John D. Minna, M.D.

The p53 Tumor Suppressor Gene and Human Cancer
(p53 as a "Molecular Policeman")

Grand Rounds, Department of Medicine

University of Texas Southwestern Medical Center
Dallas, TX 75235-8590

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Table 1

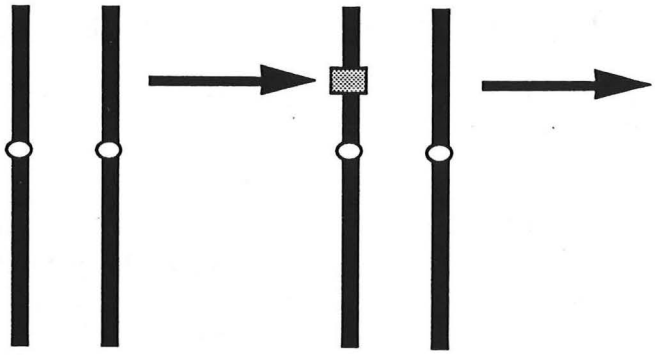
Cancer in the United States

	New Cases	Deaths
All Cases	1,170,000	526000
Lung Cancer	170,000	149,000
Colon and Rectal Cancer	152,000	57,000
Breast Cancer	183,000	46,300
Prostate Cancer	165,000	35,000
Totals	670,000	280,300

Source: ACS Statistics, 1993.

Dominant Oncogene

First Mutation

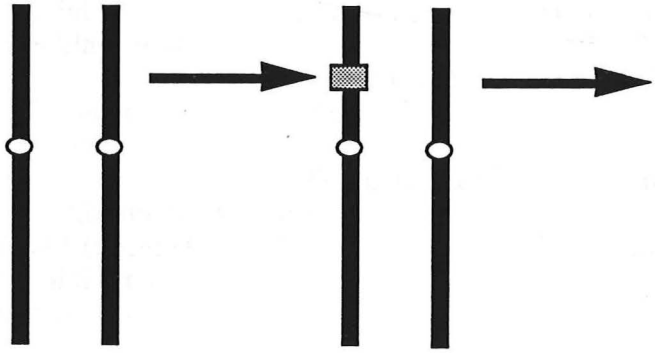


Mutant (*ras*) or Increased Amount (c-*myc*) of Dominant Oncogene Protein Produced

Small Mutation

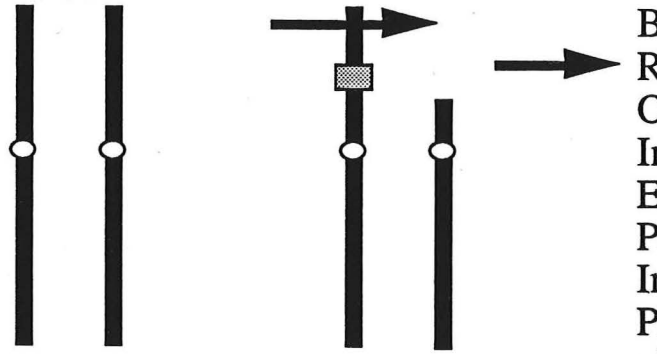
Recessive Oncogene

First Mutation



Point or Small Mutation Inactivates One Copy of Recessive Oncogene

Second Mutation



Both Copies of Recessive Oncogene Inactivated and Either No Protein or Inactive Protein Produced (p53, *rb*)

Large Lesion Cytogenetically Visable

Table 2

Tumor Suppressor Genes Involved in Human Cancers

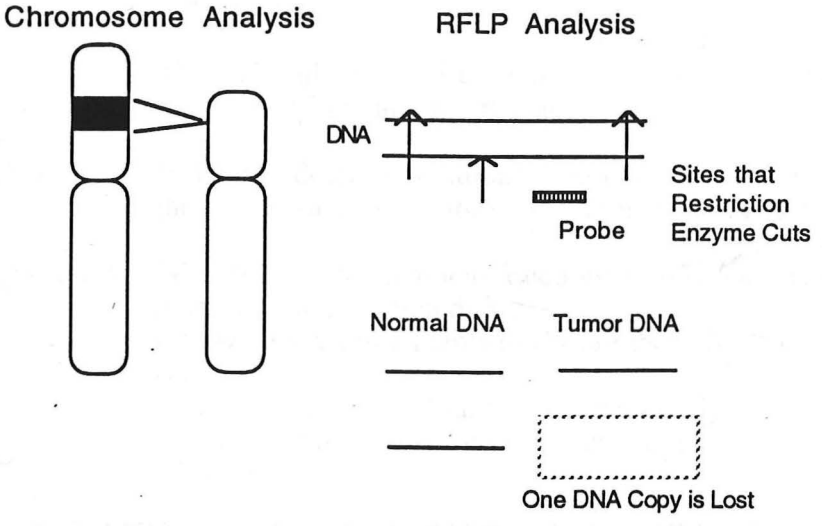
Chromosome Location	Gene	Function	Tumor Types Commonly Mutated
3p25	VHL (Von Hippel Lindau syndrome)	?	Renal
3p21.3	SCLC *(uncloned) (Familial renal cancer)	?	Lung, renal
5q21	APC Familial polyposis	?	Colon, pancreas, stomach
9p21	IGC* (uncloned) (Familial melanoma)	?	Acute leukemia, brain, lung, melanoma
11p13	WT1 (Wilms' tumor)	Transcription factor	Wilms' tumor
13q14	RB1 (Familial retinoblastoma)	Nuclear pocket protein	Retinoblastoma, osteosarcoma, breast small cell lung cancer
17p13	p53 (Li-Fraumeni syndrome)	Transcription factor	Common cancers
17q11	NF1 (Neurofibromatosis)	GTPase activating protein	Schannomas
17q21	BRCA1 (uncloned) (Familial breast & ovarian cancer)	?	Breast and Ovarian

* SCLC, small cell lung cancer or lung cancer or renal cancer gene; IGF, interferon gene cluster

Tumor Suppressor Genes are First Detected by Finding Allele Loss (Loss of Heterozygosity)

Methods to search for allele loss
- Cytogenetic analysis
- Restriction fragment length polymorphism (RFLP) analysis

Recessive Oncogenes Exhibit Deletions



Important Background Information and Questions in Approaching the Role of p53 in Human Cancer

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Fact: Cancer represents many different diseases in humans.

Question: Is there one pathway of growth control that is disrupted in many different kinds of cancer? (This pathway would provide a common denominator for understanding, preventing, and treating cancer).

