BHARATHIDASAN UNIVERSITY

Tiruchirappalli- 620024,
Tamil Nadu, India

Programme: M.Sc., Biomedical science

Course Title : Cancer Biology

Course Code : 18BMS59C16

Unit-III

TOPIC: Tumor Suppressor Genes

p53 & PTEN

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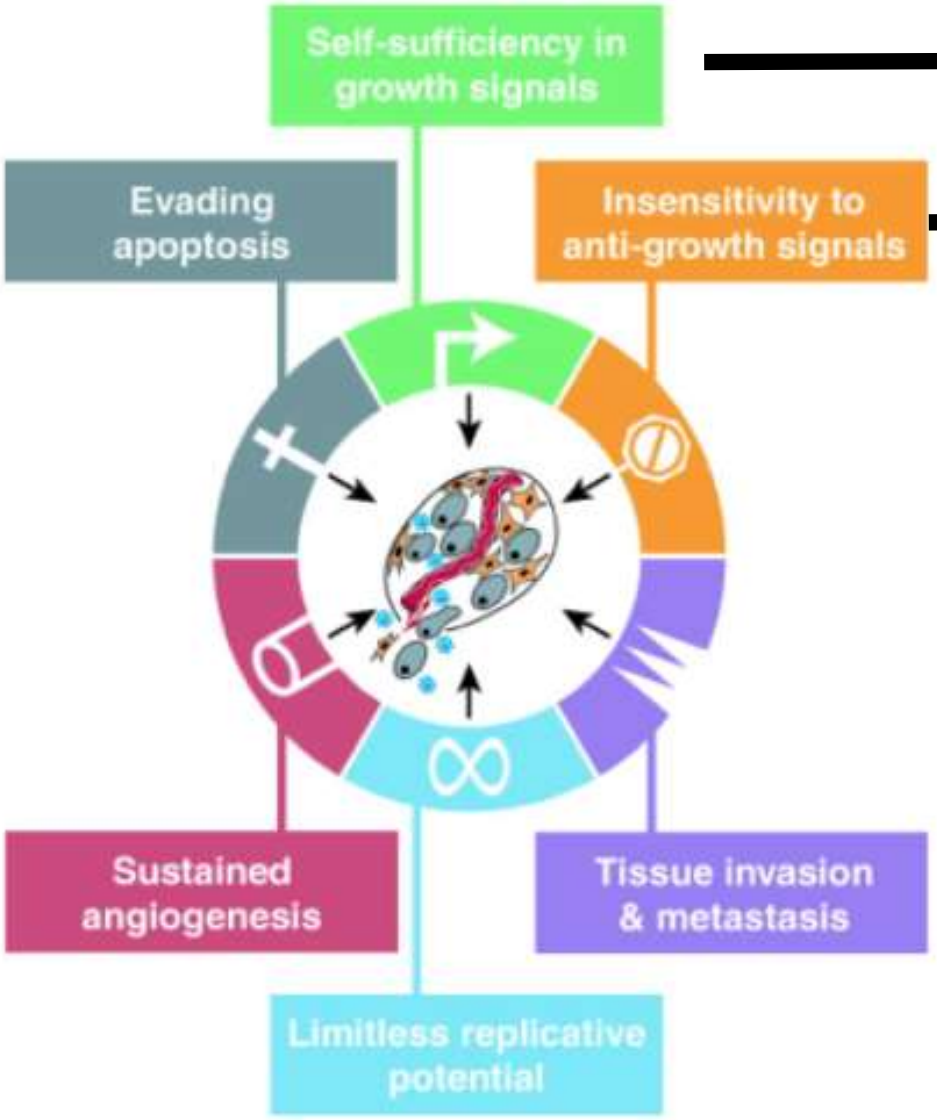
Characteristics of Cancer

