation counting were carried out as in legend to Fig. 1*A*. Results are expressed as a percentage of the amount of p53 detected by PAb246 in the absence of added metal ions. For Zn(II), identical results were obtained using $ZnCl_2$ instead of $ZnSO_4$ (not shown)

Experimental Oncology | Published: 01 February 1995

Temperature sensitivity for conformation is an intrinsic property of wild-type p53

P Hainaut, S Butcher & J Milner

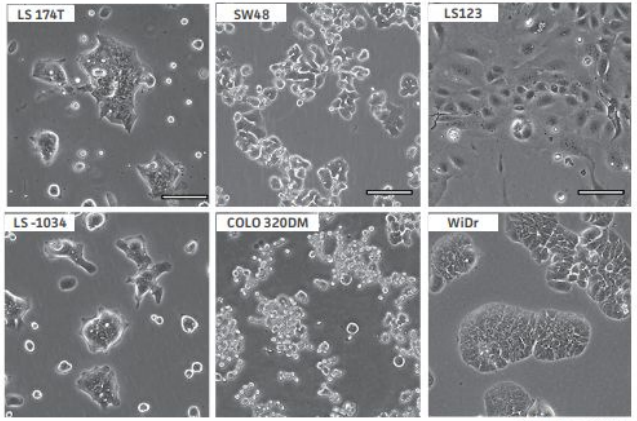
British Journal of Cancer **71**, 227–231 (1995) | Cite this article

Vision

Making cancer research computational

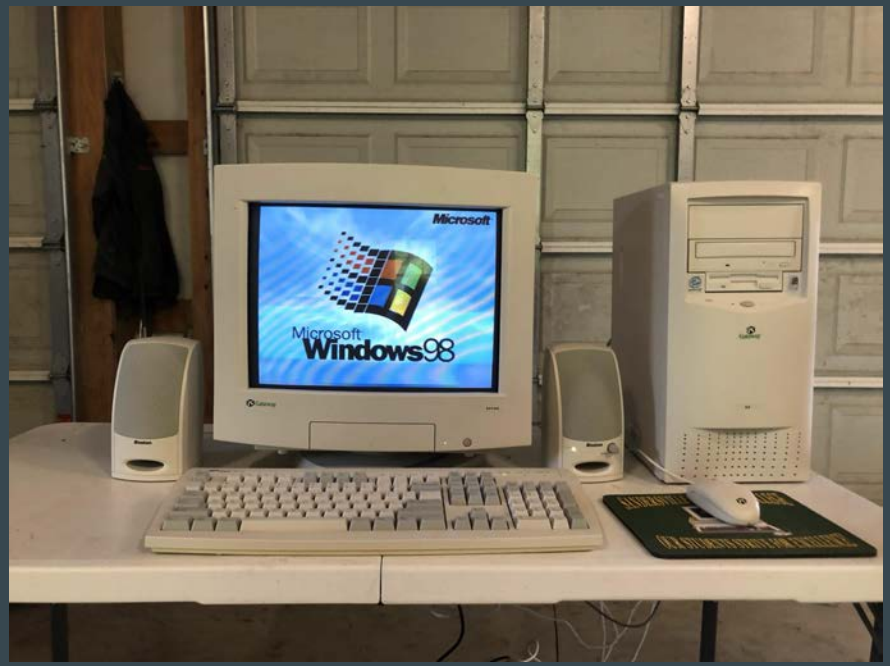
ATCC® No.	Name	Tissue	Histology	Tumor Source	TP53 status	Zygosity	Gene Mutation†	Protein Sequence†
CL-188™	LS174T	colon	adenocarcinoma	primary	WT	-	-	-
CCL-231™	SW48	colon	adenocarcinoma	primary	WT	-	-	-
CCL-255™	LS123	colon	adenocarcinoma	primary	MUT	homozygous	c.524G>A	p.R175H
CRL-2158™	LS1034	colon	adenocarcinoma	primary	MUT	homozygous	c.733G>A	p.G245S
CCL-220™	COLO 320DM	colon	adenocarcinoma	primary	MUT	homozygous	c.742C>T	p.R248W
CCL-218™	WiDr	colon	adenocarcinoma	primary	MUT	homozygous	c.818G>A	p.R273H

†For a description of the sequence variation nomenclature please refer to: den Dunnen JT and Antonarakes SE (2000), Hum. Mutat. 15:7-12.



Scale Bar = 100µm

Figure 6: Cell morphology of the six cell lines in the Colon Cancer p53 Hotspot Mutation Cell Panel. Two p53 wild-type colon cancer cell



1994 - 2011 : Development of IARC
How do you make a database?

Nucleic Acids Research, 1997, Vol. 25, No. 1 151–157

Database of p53 gene somatic mutations in human tumors and cell lines: updated compilation and future prospects

P. Hainaut, T. Soussi[1], B. Shomer[2], M. Hollstein[3], M. Greenblatt[4], E. Hovig[5], C. C. Harris[6] and R. Montesano*

International Agency for Research on Cancer, 150 cours Albert-Thomas, 69372 Lyon Cedex 08, France, [1]Unité 301 INSERM, 27 rue Juliette Dodu, 75010 Paris, France, [2]EMBL-Outstation-European Bioinformatics Institute, Wellcome Trust Genome Campus, Hinxton, Cambridge CB10 1SD, UK, [3]German Cancer Research Center, Im Neuenheimer Feld 280, D-69120 Heidelberg, Germany, [4]University of Vermont, College of Medicine, Health Sciences Complex, Burlington, VT 05405, USA, [5]Institute for Cancer Research, Norwegian Radium Hospital, 0310 Oslo, Norway and [6]Laboratory of Human Carcinogenesis, National Cancer Institute, Bethesda, MD 20892, USA

Received October 11, 1996; Accepted October 15, 1996

ABSTRACT

In recent years, there has been an exponential increase in the number of p53 mutations identified in human cancers. The p53 mutation database consists of a list of point mutations in the *p53* gene of human tumors and cell lines, compiled from the published literature and made available through electronic media. The database is now maintained at the International Agency for Research on Cancer (IARC) and is updated twice a year. The current version contains records on 5091 published mutations and is expected to surpass the 6000 mark in the January 1997 release. The database is available in various formats through the European Bioinformatics Institute (EBI) ftp server at: ftp://ftp.ebi.ac.uk/pub/databases/p53/ or by request from IARC (p53database@iarc.fr) and will be search-able through the SRS system in the near future. This report provides a description of the criteria for inclu-sion of data and of the current formats, a summary of the relevance of *p53* mutation analysis to clinical and biological questions, and a brief discussion of the prospects for future developments.

INTRODUCTION

The p53 tumor suppressor gene encodes a nuclear phosphopro-tein with cancer-inhibiting properties. The development of human cancer often involves inactivation of this suppressor function through various mechanisms, including gene deletions and point mutations. Since the identification of tumor-specific, missense p53 mutations in 1989, there has been a widespread interest in the possibility that the localisation and the characteris-tics of these mutations may reveal clues about the etiology and the molecular pathogenesis of human cancer (reviewed in 1–3). Point mutations are scattered over more than 250 codons and are common in many forms of human cancer. In this respect, the p53 gene differs from other tumor suppressor genes such as Rb, APC and p16^{MTS-1}, which are frequently inactivated by deletion or nonsense mutations, and from the oncogenes of the *ras* family, which are activated by mutation at a small number of well-defined codons.

The p53 protein is a multi-functional transcription factor involved in the control cell cycle progression, DNA integrity and cell survival in cells exposed to DNA-damaging agents. DNA-damage induces a transient nuclear accumulation and activation of the p53 protein, with transcriptional activation of target genes such as the cyclin kinase inhibitor p21 waf-1 (a negative regulator of cell-cycle) and the regulator of apoptosis bax-1 (a dominant-negative inhibitor of bcl-2). Most mutations impair the specific DNA-binding capacity of p53, therefore allowing cells to proliferate in conditions where cells with intact p53 function are suppressed or eliminated. Mutation of p53 may thus provide a selective advantage for the clonal expansion of pre-neoplastic or neoplastic cells. However, all mutations are not equivalent. Mutant proteins differ by the extent of their loss of suppressor function and by their capacity to inhibit wild-type p53 in a dominant-negative manner. In addition, some p53 mutants apparently exert an oncogenic activity of their own, but the molecular basis for this gain-of-function phenotype is still unclear (reviewed in 4).