Fact: The p53 pathway is directly or indirectly involved in the majority of human malignancies.

Fact: If the p53 defect in a human tumor is corrected you can "cure" the cancer in tissue culture or in mouse xenografts.

Questions: What are the biochemical functions of p53 and the effect of mutation on these functions?
Can we design treatments to restore these functions in tumor cells?
Can we detect p53 mutations at a very early stage in carcinogenesis to institute very early treatment or prevention efforts?

Methods of detecting p53 mutations

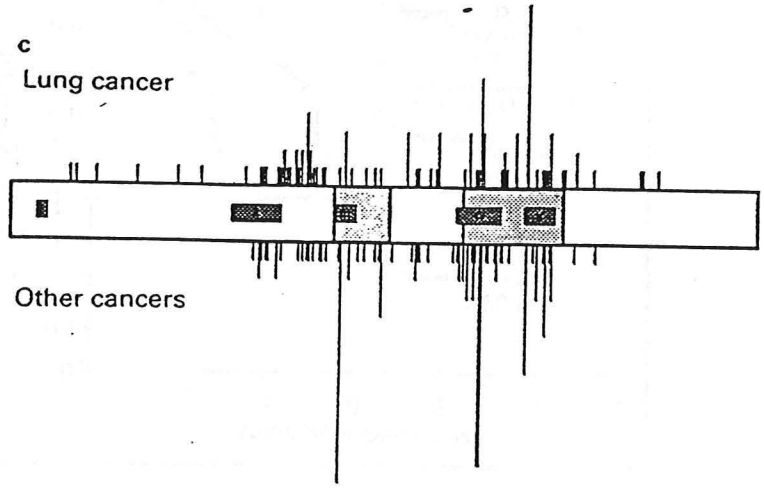
cDNA/PCR
SSCP (single strand conformation polymorhism analysis)
DNA sequencing
Immunostaining

Table 3

Frequency of p53 Mutations in Common Human Epithelial Tumors

Tumor Type		% with p53 mutation
Lung		
	SCLC	90
	Non-SCLC	50
Breast		46
Colon		50
Ovarian		80
Esophagus		39
Brain		
	Grade 3	38
	Grade 4	65
Gastric		
	Primary	20
	Metastases	100

(Baker et al., 1989; Baker et al., 1990; Bartek et al., 1990; Callahan et al., 1992; Chiba et al., 1990; D'Amico et al., 1992; Hollstein et al., 1991; Iggo et al., 1990; Mitsudomi et al., 1992; Takahashi et al., 1989); Hollstein, 1990 #315; Osborne, 1991 #316; Jaros, 1990 #317



The Presence of p53 Mutations Detected by Increased Levels of p53 Protein is a Negative Prognostic Factor in Several Tumors Including Breast, Lung, and Prostate Cancer

(Allred et al., 1993; Quinlan et al., 1992; Thor et al., 1992; Visakorpi et al., 1992)

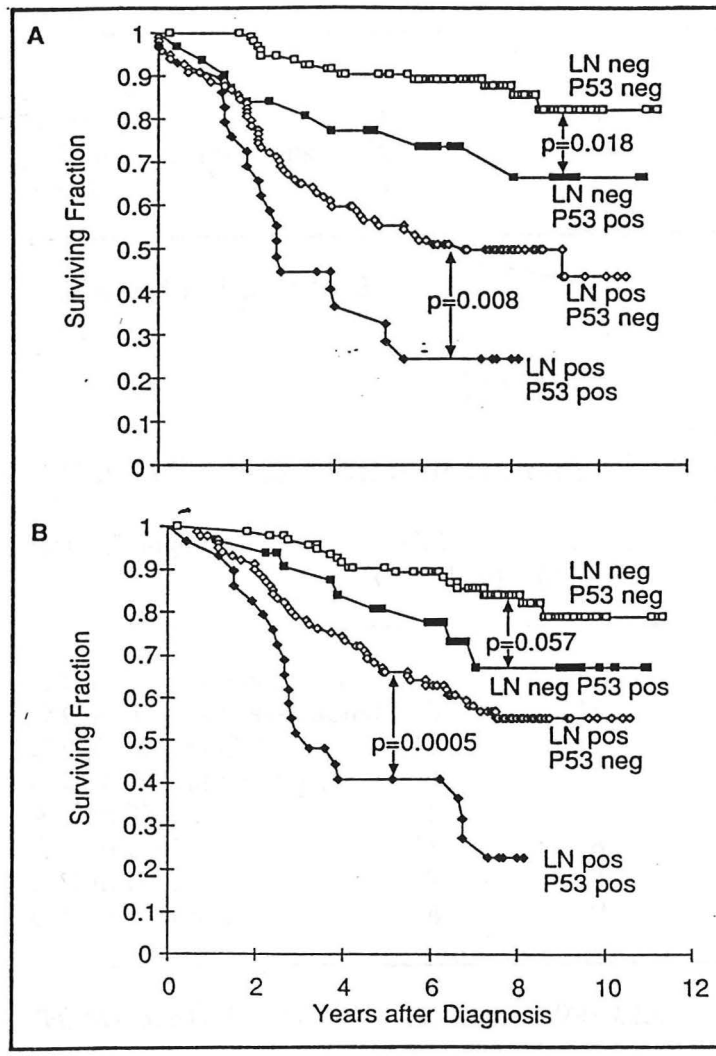


Fig. 3. Kaplan-Meier survival curves by lymph node and p53 status. The number of patients represented by each curve are as follows. A) Metastasis-free survival: lymph node (LN) neg, p53 neg (n = 96); LN neg, p53 pos (n = 31); LN pos, p53 neg (n = 98); LN pos, p53 pos (n = 28). B) Overall survival: LN neg, p53 neg (n = 96); LN neg, p53 pos (n = 32); LN pos, p53 neg (n = 102); LN pos, p53 pos (n = 29). Results of pairwise comparison of survival curves based on the Cox-Mantel test (42) are shown as *P* values. Symbols on the same horizontal line indicate patients alive at the end of the study or alive when lost to follow-up. Symbols on dropping portions of lines represent failures (i.e., metastasis or death). Each symbol represents one patient.