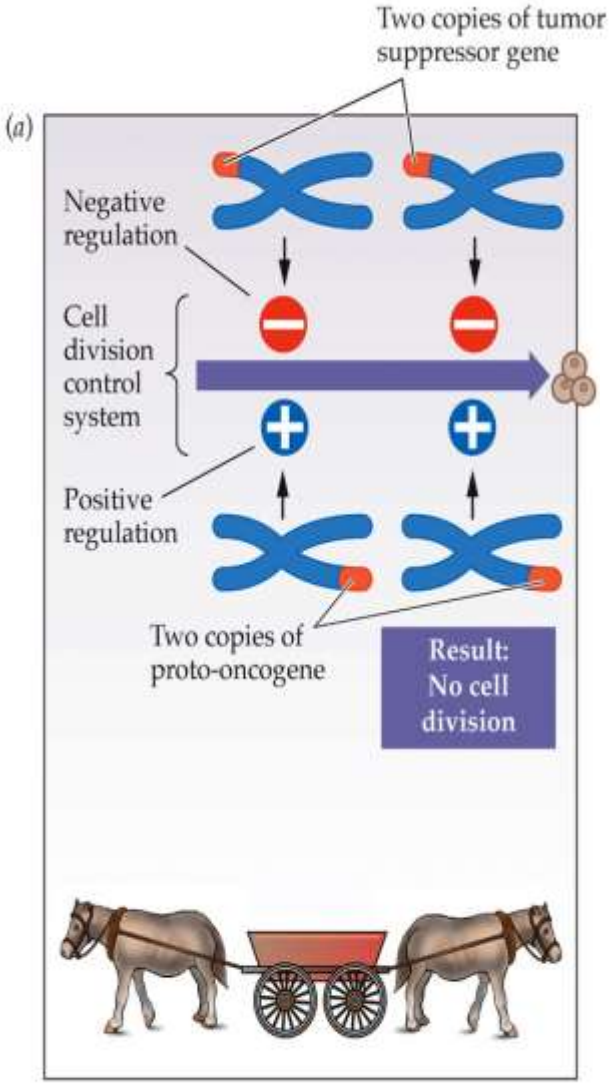


Activated proto-oncogene

Inactivated tumour suppressor gene

For cancer: two classes of cellular genes are targets for mutations

- ***PROTO-ONCOGENES***
- ***TUMOUR SUPRESSOR GENES***



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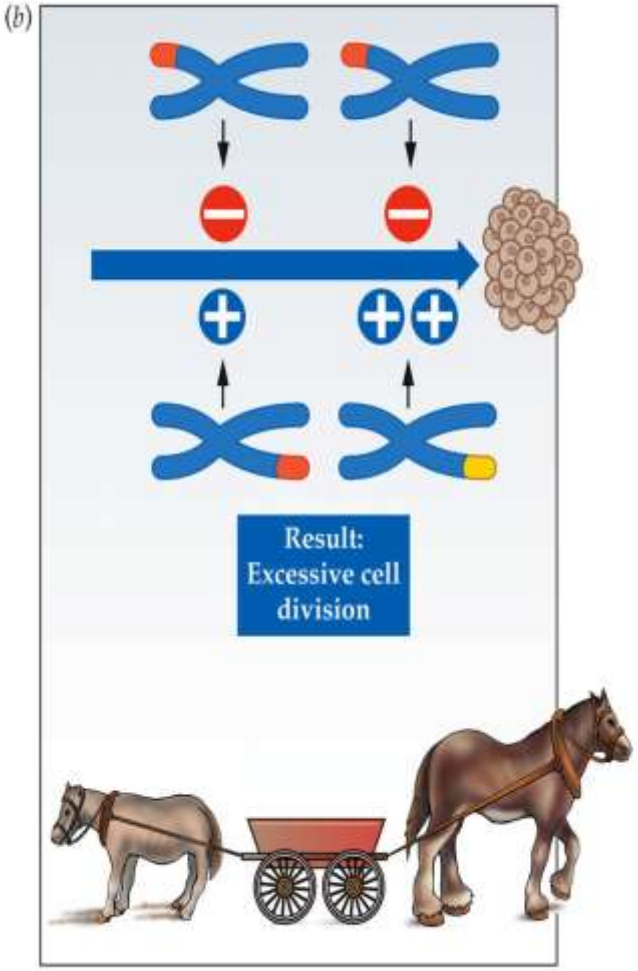