The diversity of p53 mutations provide a valuable tool to identify important sources of cancer-causing agents in the human setting. Mutagens and carcinogens damage the genome in characteristic ways, leaving 'mutagen fingerprints' in DNA. Specific DNA changes can also be brought about by endogenous biological processes. DNA-repair and bioselection of mutants with specific properties act as additional 'filters' to generate the final mutation spectrum observed in any particular tumor type. Thus, p53 mutations can provide clues to the nature of exogenous

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A	B	C	D	E	F	G	H	I	J	K	L	M	N
1	143	GTG to GCG	428	T to C	Cx3	Colon	1				Val->Ala		
2	175	CGC to CAC	524	G to A	Cx1	Colon	1			yes	Arg->His		
3	132	AAG to CAG	394	A to C	BT 20	Breast	2		L		Lys->Gln		
4	249	AGG to AGC	747	G to C	BT 549	Breast	2		L		Arg->Ser		
5	280	AGA to AAA	839	G to A	MDA231	Breast	2		L		Arg->Lys		
6	285	GAG to AAG	853	G to A	BT 474	Breast	2		L		Glu->Lys		
7	157	GTC to TTC	469	G to T	OZ1	HCC	3				Val->Phe		
8	249	AGG to AGT	747	G to T	OZ2	HCC	3				Arg->Ser		
9	249	AGG to AGT	747	G to T	OZ3	HCC	3				Arg->Ser		
10	249	AGG to AGT	747	G to T	OZ4	HCC	3				Arg->Ser		

Figure 1. Section of the database in Excel spreadsheet format showing record 1 as an example.

Notes to authors

When tumor mutations are reported for the second time in a new publication we recommend this be stated in a footnote to the table where the mutations are re-listed, also indicating which tumor mutations were reported previously. Providing tumor samples with unique case numbers would also help to avoid redundancies in the database.

The inherent inconsistencies we have detected (~2% of all records) in reported mutations were usually traceable to typo-graphical errors in the publication, to reading the genetic code from the wrong DNA strand or to misnumbering the codons in sequencing film illustrations.

The electronic form of the database may be cited by referencing this *Nucleic Acids Research* article.

THE p53 MUTATION DATABASE

Codon	substitution	Number	Total	Frequency (in %)
all cancers				
273	Arg->Cys	130	348	37,4
273	Arg->His	157	348	46.3
273	Arg->Gly	2	348	,6
273	Arg->Pro	8	348	2,3
273	Arg->Leu	41	348	11,8
273	Arg->Ser	4	348	1,1
273	Arg->Asn	1	348	,3
273	frameshift	1	348	,3
lung cancers				
273	Arg->Cys	7	41	17,1
273	Arg->His	7	41	17,1
273	Arg->Pro	5	41	12,2
273	Arg->Leu	18	41	43,9
273	Arg->Ser	3	41	7,3
273	Arg->Asn	1	41	2,4
Colon cancers				
273	Arg->Cys	20	48	41,7
273	Arg->His	28	48	58,3

Bravery

Facing the tobacco industry

How do you stand up to a multi-billion dollar industry?


BRITISH AMERICAN
TOBACCO

Pierre publishes evidence of smoking/p53

A Specific Spectrum of *p53* Mutations in Lung Cancer from Smokers: Review of Mutations Compiled in the IARC *p53* Database

Tina M. Hernandez-Boussard and Pierre Hainaut

International Agency for Research on Cancer, Lyon, France

Mutations in the *p53* gene are common in lung cancer. Using data from the the International Agency for Research on Cancer *p53* mutation database (R1), we have analyzed the distribution and nature of *p53* mutations in 876 lung tumors described in the literature. These analyses confirm that G to T transitions are the predominant type of *p53* mutation in lung cancer from smokers. The most frequently mutated codons include 157, 158, 179, 248, 249, and 273, and several of them (157, 248, and 273) have been shown to correspond to sites of *in vitro* DNA adduct formation by metabolites of polycyclic aromatic hydrocarbons (PAHs) such as benzo(*a*)pyrene. Furthermore, most of the base changes at codons 248, 249, and 273 in lung cancer differ from those commonly observed at these codons in other cancers reported in the database. Thus, lung cancer from smokers shows a distinct, unique *p53* mutation spectrum that is not observed in lung cancer from nonsmokers. These results further strengthen the association between active smoking, exposure to PAHs, and lung cancer. They also indicate that a different pattern of mutations occurs in nonsmokers, and this observation may help to identify other agents causally involved in lung cancer in nonsmokers. *Key words:* benzo(*a*)pyrene, lung cancer, nonsmokers, *p53* mutations, tobacco. *Environ Health Perspect* 106:385–391 (1998). [Online 10 June 1998] *http://ehpnet1.niehs.nih.gov/docs/1998/106p385-391hernandez/abstract.html*

Lung cancer is the leading cause of death in developed countries and is considered as one of the most common cancers worldwide (*1–3*). Tobacco smoking has been identified as a major risk factor for the development of this cancer (*4–6*). Overall, recent cohort studies show that the risk of death from lung cancer in smokers of two or more packs of cigarettes per day is about 20 times that of nonsmokers (*5,6*). Tobacco smoke is a complex mixture that contains about 3,800 different potentially harmful chemicals (*7*). Several of these chemicals are proven carcinogens and occur at significant concentrations in tobacco smoke. These chemicals include benzo(*a*)pyrene [BaP, a polycyclic aromatic hydrocarbon (PAH)] at 20–40 ng/cigarette, *N*-nitroso compounds at up to 200–3,000 ng/cigarette, 4-aminobiphenyl (an aromatic amine) at 2.4–4.6 ng/cigarette, and vinyl chloride at 1.3–16 ng/cigarette (*8,9*). The exact contribution of each of these various carcinogens to lung cancer induced by tobacco smoke is poorly understood.

Deletion and point mutations in the *p53* tumor suppressor gene are common in most types of human cancers, including lung cancer. Missense mutations occur at about 300 distinct positions within the *p53* coding sequence. The diversity of positions and chemical natures of these mutations allows the determination of tumor-specific mutation spectra that can provide clues to the nature of the mutagenic agents which are involved as causative agents (*10–12*). To facilitate the analysis and interpretation of these mutations, a database of *p53* mutations in human tumors and cell lines is maintained at the International Agency of Research on Cancer (IARC). This database, initiated in 1991 by Hollstein et al. (*13*), is exclusively based on published material and contains about 8,000 somatic mutations in an electronic format (*14*).

To determine whether the spectrum of *p53* mutations may aid in understanding the role of carcinogens associated with tobacco smoke, we have carried out detailed analyses of the mutations associated with lung cancer compiled in the IARC *p53* mutation database (849 cases). We have reviewed these data in the light of recent progress in our understanding of the mechanisms of 1) selective adduct formation in the *p53* coding sequence, 2) strand-specific and position-specific DNA repair, and 3) selection of mutant proteins with specific functional properties. Our analyses confirm and extend previous reports that G to T transversions are specifically found in tumors from smokers (*11*). Moreover, we have retrieved data on *p53* mutations in 36 nonsmokers; analysis of these mutations shows a unique spectrum of mutations, different from lung cancer from smokers as well as from all other cancers.

Methods

Point mutations in the *p53* gene of human tumors and cell lines were extracted from the IARC *p53* mutation database. This database is updated twice a year, and for this analysis we used the January 1998 update (R1; 8,000 mutations). The database exists in different electronic formats available on the World Wide Web (*http://www.iarc.fr/p53/homepage.html*) and is deposited at the European BioInformatic Institute (EBI; *http://www.ebi.ac.uk*). For analysis of the database, we developed a program using FileMaker Pro 3.0 (Claris Corporation, Santa Clara, CA) that is described on our database web site and published elsewhere (*14*). The database contains information on 876 mutations in lung tumors. These mutations were compared with those found in breast tumors (729 cases) and colon tumors (900 cases) because they occur at high frequencies in the general population, they frequently contain *p53* mutations [approximately 50% in colon cancer (*11*), and 15–40% in breast cancer (*15*)], and they are well represented in the IARC *p53* database.

The database is based on published records and does not contain information on tumors without *p53* mutations. When information was provided on the smoking status of individual patients with lung cancer, the tumors were classified into two groups: ever smoked (236 cases) and never smoked (36 cases). Information on sex was available for 13% of the cases (81 males and 32 females). For the classification of the different lung cancer pathologies, we used the terminology given in each individual paper. The classification of the 876 lung tumors, as well as the availability of information on smoking, is given in Table 1.

The χ^2 test was used for statistical analyses. When expected values in the χ^2 test were less than 5, Fisher's exact test was used.