Table 4

Comparison of p53 and APC Somatic Mutations in Human Cancer

Mutation Type	p53 mutations (%) (N= 1,312)	APC mutations (%) (N = 37)
Missense	83	9
Nonsense	6	37
Deletions & insertions	10	54
Silent	1	<1

(Harris and Hollstein 1993)

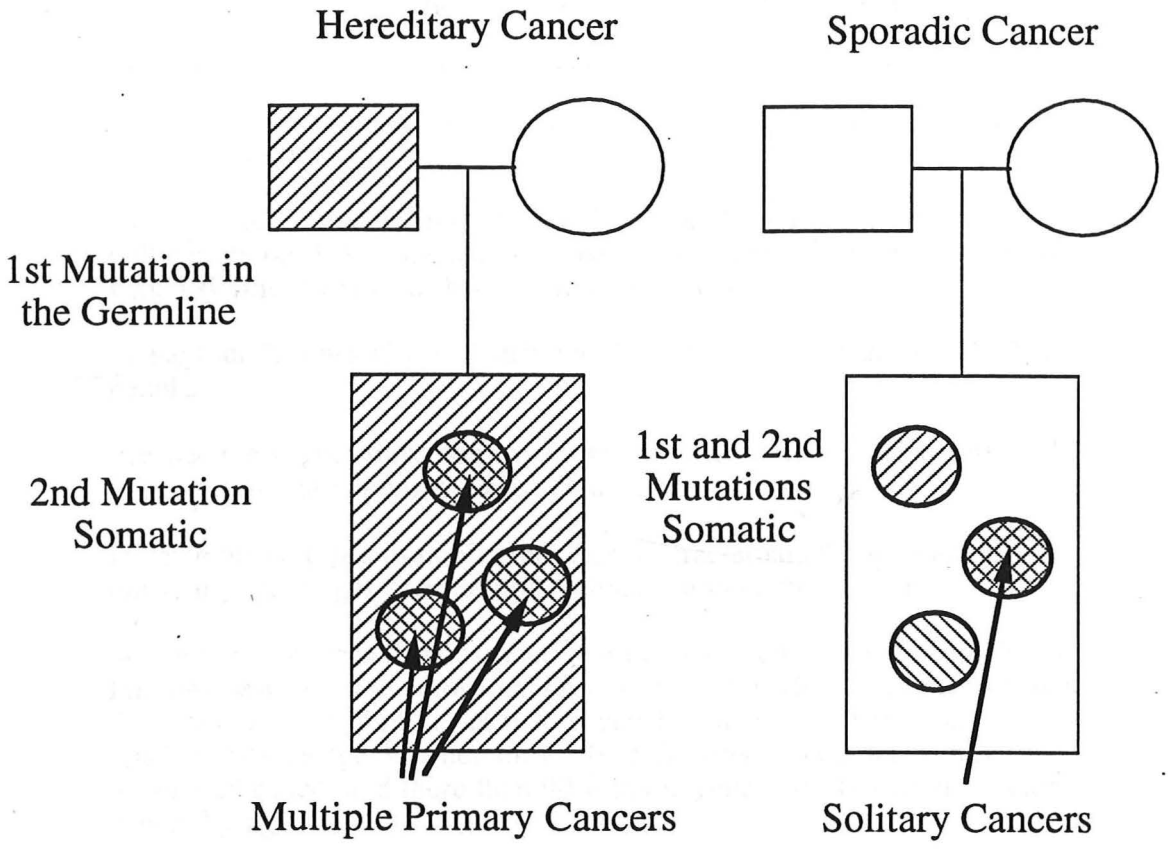
Table 5

Base Changes Found in Human p53 Somatic Mutations

Base Change	Colon (N = 190)	Lung (N = 263)	Breast (N = 128)
G:C to C:G (transitions)	8	11	11
G:C to T:A (transversions)	8	38	18
G:C to A:T at CpG	41	7	20
G:C to A:T at non CpG	24	16	17
A:T to T:A	5	5	6
A:T to G:C	11	8	11
A:T to C:G	2	4	8
del + insertions	4	10	9

(Harris and Hollstein 1993); Hollstein, 1991 #235

Knudson Two Mutation Model of Cancer



Recessive Oncogenes ("Tumor Suppressor Genes ") are Inherited as a Dominant Predisposition to Cancer in a Family but Function as a Recessive Trait on the Cellular Level

The Li-Fraumeni Syndrome of Inherited Predispostion to Multiple Cancer Types is Frequently Caused by a Germline Mutation in the p53 Gene

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An autosomal dominant disorder that predisposes individuals to multiple forms of cancer

1969 Li and Fraumeni reviewed medical reocrds and death certificates of 648 childhood rhabdomyosarcoma patients and identified four families in which siblings or cousins had a childhood sarcoma.

These four families also had striking histories of breast cancer and other neoplasms

Prospective studies have confirmed the high risk in family members of the tumor types that comprise the Li Fraumeni Syndrome

Diverse tumor types in family members characteristically develop at unusually early ages and multiple primary tumors are frequent

Segregation analysis demonstrated that the observed cancer distribution in families best fit a rare autosomal dominant gene model which predicts that the probability for families at risk of developing any invasive cancer reaches 50% by age 30 when only 1% of the general population has developed cancer and more than 90% of the gene carriers would develop cancer by age 70.

Component Tumor types: Breast cancer, soft tissue sarcomas, brain tumors, osteosarcomas, leukemia, adrenocortical carcinoma

Possible Component Tumors: lung cancer, pancreas, prostate, melanoma, gonadal germ cell tumors

Transgenic mice carrying a human mutant p53 gene have an incrased incidence of osteosarcomas, soft tissue sarcomas, lung adenocarcinoma, adrenal and lymphoid tumors

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(Malkin et al., 1990; Srivastava et al., 1990); Li, 1969 #312; Garber, 1991 #313; Lavigueur, 1989 #82

Soft-Tissue Sarcomas, Breast Cancer, and Other Neoplasms

A Familial Syndrome?

FREDERICK P. LI, M.D., and JOSEPH F. FRAUMENI, JR., M.D., F.A.C.P.
Bethesda, Maryland

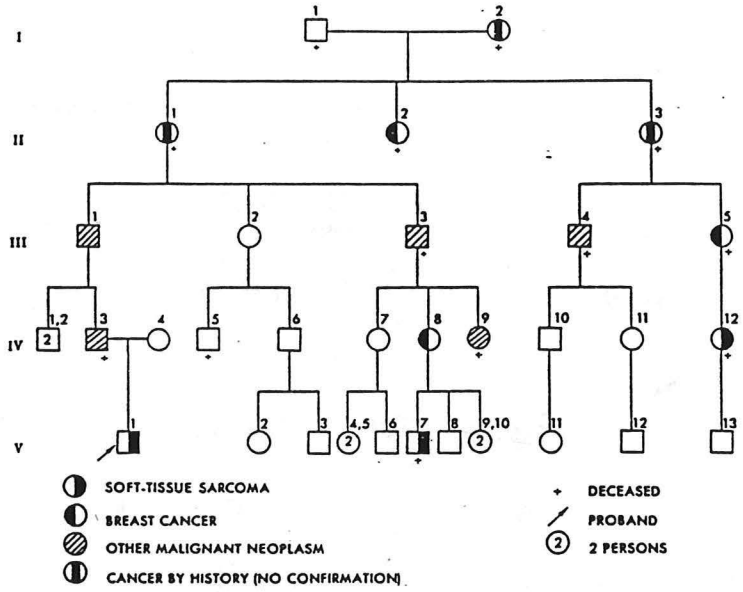


FIGURE 1. Pedigree of Family A.