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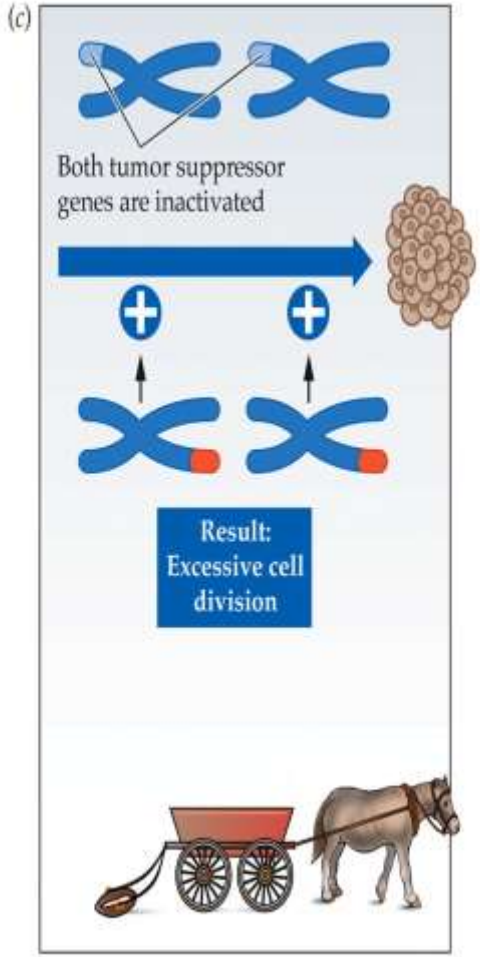

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Tumour suppressor genes

Act as a brake for cell division

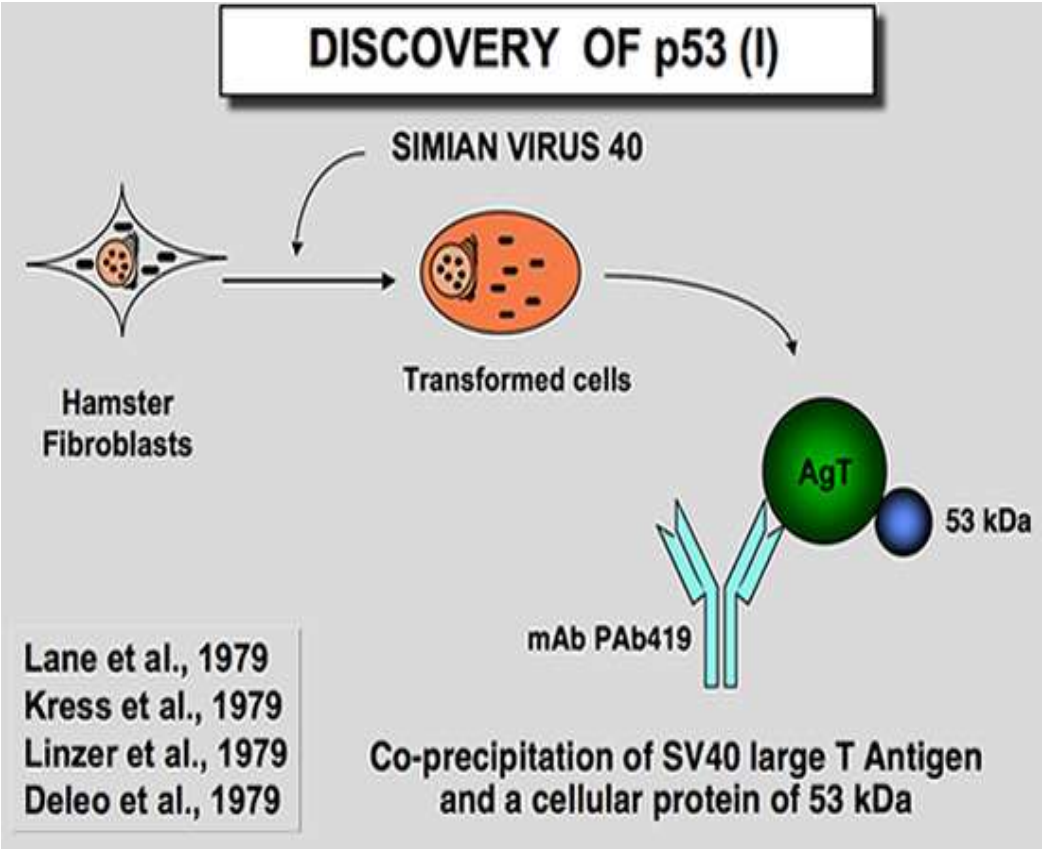
"GUARDIAN OF THE GENOME"

Mutation in tumour suppressor genes = brakes don't work, or there is an accumulations of mutations (DNA repair enzymes)

The mutated gene product is not functional = *NULL ALLELE*

Discovery of p53

The discovery in 1979 of the p53 protein was the culmination of two types of studies involving a virologic approach and a serologic approach.