Results

High frequency of G to T transversions. In lung cancer, *p53* missense mutations are detected in about 60% of the tumors, and about one-third of these mutations have been reported as G to T transversions (*11*). Figure 1 compares the spectrum of mutations in lung cancer with all other cancer types. Lung cancer shows a significantly higher proportion of

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The authors would like to thank R. Montesano, M. Denisenko, and G. Pfeifer for their critical reviews of this manuscript and helpful suggestions and J. Esteve for aid with the statistical analyses. T.H.-B. is a recipient of a Special Training Award from the International Agency for Research on Cancer.

Received 13 December 1977; accepted 9 February 1998.

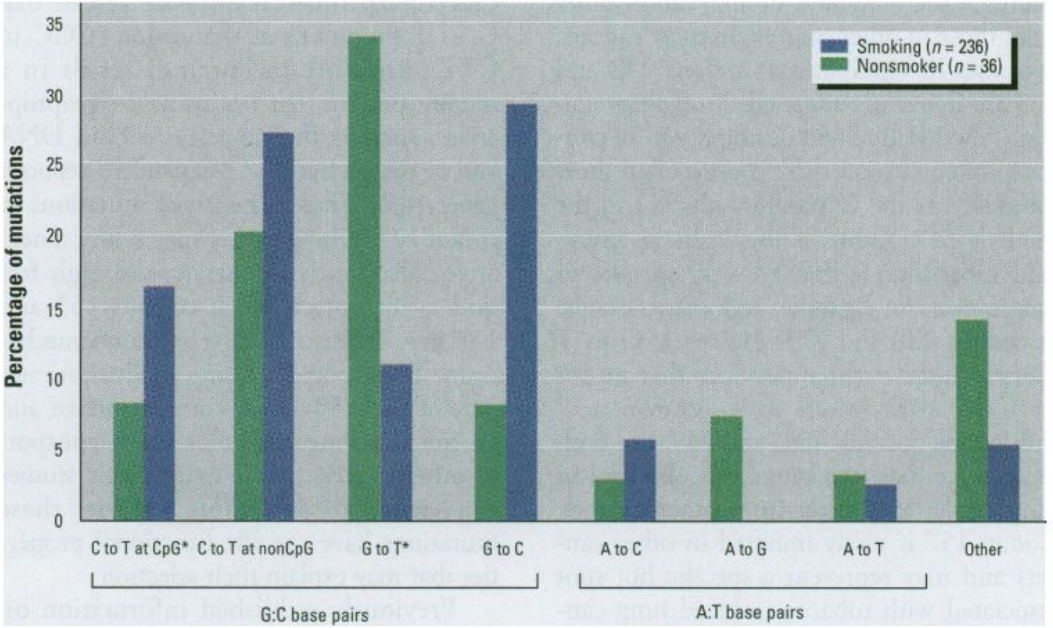


Figure 6. Comparison of the mutation spectra of *p53* mutations in lung cancer of smokers and nonsmokers. Mutations are classified as transitions or transversions affecting G:C base pairs or A:T base pairs. Abbreviations: C to T, GC to AT transitions; G to T, GC to TA transversions; A to C, AT to CG transversions; A to G, AT to GC transitions; A to T, AT to TA transversions; Other, insertions, deletions, and complex mutations.
*Significant at $p<0.001$.

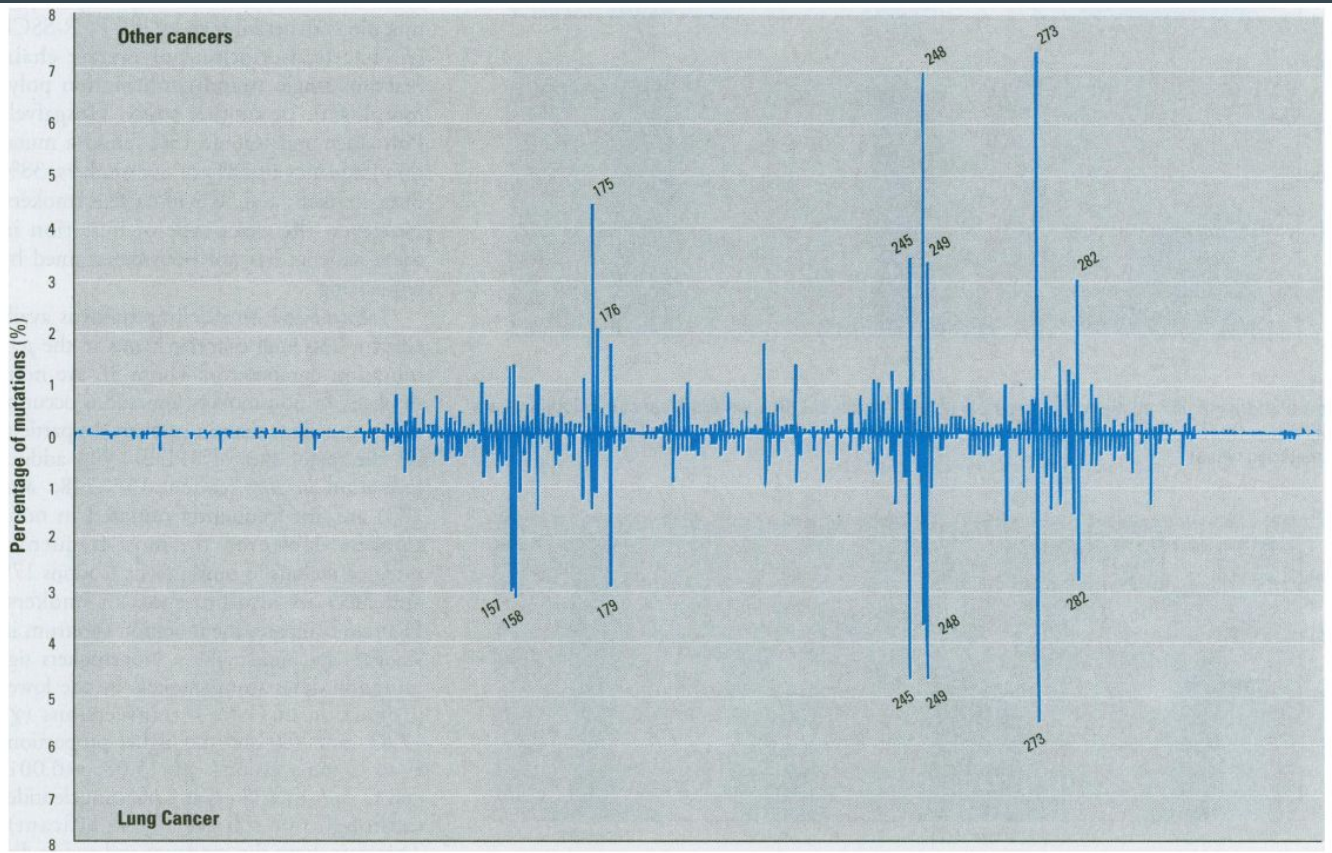


Figure 3. Comparison of the *p53* hot spots in lung cancer with all cancers in the IARC *p53* database. The distribution of mutations in the *p53* coding sequence is shown, with the size of bars representing the frequency of mutations at a particular codon. Hot spot codon numbers are shown (*11*).

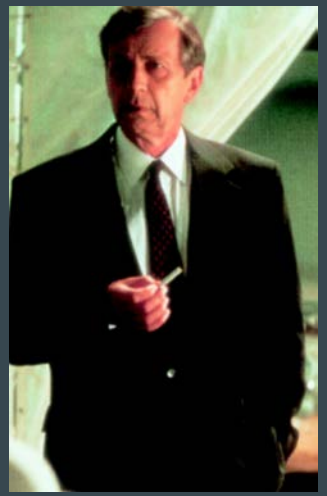
The tobacco industry's insiders

BAT established the "Scientific Research Group" to monitor research on p53 in the late 1980s.

BAT funded scientists across the globe and encouraged them not to mention support.