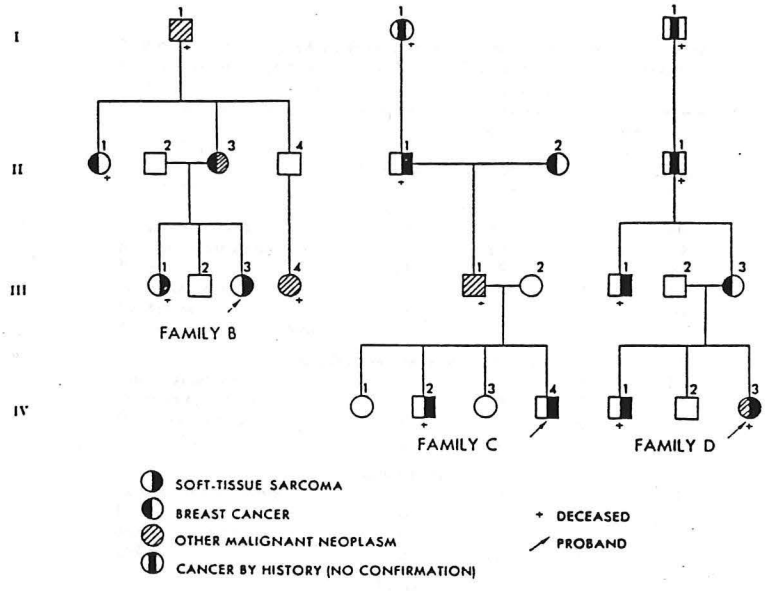


FIGURE 2. Pedigrees of Families B, C, and D.

Germ Line p53 Mutations in a Familial Syndrome of Breast Cancer, Sarcomas, and Other Neoplasms

David Malkin, Frederick P. Li, Louise C. Strong, Joseph F. Fraumeni, Jr., Camille E. Nelson, David H. Kim, Jayne Kassel, Magdalena A. Gryka, Farideh Z. Bischoff, Michael A. Tainsky, Stephen H. Friend*

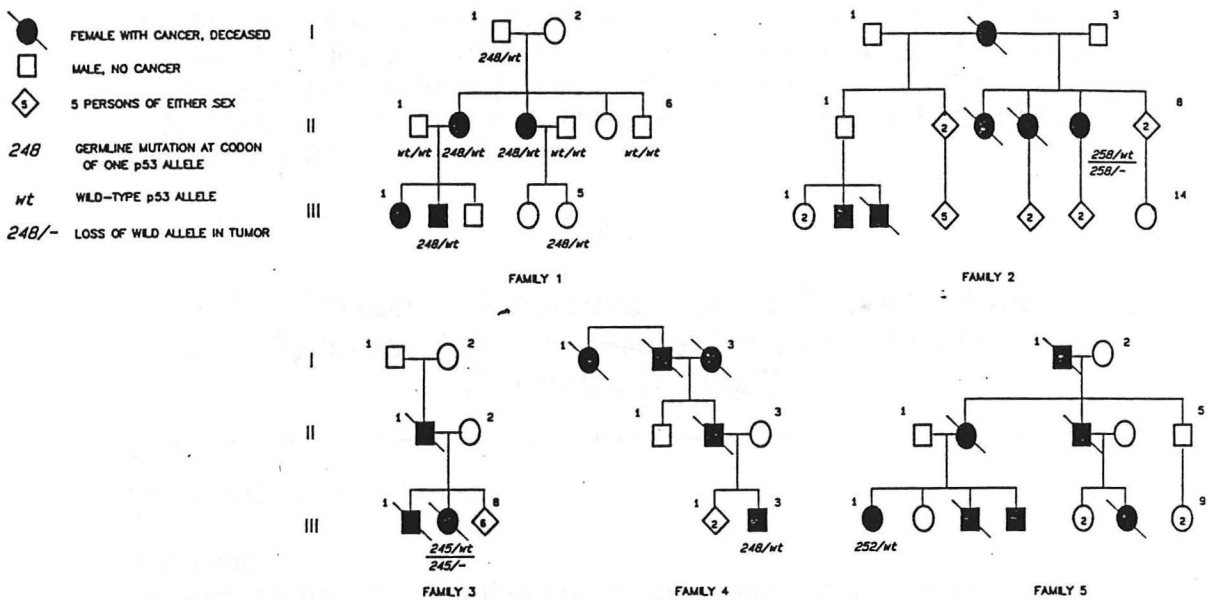


Table 1. Cancers in 43 families with Li-Fraumeni Syndrome (LFS), by tumor type and age at diagnosis. All families were ascertained through a proband with sarcoma, who is excluded from the tabulations.

Tumor type	Age at diagnosis (years)*			
	0–14	15–44	>44	All ages
Component tumors of LFS				
Breast carcinoma	0	49	11	60
Soft tissue sarcoma	13	12	4	29
Brain tumors	12	15	1	28
Osteosarcoma	6	6	2	14
Leukemia	8	4	2	14
Adrenocortical carcinoma	5	0	0	5
Possible component tumors of LFS				
Lung carcinoma	0	7	12	19
Prostate carcinoma	0	0	8	8
Pancreas carcinoma	0	1	6	7
Melanoma	0	1	2	3
Other tumors				
Colorectal carcinoma	1	3	4	8
Lymphoma	0	5	1	6
Stomach carcinoma	0	3	1	4
Other	5	13	8	26
All cancers				
	50	119	62	231

*Only first cancer was counted in patients with multiple tumors.

Table 6
Many Codons are Involved in Germline p53 Mutations:

120, 133, 181, 242, 242, 245 (4), 248 (6), 252, 258, 272, 273, 282 (2), 286, 307, 325, insertions (2), deletion

Malkin (1990) Science 250:1233, 1990; Srivastava Nature 348:747, 1990; Metzger PNAS 88:7824, 1991; Birch Med Ped Onc 19:341, 1991; Law Can Res 51:6385, 1991; Felix JCI 89:640, 1992; Borenson Can Res 52:3234, 1992; Malkin NEJM 326:1309, 1992; Toguchida NEJM 326:1301, 1992; Sameshima JNCI 89:703, 1992.

Table 7

Ethical Issues in Predictive Testing for Germ Line Mutations Among Cancer-Prone Individuals ("Genetic Testing")

Ethical Principles of Respect:

Autonomy
Freedom from coercion, full understanding of the implications of an action, respect for an individual's right to decide about something which may have a profound effect on his or her life

Beneficence
"first do no harm"

Confidentiality
Avoid inadvertent disclosure of information to third parties

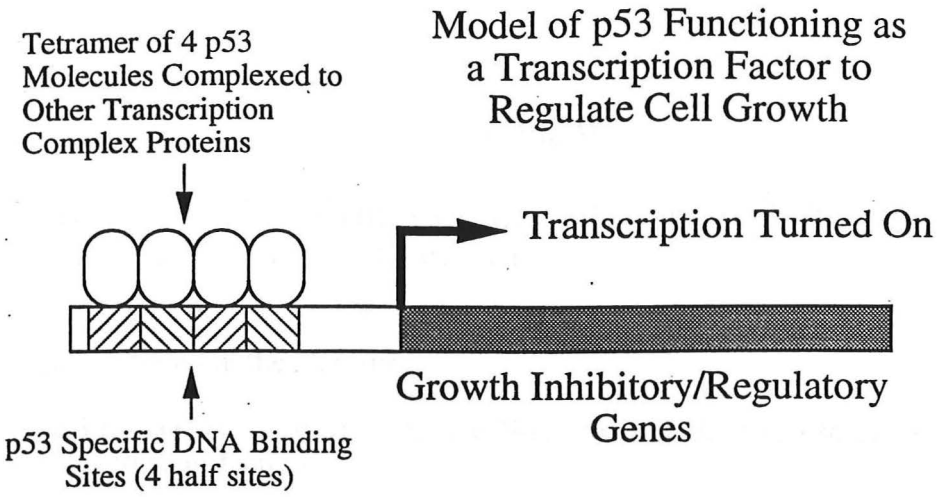
Justice
Implies fairness, including access to health care and freedom from discrimination based on predictive testing results

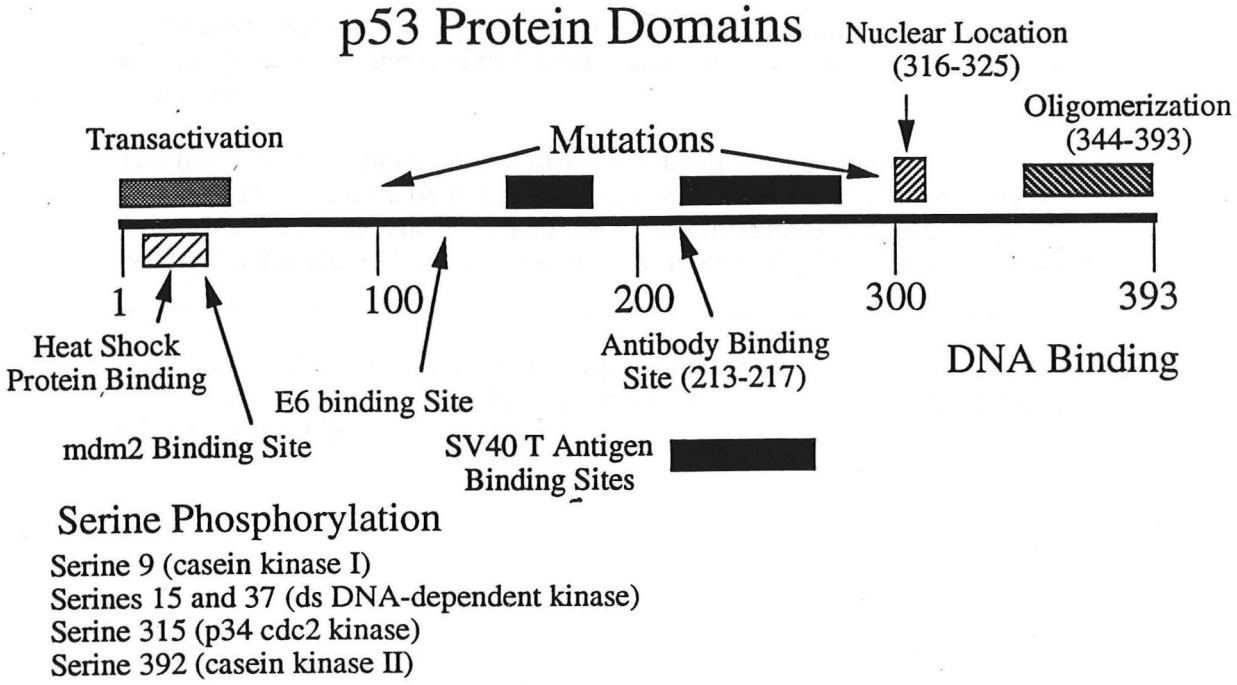
Table 8

Biologic Functions of Wild-type p53 Protein

Wild-type p53 suppresses tumor growth and tumorgenicity of tumors bearing p53 mutation despite multiple other mutations. This probably occurs through inducing apoptosis
Wild-type p53 suppresses transformation mediated by other oncogenes
Increases in wild-type p53 causes cells to arrest in G1/S
Mutant p53 can immortalize normal cells
Mutant p53 + a mutant *ras* gene can transform primary normal rodent cells to malignancy (even in the presence of a normal endogenous wild-type p53)

(Levine et al., 1991; Oren 1992; Vogelstein and Kinzler 1992)





Serine Phosphorylation

Serine 9 (casein kinase I)
Serines 15 and 37 (ds DNA-dependent kinase)
Serine 315 (p34 cdc2 kinase)
Serine 392 (casein kinase II)

Table 9

Biochemical Activities of the Wild-type p53 Protein: p53 is a Transcription Factor

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p53 is found in the Nucleus

p53 has an acidic domain near the N-terminus simlar to those of other transcription factors

Gal4:p53 fusion proteins can activate transcription from a Gal4 operon

Specific DNA sequences can bind to p53 in vitro

These sequences contain two copies each with a half sites suggesting p53 binds as a tetramer