"*BAT and p53*
More papers are currently published on p53 than any other topic on cancer research . . . The SRG identified p53 as an important area some four years ago and the SRG currently supports two research projects relating to p53. Through one connection in particular we are often aware of work before it is published

p53 and Litigation
. . . Attempts to implicate tobacco by analysis of mutational spectra in p53 isolated from lung or other cancers may be foreseen."[11]



A coordinated effort by the tobacco industry

Another study was carried out by Thilo Paschke, an employee of the Verband der Cigarettenindustrie (VdC), the German association of cigarette manufacturers, from at least June 1999.[42,43] The VdC includes German companies as well as PM, BAT, RJR, Lorillard.[44] A June 13, 2000 e-mail from Paschke to Chris Coggins, Lorillard Senior Vice President of Research and Development, reports:

> "I published my analysis of the [IARC p53] database at a German conference on environmental mutagenesis . . . and submitted it to a journal on mutagenesis. I'll send you a preprint of the paper, if the referees accept it for publication".[43]

Paschke's paper was published in the November, 2000, issue of *Mutagenesis*. Analysing changes in the

Mutagenesis vol.15 no.6 pp.457–458, 2000

DISCUSSION FORUM

Analysis of different versions of the IARC *p53* database with respect to G→T transversion mutation frequencies and mutation hotspots in lung cancer of smokers and non-smokers

Thilo Paschke

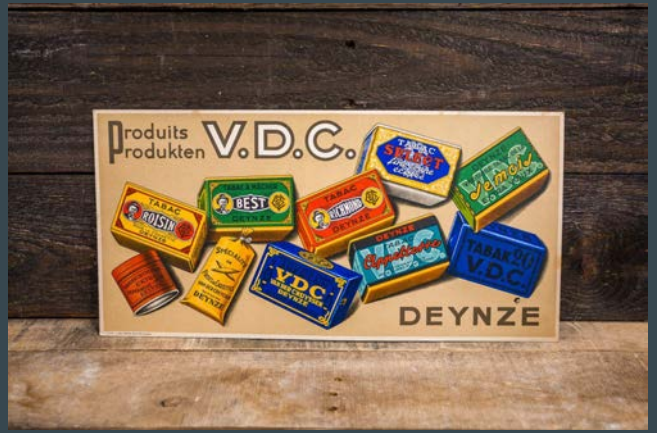
Analytisch-Biologisches Forschungslabor, Goethestrasse 20, D-80336 Munich, Germany

Analysis of the IARC *p53* database revealed a large number of discrepancies in the classification of smoking status for identical lung cancer entries in different versions of the database. In addition, no statistically significant differences in G→T transversion mutation frequencies or in mutational hotspots at codons 157, 248 and 273 were found in the R3 version of the database between p53 sequences from smoking and non-smoking lung cancer patients. The possible influence of confounding factors on *p53* mutation spectra was demonstrated as illustrated by the impact of ethnicity on G→T transversion mutation frequencies.

tion had been reported in two separate publications, reanalysis of the R1 version of the database showed a statistically significant difference in G→T transversion frequencies in smokers and non-smokers ($\chi^2 = 3.98$; $P < 0.046$), although the difference was less clear than that reported by Hernandez-Boussard and Hainaut (1998).

The R1 version of the IARC *p53* database was updated in July 1998. The updated version, R2, contained information from 1046 lung cancer cases, whereas the earlier version had 900 entries. Surprisingly, only 221 of the entries in the R2 version were classified for smoking status (131 smokers, 90 non-smokers), whereas in the earlier R1 version 379 lung cancer cases with information on smoking status had been included (284 smokers, 95 non-smokers).

Careful analysis of the R2 version of the database revealed



A coordinated effort by the tobacco industry

Human lung cancer and *p53*: The interplay between mutagenesis and selection

Sergei N. Rodin*†‡ and Andrew S. Rodin§

*Biology Department, Beckman Research Institute of the City of Hope, 1450 East Duarte Road, Duarte, CA 91010; †Institute of Cytology and Genetics, Siberian Branch of Russian Academy of Sciences, Novosibirsk, 630090, Russia; and §Human Genetics Center, School of Public Health, University of Texas–Health Science Center, P.O. Box 20334, Houston, TX 77225

Communicated by Eugene Roberts, Beckman Research Institute of the City of Hope, Duarte, CA, July 11, 2000 (received for review February 2, 2000)

It is an almost consensus opinion that the major carcinogenic risk of tobacco smoke is in its direct mutagenic action on DNA of cancer-related genes. The key data supposedly linking smoke-induced mutations to lung cancer were obtained from the adduct spectrum of the *p53* tumor suppressor gene. Results of our analysis of p53 mutations compiled from the International Agency for Research on Cancer *p53* database (April 1999 update) and from the literature point to a different causative link. Our new analytical tests focused on complementary base substitutions and showed that it is strand-specific repair of primary lesions and site-specific selection of the resultant mutations that determine the lung cancer-specific hot spots of G:C to T:A transversions along the *p53* gene and also their increased abundance in lung tissues as compared with smoke-inaccessible tissues. However, on each of the two strands of p53 DNA, our tests revealed no significant difference between smokers and nonsmokers, either in the frequency of different types of mutations or in the frequency of their occurrence along the *p53* gene. Moreover, in both smokers and nonsmokers, there was the same frequency of lung tumors with silent p53 mutations. Accordingly, we offer here a selection-based explanation of why lung cancers with nonsilent p53 mutations are more common in smokers than in nonsmokers. We conclude that physiological stresses (not necessarily genotoxic) aggravated by smoking are the leading risk factor in the p53-associated etiology of lung cancer.

mutational hot spots in human lung cancer. This coincidence led the authors (10) to conclude that "targeted adduct formation rather than phenotypic selection appears to shape the *p53* mutational spectrum in lung cancer." Whereas codons 248 and 273 are among major mutational hot spots in virtually all cancers, codon 157 was claimed to be a hot spot unique to lung cancer (10).

In mutationally characterizing the *p53* gene, one way is to plot the frequency of each of 12 different types of base substitutions in the gene as a whole (Fig. 1*A*), which we will call hereon a *p53* mutational pattern. Another way (Fig. 2) is to plot a frequency of the particular 1 of these 12 types as a function of a position in the *p53* gene, which we refer to as a *p53* mutational spectrum.

Thus, the previously proposed causative chain was as follows: (*i*) the major risk factor for lung cancer is smoking, (*ii*) smoke contains benzo[*a*]pyrene, (*iii*) BPDE forms bulky adducts at G bases of DNA, (*iv*) the adducts cause G→T transversions, (*v*) these transversions are a hallmark of mutant *p53* genes from lung cancers, and (*vi*) hot spots of G→T transversions coincide with preferential sites of p53 DNA-BPDE adducts. However, as noted in ref. 11, some pivotal epidemiological evidence required was missing. If BPDE is a major initiating mutagen that shapes the lung cancer *p53* mutational pattern and spectrum of smokers, then one would expect both the pattern and the spectrum to be essentially different in the lung tumors of confirmed nonsmokers. Until recently, published p53 mutational data in nonsmokers

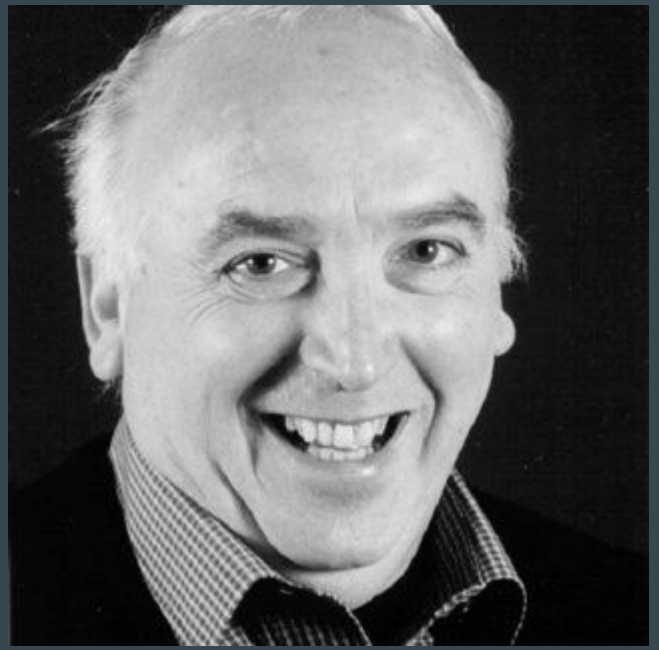
In addition to addressing these technical issues, Hainaut and colleagues noted Paschke's ties to the tobacco industry. Their response, as initially submitted to the journal stated that

> ". . . the paper by Paschke comes from a private institute of the German Association of Cigarette Manufacturers which has a long and proven history of participating in campaigns by the tobacco industry to subvert the normal scientific process of the evaluation of effects of tobacco smoke."[46]

James M Parry, editor of *Mutagenesis*, responded to Hainaut and colleagues:

> "I am not willing to approve the publication of your . . . point about the scientific integrity of Dr. Paschke. I am not willing to allow the pages of Mutagenesis to be used for non-scientific purposes . . . I now intend to forward your reply to Dr. Paschke together with a copy of this letter and indicate that he may provide a response to your comments. However, in any response from Dr. Paschke I will request that he provides an acknowledgement to any financial support to his work."[48]