Co-transfection of wild-type p53 and a reporter plasmid with p53 binding site(s) upstream of the reporter gene results in high level of reporter gene activation

The transcription appears to be directly related to p53 since:
The amount of transactivation correlates with strength of p53 binding to these specific DNA sequences and the transactivation is also seen in yeast (without a known p53 homologue and with purified p53 in in vitro systems

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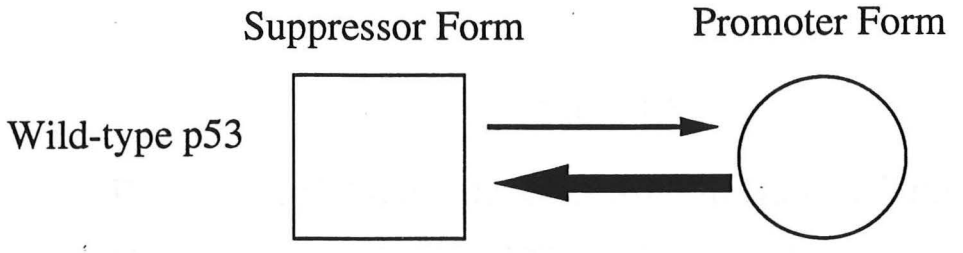
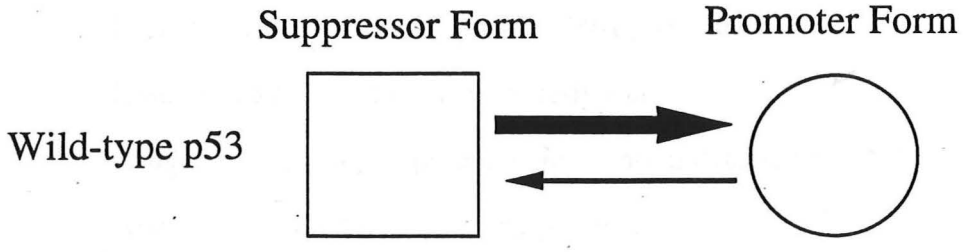
(El-Deiry et al., 1992; Fields and Jang 1990; Funk et al., 1992; Kern et al., 1991; Raycroft et al., 1990; Scharer and Iggo 1992; Unger et al., 1992); Farmer, 1992 #298

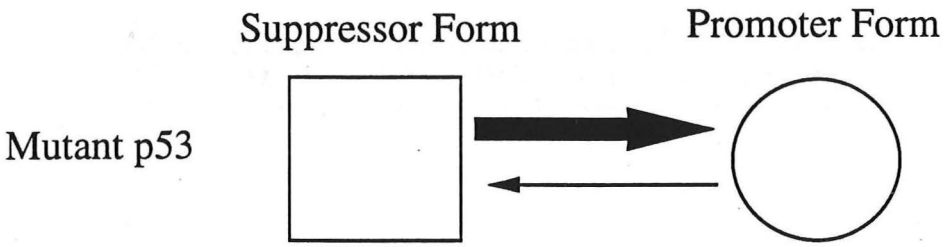
Changes in p53 Protein Conformation Between Suppressor and Promoter Form



Resting Cells



Growth Stimulation



All Growth Conditions

Milner Curr Opin Cell
Biol 3:282, 1991

Summary of Multiple Studies:

Subtle mutations in p53 can affect the conformation of the entire protein altering the structure of domains far removed from the sites of mutation

Table 10

Effect of p53 Mutations Occurring in Human Tumors

Single amino acid substitutions resulting in missense mutations cause p53 to:

Lose its ability to bind to specific DNA p53-binding sites

Lose the ability to stimulate transcription

Cause a change in p53 protein conformation detected by antibodies

Allow p53 to bind to heat shock proteins

Disrupt transcriptional function even when fused to a Gal4 DNA binding domain

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(El-Deiry et al., 1992; Fields and Jang 1990; Raycroft et al., 1991; Scharer and Iggo 1992; Unger et al., 1992; Vogelstein and Kinzler 1992); Chen, 1993 #300; Sturzbecher, 1987 #154; Stephen, 1992 #301

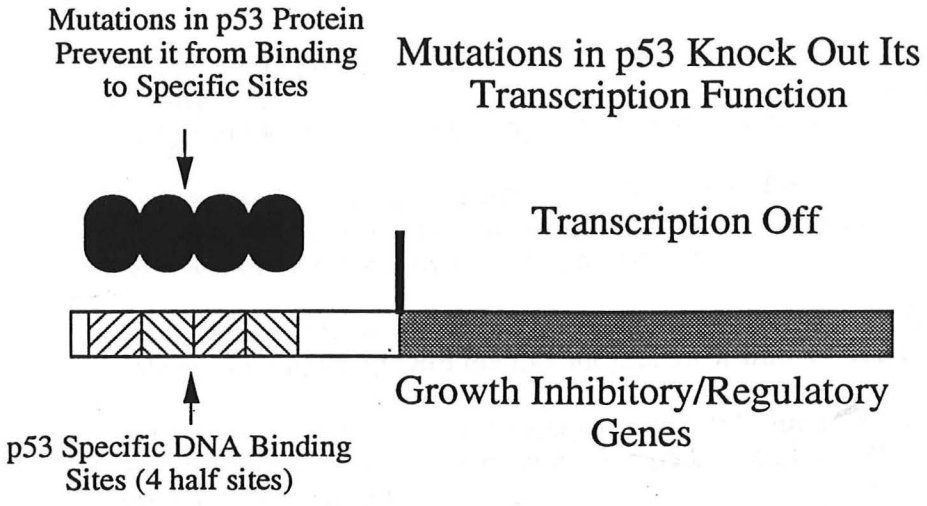
Mutations in p53 Protein
Prevent it from Binding
to Specific Sites
Mutations in p53 Knock Out Its
Transcription Function
Transcription Off
Growth Inhibitory/Regulatory
Genes
p53 Specific DNA Binding
Sites (4 half sites)

Table 11

Effect of Mutation on the Function of Mixtures of Wild-type and Mutant p53 Proteins

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Wild-type p53 protein has a half life of 30 minutes while mutant p53 proteins have half lives of 2-3 hours resulting in a great increase in the steady state level of mutant compared to wild-type protein.