A coordinated effort by the tobacco industry



Pierre responds

Mutagenesis vol.16 no.6 pp.551–553, 2001

LETTER TO THE EDITORS

TP53 mutation spectrum in lung cancers and mutagenic signature of components of tobacco smoke: lessons from the IARC TP53 mutation database

Pierre Hainaut[2], Magali Olivier and Gerd P.Pfeifer[1]

Group of Molecular Carcinogenesis, International Agency for Research on Cancer (WHO), 150 Cours Albert Thomas, 69372 Lyon Cedex, France and [1]Department of Biology, Beckman Research Institute of the City of Hope, Duarte, CA 91010, USA

A database of all published *TP53* mutations in human cancer is maintained at the International Agency for Research on Cancer (IARC). In lung cancers, *TP53* mutation patterns show an exceptionally high prevalence of G→T transversions, mostly occurring at codons demonstrated to be sites of adduction of metabolites of polycyclic aromatic hydrocarbons such as benzo[*a*]pyrene, one of the major carcinogens of tobacco smoke. These observations have been challenged in a recent 'Discussion Forum' by T.Paschke, who claimed that a large number of discrepancies existed in the classification of smoking status between successive releases of the IARC TP53 mutation database and that no statistically significant differences could be found in G→T transversion frequencies between smoking and non-smoking lung cancer patients. In the present Letter we question the methods and the conclusions of the analysis presented by Paschke. Based on an assessment of all published data, we confirm the existence of a highly significant difference in the prevalence of G→T transversions between smoking and non-smoking lung cancer patients.

Missense *TP53* mutations are common in human cancers. These mutations are scattered over many different codons and differ by their position and their chemical nature. This variability has allowed one to draw tumour-specific mutation patterns which, in some instances, are consistent with mutagenic mechanisms thought to be involved in the aetiopathogenesis of cancer (for a recent review see Hainaut and Hollstein, 2000). In 1991 a database of published *TP53* mutations was established by C.C.Harris and M.Hollstein in order to facilitate the retrieval and analysis of *TP53* mutations. Since 1994 this database has been maintained at the International Agency for Research on Cancer (IARC) and is made freely available as a service to the scientific community (http://www.iarc.fr/p53/Index.html).

In lung cancers a large fraction of *TP53* mutations (>30%) are G→T transversions, a type of mutation which is infrequent in tumours other than lung cancers (<12%, aside from hepatocellular carcinoma linked with exposure to aflatoxins) as well as in lung cancers from non-smoking patients (~10%) (Greenblatt *et al.*, 1994; Bennett *et al.*, 1999; Hussain *et al.*, 1999). Previous studies have shown that mutations often occur at bases known to be sites of formation of polycyclic aromatic hydrocarbon (PAH) adducts in the coding sequence of *TP53*. Codons 157, 248 and 273, the three strongest binding codons for benzo[*a*]pyrene diol epoxide adducts *in vitro*, contain 21% of all G→T mutations in lung cancers, versus <10% in all other cancers (Denissenko *et al.*, 1996, 1998; Hernandez-Boussard and Hainaut, 1998; Smith *et al.*, 2000). These observations support the notion that patterns of G→T transversions in lung cancers reflect the primary mutagenic signature of DNA damage inflicted by components of tobacco smoke.

The origin of *TP53* mutations in lung cancers has recently been questioned in two publications claiming that there were no significant differences in the prevalence of G→T transversions in lung cancers from smoking and non-smoking patients. The first of these reports was published in *Proceedings of the National Academy of Sciences* (Rodin and Rodin, 2000) and prompted us to reassess the evidence available on *TP53* mutations in smokers and non-smokers. This reassessment, which has been published recently, has fully confirmed our previous conclusions (Hainaut and Pfeifer, 2001). The second of these reports, by Paschke, appeared as a 'Discussion Forum' in *Mutagenesis* (Paschke, 2000). Paschke has compared three successive versions of the IARC TP53 database released between 1997 and 1999 (versions R1, R2 and R3) and has identified a large number of discrepancies in the classification of smoking and non-smoking status. In addition, he did not find any statistically significant difference between smoking and non-smoking lung cancer patients with respect to G→T transversion mutation frequencies or in mutational hotspots at codons 157, 248 and 273 using the R3 version (1999) of the database. In conclusion, he raised the hypothesis that previously reported differences might result from the influence of confounding factors such as ethnicity on *TP53* mutation spectra. We strongly disagree with these conclusions and with the way Paschke has used the contents of the database.

First, on the issue of G→T transversions in lung cancers, the literature referenced in PubMed (National Library of Medicine, Bethesda, MA) demonstrates a highly significant difference between smokers and non-smokers. The current version of the IARC TP53 mutation database reflects the literature published until December 2000 and contains a total of 1697 mutations in primary lung cancers, including 349 in ascertained smokers and 99 in ascertained non-smokers. The prevalence of G→T transversions in smokers is 29%, compared with 10% in non-smokers ($P < 0.0001$, χ^2 test). In tumours not related to tobacco smoke the prevalence of G→T transversions varies between 8 and 12% (Hainaut and Pfeifer, 2001). As far as 'hotspot' mutations are concerned, Paschke (2000) fails to mention that the specificity of mutational hotspots in lung cancers is not at the *codon* level, but at the *base* level (Denissenko *et al.*, 1996; Hernandez-Boussard and Hainaut,

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P.Hainaut, M.Olivier and **G.P.Pfeifer**

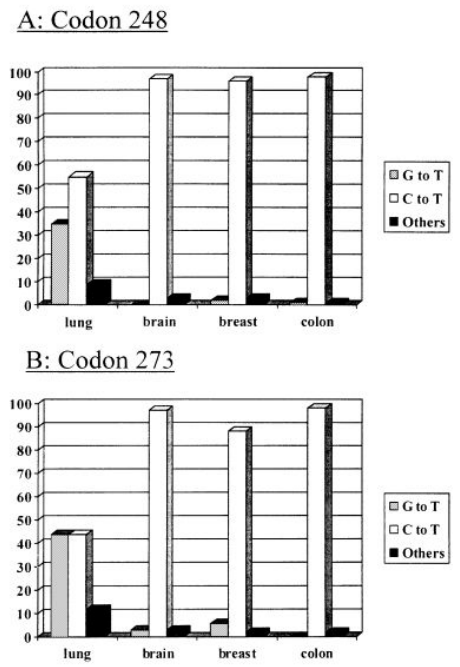


Fig. 1. Patterns of mutations at two common 'hotspot' codons in lung, brain, breast and colon cancers. Percentages of the total numbers of mutations are given. Total numbers at codon 248: 90 (lung), 62 (brain), 78 (breast) and 165 (colon). Total numbers at codon 273: 93 (lung), 107 (brain), 69 (breast) and 102 (colon). Data from IARC TP53 mutation database, version R4. (See also Hainaut and Pfeifer, 2001.)

Network of tobacco-funded scientists revealed

Scientist was paid consultant to tobacco firm

Serious concerns were raised last night about a scientific journal that published influential studies questioning the links between smoking and lung cancer.

Anti-smoking scientists said they were alarmed that the former editor of Mutagenesis, a genetics journal published by Oxford University Press, failed to disclose his position as a paid consultant to the tobacco industry. Throughout the 1990s, leading scientists published research in the peer-reviewed journal which cast doubt on studies showing that benzoapyrene, a powerful carcinogen found in tobacco smoke, was a primary factor behind genetic mutations which cause cancerous tumours.

Professor James Parry, who was paid £5,000 for consultancy work by British American Tobacco (BAT) when he was editing the journal in 1993, stepped down from its board this month as allegations of the conflict of interest surfaced.

His contract with BAT stipulated: 'The consultant shall use his best endeavours to safeguard the best interests of the company and shall not expose the company to any liability or commit the company to any obligation without the company 's prior written consent.'

Parry denies his decision to leave the board was due to concern about his links with the tobacco industry. But Dr Helen Wallace of campaign group GenewatchUK, which has spent months documenting Parry's links to the industry, said: 'Geneticists working secretly for the tobacco industry threaten the integrity of science as well as public health. Universities should not allow their medical researchers to take tobacco money, nor should journal editors receive tobacco funding.'

Studies published in Mutagenesis have been used by the tobacco industry to suggest that links between cigarette smoke and lung cancer are not as explicit as the wider scientific community has made out.

Researchers at the Centre for Tobacco Control Research and Education at the University of California raised concerns about Parry's tobacco industry links in the latest edition of the Lancet. The team note that evidence of a direct link between smoking and lung cancer 'is a potentially powerful tool that can connect a patient's disease to its specific cause... such a tool could be useful in litigation and regulation concerning tobacco use, as it provides genetic proof of the health effects of tobacco, both for the individual smoker and those exposed to second-hand smoke'.

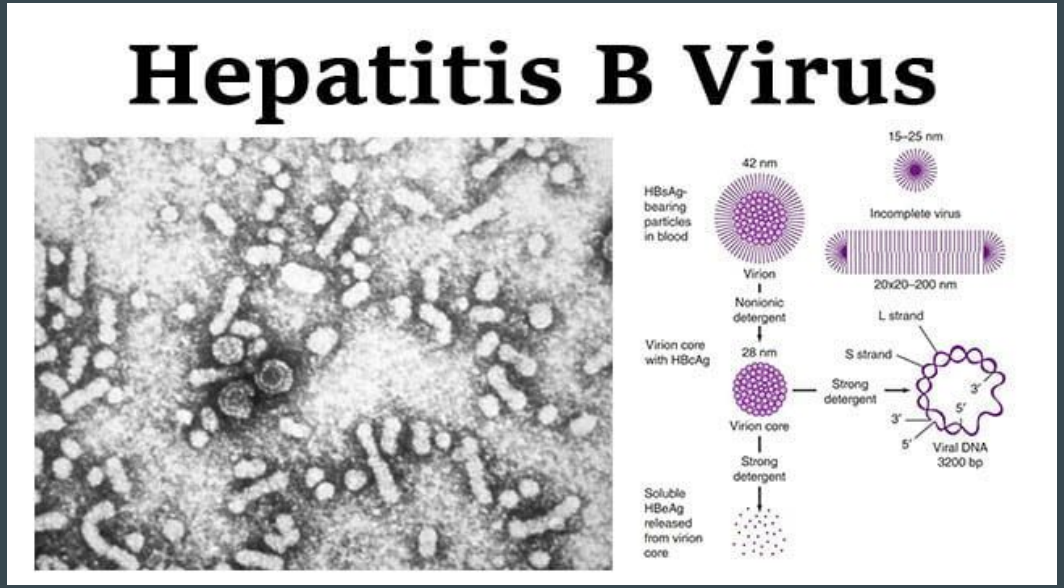
Parry said it was not the journal's policy for staff to list potential conflicts of interest until 2001, when he stepped down as editor, although he remained on the editorial board until this month.

'In 2001, Oxford University Press decided to request editors for a declaration of interests towards the tobacco industry,' he said. 'This topic had never been raised before, but was irrelevant to me, as I was standing down anyway under our long-term agreement. I think it is fairly clear that the message of the Lancet article that there was a correlation between my standing down from Mutagenesis and declarations of interest is wrong.'

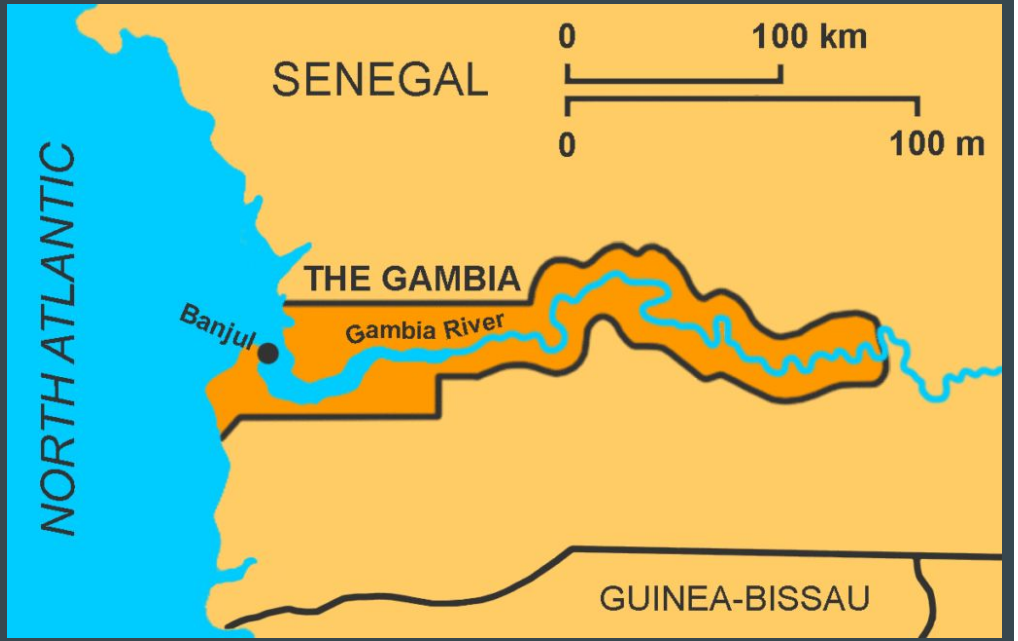
A spokeswoman for the OUP said it had a 'strict conflict of interest policy in all its journals', but as it was only the publisher of the journal, it was unable to act.can

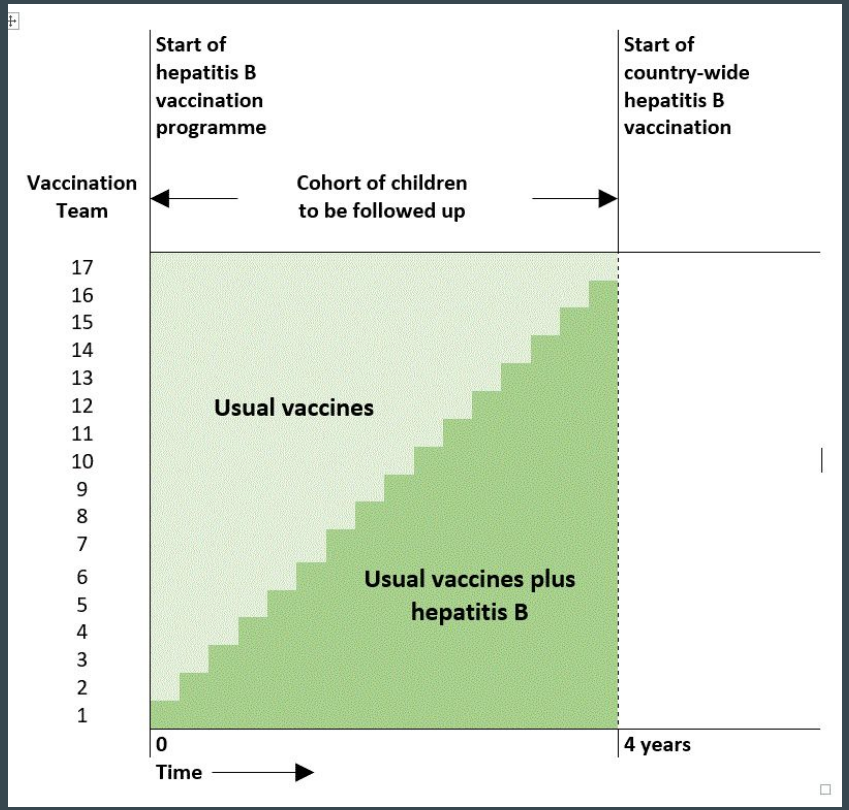
Empathy

The Gambia Hepatitis Intervention Study



The Gambia Hepatitis Intervention Study

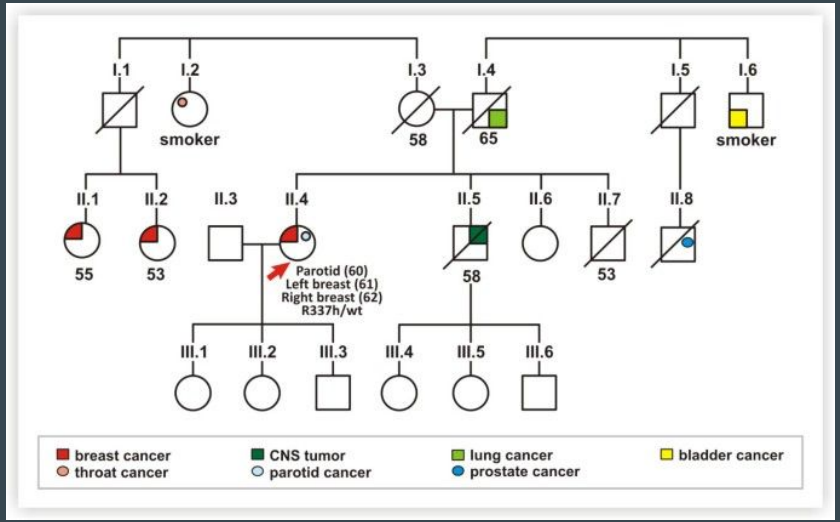


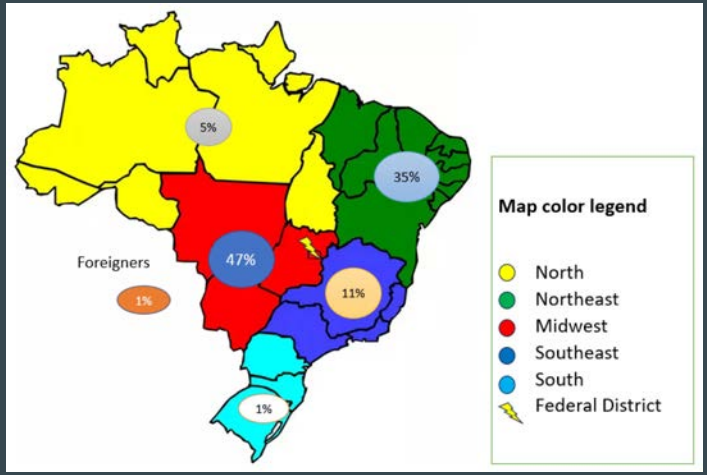


Why does cancer stalk this family?