The increased steady state levels allow the mutant form of the protein to be easily detected by immunohistochemistry while the wild-type steady state levels are so low that they do not immunostain

Changes in conformation of mutant p53 proteins can effect wild-type molecules complexed with the mutant protein within the tetramer

Mutant p53 protein expressed together with wild-type p53 protein in the same cell inhibits the ability of wild-type p53 to bind to DNA and to stimulate transcription

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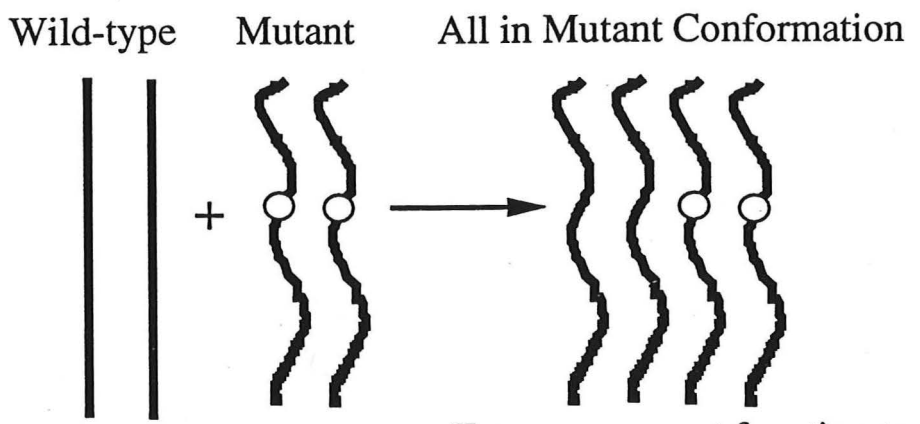
Mutant p53 Proteins Alter the Conformation of Wild-type Molelcules



Milner & Medcalf
Cell 65:765, 1991

Tetramer cannot function to bind to DNA or Stimulate Transcription

The formation of oligomers of wild-type and mutant p53 proteins provides and explanation why some p53 mutants can transform normal cells by inhibiting activity of endogenous wild-type p53 proteins in a "dominant negative" fashion

Since the vast majority of tumors go onto lose the wild-type p53 allele it is apparent that tumor cells don't tolerate even small amounts of wild-type p53

Are some p53 mutants worse than others?

Other Genetic Alterations Affecting the p53 Pathway

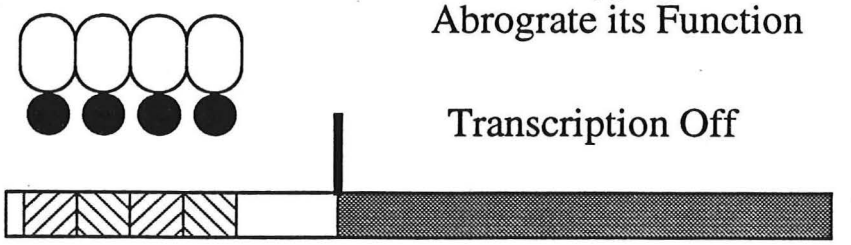
Table 12

Viral Oncoproteins and Cellular Proteins That Bind to p53 and Inhibit its Function

	Protein	Human Tumors
Viral Oncoproteins		
SV40	Large T Antigen	None known
Adenovirus	E1B	None known
Human Papilloma Virus (HPV) (>98%)		E6 * Cervical Cancer
Cellular Oncogene (36%)	mdm2†	Soft tissue sarcomas

* E6 also facilitates the degradation of p53 through a ubiquiton pathway.

† mdm2 is a cellular gene located on chromosome 12q that is amplified and over expressed in a mouse tumor and some human soft tissue sarcomas. When mdm2 is overexpression p53 is not mutated.
(Fakharzadeh et al., 1991; Momand et al., 1992; Oliner et al., 1992)



mdm2 or HPV E6 Proteins bind to wild-type p53 and prevent it from functioning. (E6 also facilitates p53 degradation),

Table 13

What is Regulated by p53?
p53 is Not Required for Normal Development or Cell Maintenance

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p53 germ line mutation in the Li-Fraumeni syndrome have no effect on development (although one wild-type p53 copy remains)

Tumor cell lines exist with homozygously deleted p53 producing no p53 mRNA or protein

Wild-type p53 is normally expressed at very low levels in most normal cells
p53 homozygous knockout mice develop normally but >70% will develop tumors by the age of 6 months

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(Takahashi et al., 1989); Donehower, 1992 #306; Malkin, 1990 #158

p53: a "Molecular Policeman" to Control the Growth of "Stressed" Cells

p53 Functions as a Transcription Factor to Provide G1 Checkpoint Control for DNA Damage and p53 Mutations Give Rise to Genetically Unstable Cells

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With x-ray or drug induced DNA damage normally there is a rapid increase in p53 protein which serves to arrest the damaged cell in G1 presumably until damage is repaired

If repair fails p53 can trigger cell suicide by apoptosis

Cells with mutant or absent p53 do not arrest in G1 and thus can accumulate other mutations

Cells (like tumor cells) with mutant or abnormal p53 cannot carry out arrest and are genetically less stable and will accumulate mutations and chromosomal rearrangements to give rapid evolution of malignant clones