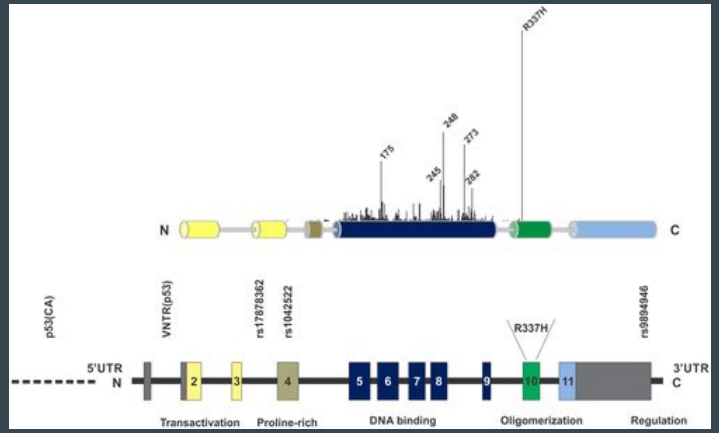
These relatives all have a genetic mutation that makes their odds of getting the disease frighteningly high. Stephanie Nolen tracks down the researcher who discovered the same thing in families throughout Brazil and explores what their genes can teach us about how cancer works

Dr. Maria Isabel Achatz, centre, a cancer geneticist, sitting with, from left to right: Pietro Freire de Oliveira, 17, his grandmother Ernestina de Jesus de Oliveira, 70, Andrea Domingues da Silva, 34, Andrea's cousin Rosimeire Domingues, 32, her uncle Benedito Batista Filho, 73, and his sister Angelina Domingues da Silva, 61, in Sao Paulo. The families carry a hereditary genetic mutation that makes them highly vulnerable to cancer.





A perfect meeting



Together, he and Achatz would go on to do research that found that a mutation in a key cancer-fighting gene, once thought to be incredibly rare, may occur in as many as 300,000 Brazilians. It causes them to get multiple primary cancer tumours – mainly sarcomas, leukemia, breast and brain cancer and adrenal cortical cancer. This is both a massive public-health challenge for Brazil and a dizzyingly rich research opportunity for cancer researchers worldwide.

Figure 2. A gathering of subjects from cancer prone families, proudly showing their familial tree spanning 8 generations

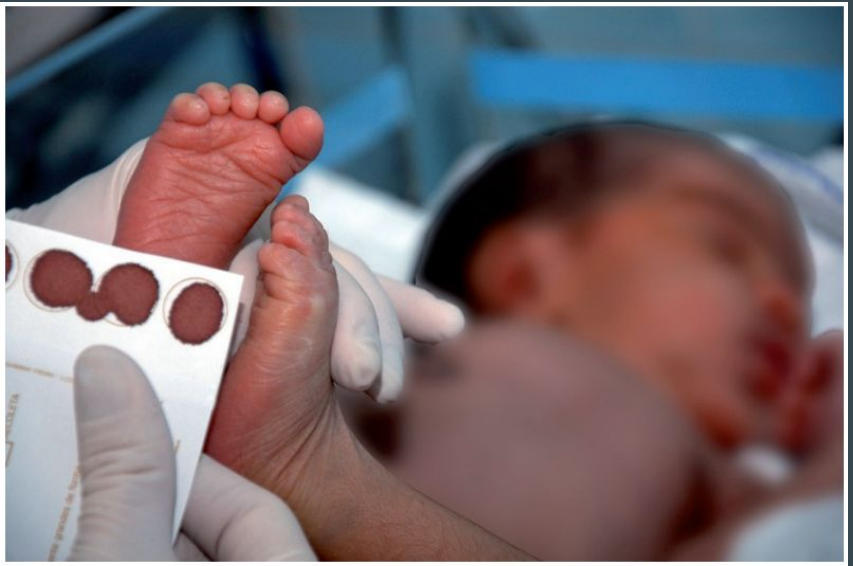


Figure 3. TP53 R337H mutation and Newborn screening

Presence of a common TP53 mutation in 0.3% of the population of South Brazil has led to questions about whether it was appropriate to screen newborns for this mutation in order to better detect subjects at high risk of cancer. Studies by IARC and collaborators have argued against this approach, considering that there is not enough evidence to predict the risk of cancer over lifetime. Childhood predictive genetic testing for R337H should not be carried out in mass screening programs, although it may represent a suitable approach in some families, on a case-by-case basis and within counseling and follow-up strategies that take into account the wide diversity of tumour patterns in mutation carriers

LI-FRAUMENI SYNDROME

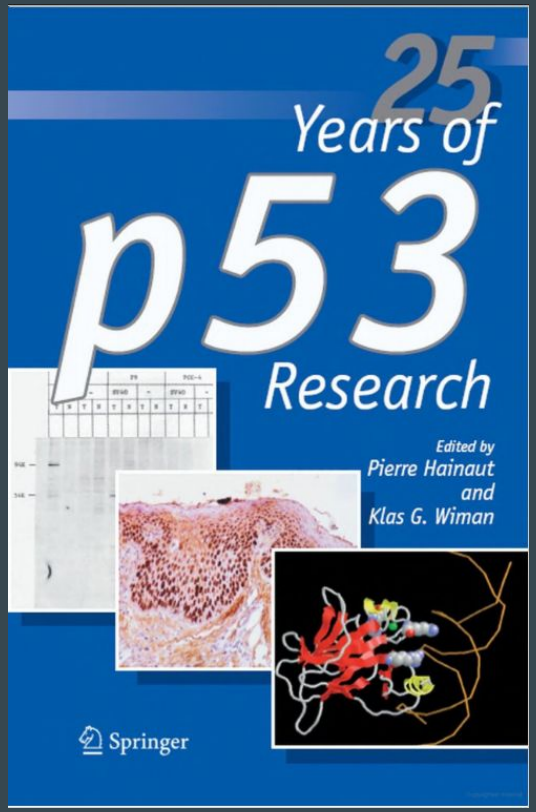
LFSA

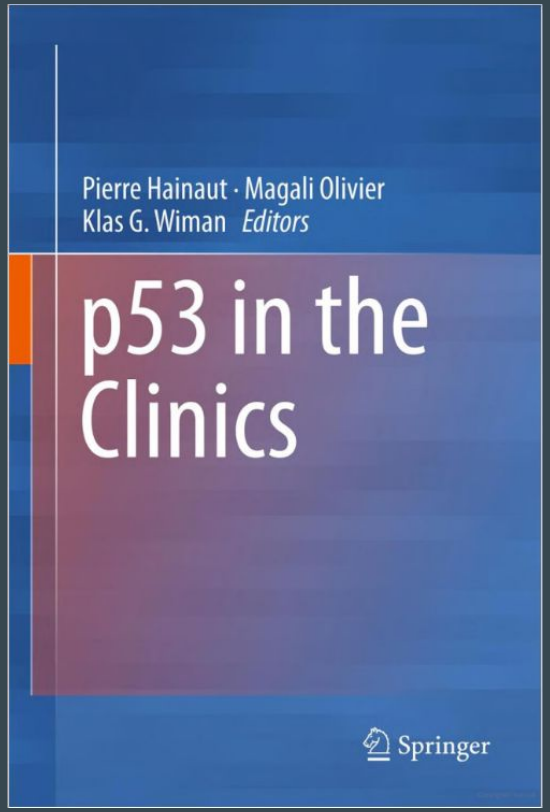
ASSOCIATION

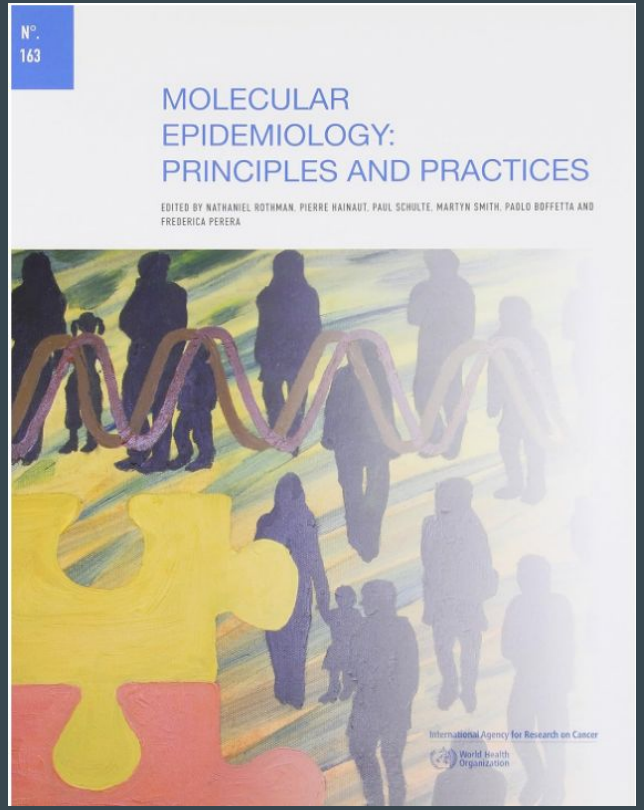
Devoted to a world without inherited cancer

Legacy

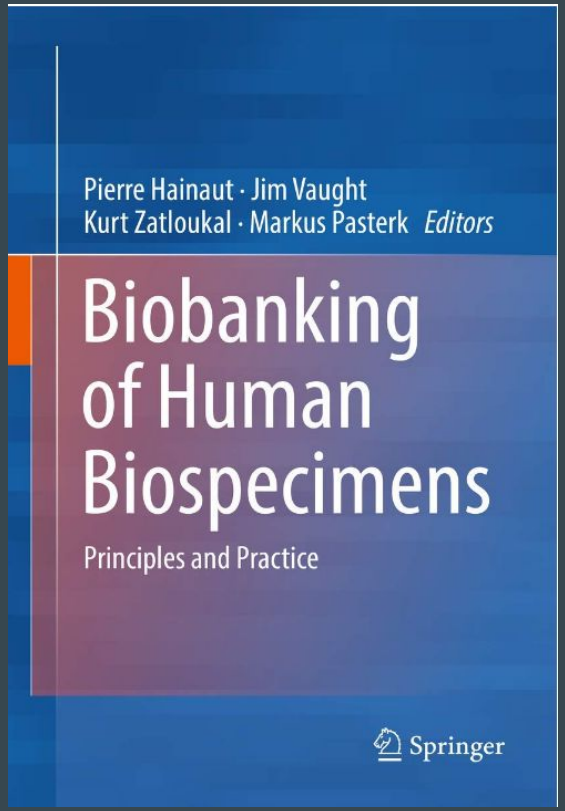
A prolific writer (2005 - 2018)

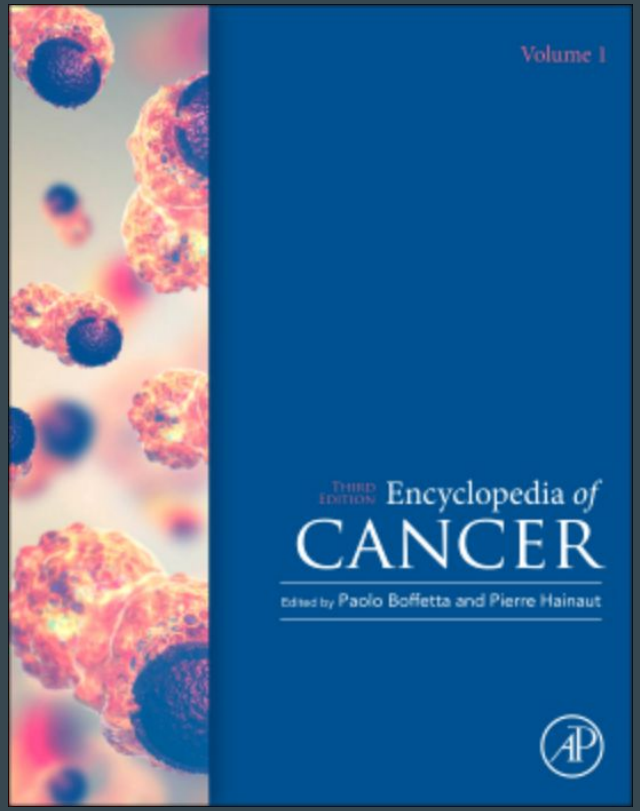






A prolific writer (2005 - 2018)



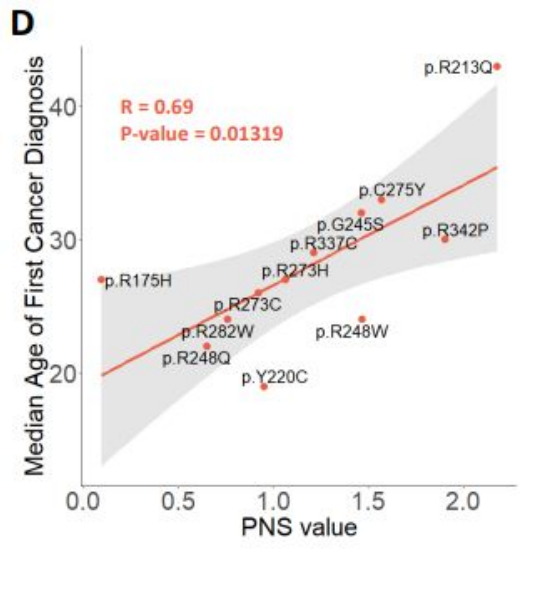


p53: Ariadne's thread in the maze of cancer biology

After 25 years of research, p53 has had a tremendous impact on our understanding of the molecular biology of cancer. As this knowledge develops to reveal more and more intricate pathways, the p53 protein will continue to be the « Ariadne thread » pointing out new routes in the maze of cancer biology. But the greatest hope for the 25 years to come is that concerted efforts to remove roadblocks for clinical applications will result in the effcicient transfer of p53 know-how from he lab to the bedside. We hope that this book will, it its own way, contribute to this objective by opening up the « p53 box » to the scientific and medical community.

Continued contributions

Validation of neoantigenic properties in LFS patients.



Neoantigenic properties of *TP53* variants modify cancer risk in individuals with Li-Fraumeni syndrome

Emilie Montellier, Olivier Manches, Jonathan Gaucher, Sandrine Blanchet, David Hoyos, Murielle Verboom, Christina M. Dutzmann, Sophie Coutant, Jacqueline Bou, Bertrand Fin, Robert Olaso, Jean-François Deleuze, Thierry Frebourg, Benjamin D. Greenbaum, Arnold J. Levine, Christian P. Kratz, Gaëlle Bougeard, Pierre Hainaut

doi: https://doi.org/10.1101/2025.06.24.25328545

Posted June 24, 2025.

STRUCTURED ABSTRACT

Importance Li-Fraumeni Syndrome (LFS) is an heterogenous cancer predisposition caused by pathogenic *TP53* variants, characterized by a lifelong high risk of a broad spectrum of cancers. At least certain pathogenic *TP53* variants have been shown be immunogenic in a somatic context. Whether neoantigenicity contributes to the heterogeneity of LFS is unknown.

Objective To analyze the correlations between predicted neoantigenic properties of pathogenic *TP53* missense variants and LFS patterns.

Design Association study between predicted MHC-I presentation scores for *TP53* hotspot variants, LFS presentation and individual HLA-I genotyping in carriers of *TP53* germline pathogenic variants using data from mutation databases and clinical registries.

The tree #. 356586

Explanations

Choerospondias axillaris

Planted 2 days ago by P.-F. TITEUX-LECOQ srl

Features

The tree #. 356586 : Choerospondias axillaris
Common names: **Lapsi, Hog Plum**

Family:
Type of foliage:
Life expectancy: 45 years
Total weight of CO2 captured: 220 Kg
Height: 20 m

Personal stories

“The work you do,

no one else can do,

but we need the most.”

Dr. Pierre Hainaut

1958-2025

Scientist, Mentor, and Friend to the LFS Community

“ The wind blows where it wishes; you hear its voice,
but you do not know where it comes from or where it goes.
So it is with everyone who is born of the breath of the Spirit .”