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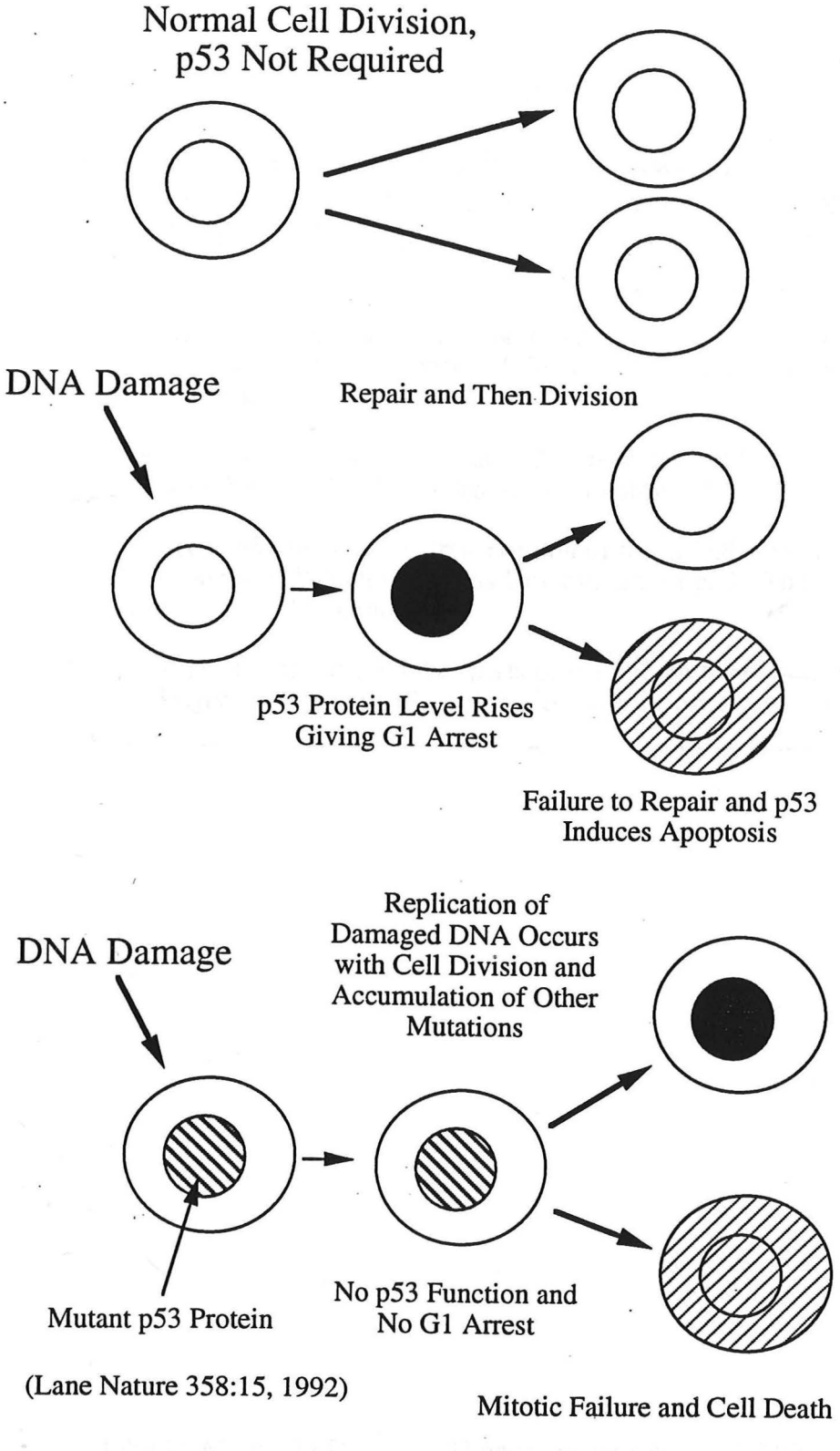
(Kastan et al., 1991; Kastan et al., 1992; Lane 1992; Maltzman and Czyzyk 1984; Yonish-Rouach et al., 1991); Hartwell, 1992 #311; Livingstone, 1992 #310; Yin, 1992 #309



(Lane Nature 358:15, 1992)

Table 14

The DNA Damage:Repair p53 Pathway Involves the Ataxia Telangectasia Gene Product as an Upstream Event while the GAD45 Gene Product is a Downstream Function

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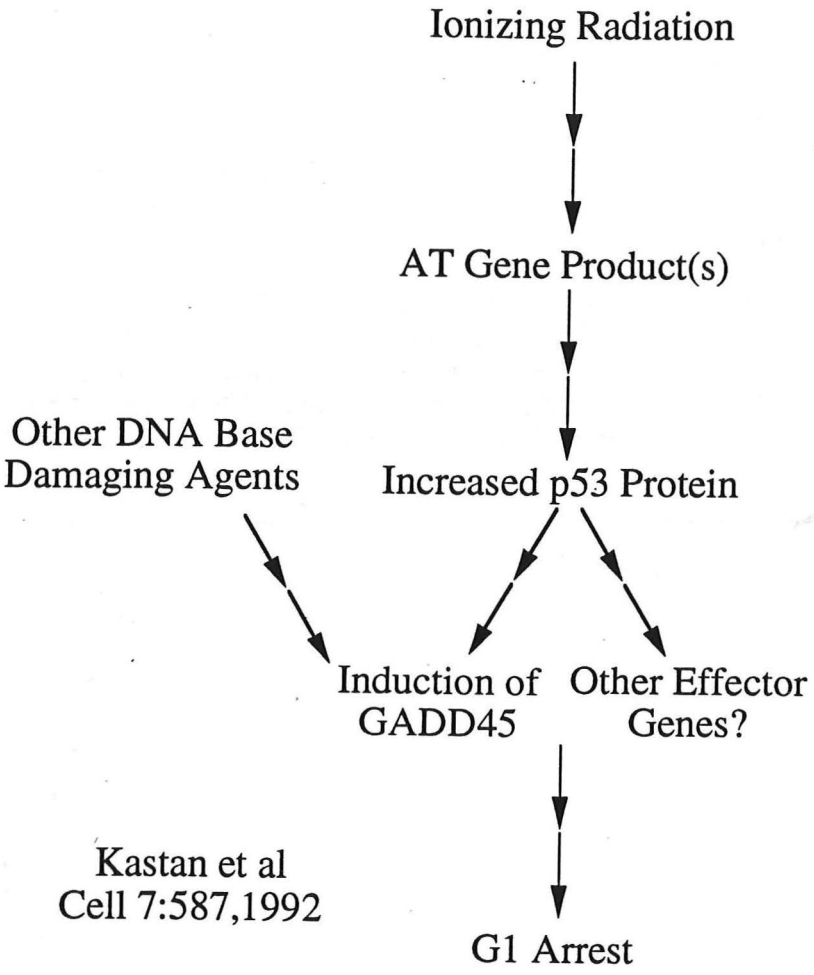
The DNA damage pathway has the Ataxia Telangectasia gene product as a proximal step and thus absence of the the AT product leads to a failure of p53 induction

The increase incidence of cancer in AT patients could thus be related to a defect in the "p53 DNA Damage Control Pathway"

Wild-type p53 induces the transcription of the GADD45 gene (G1 arrest DNA Damage) Which Functions Downstream of p53. The role GADD45 plays is currently unknown

Could genetic lesions in other parts of the p53 pathway proximal or distal to p53 give rise to sporadic or inherited predisposition to cancer?

The p53 G1 Arrest Pathway After DNA Damage



Kastan et al
Cell 7:587,1992

Table 15

Clinical Applications

Testing for Germ Line Mutations
Early Detection of p53 Somatic Mutations in Persons at Risk
Use of p53 as a Molecular Monitor for Chemoprevention and Intervention Trials
Detection of p53 Mutations in Tumors as a Prognostic Factor
Development of Drugs to Replace p53 Function
Exploit Presence of p53 Mutation in Tumors
Detection of Immune Responses Against p53 for Early Detection
Development of Cytotoxic T-Cell Therapy Directed Against Mutant Epitope

References

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