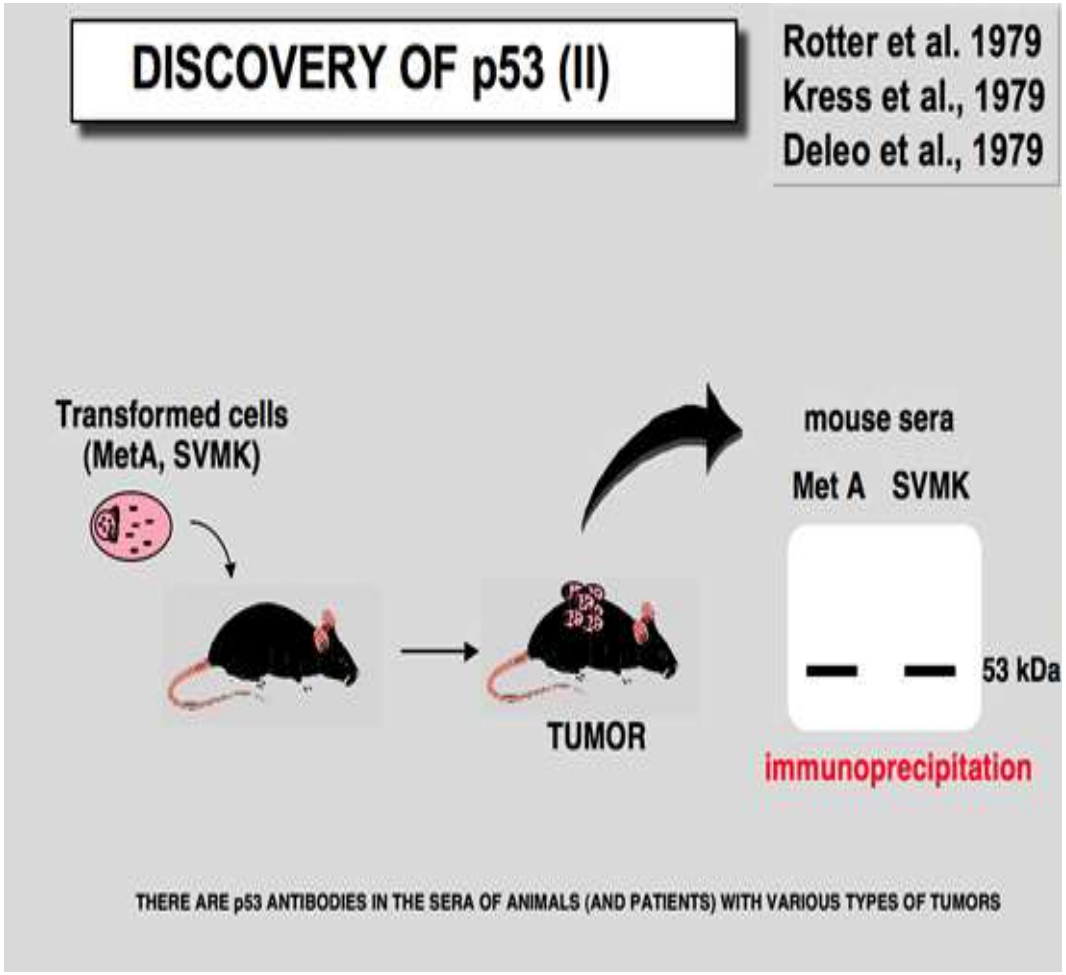


53-kDa protein was overexpressed in a wide variety of murine SV40 transformed cells, but also in uninfected embryonic carcinoma cells.

A partial peptide map from this 53-kDa protein was identical among the different cell lines, but was clearly different from the peptide map of SV40 large-T antigen (Kress et al. 1979; Linzer and Levine 1979).

It was then postulated that SV40 infection or transformation of mouse cells stimulates the synthesis or stability of a cellular 53-kDa protein.

Discovery of p53



- methylcholanthrene-induced tumor cell line such as MethA was directed toward the p53 protein.

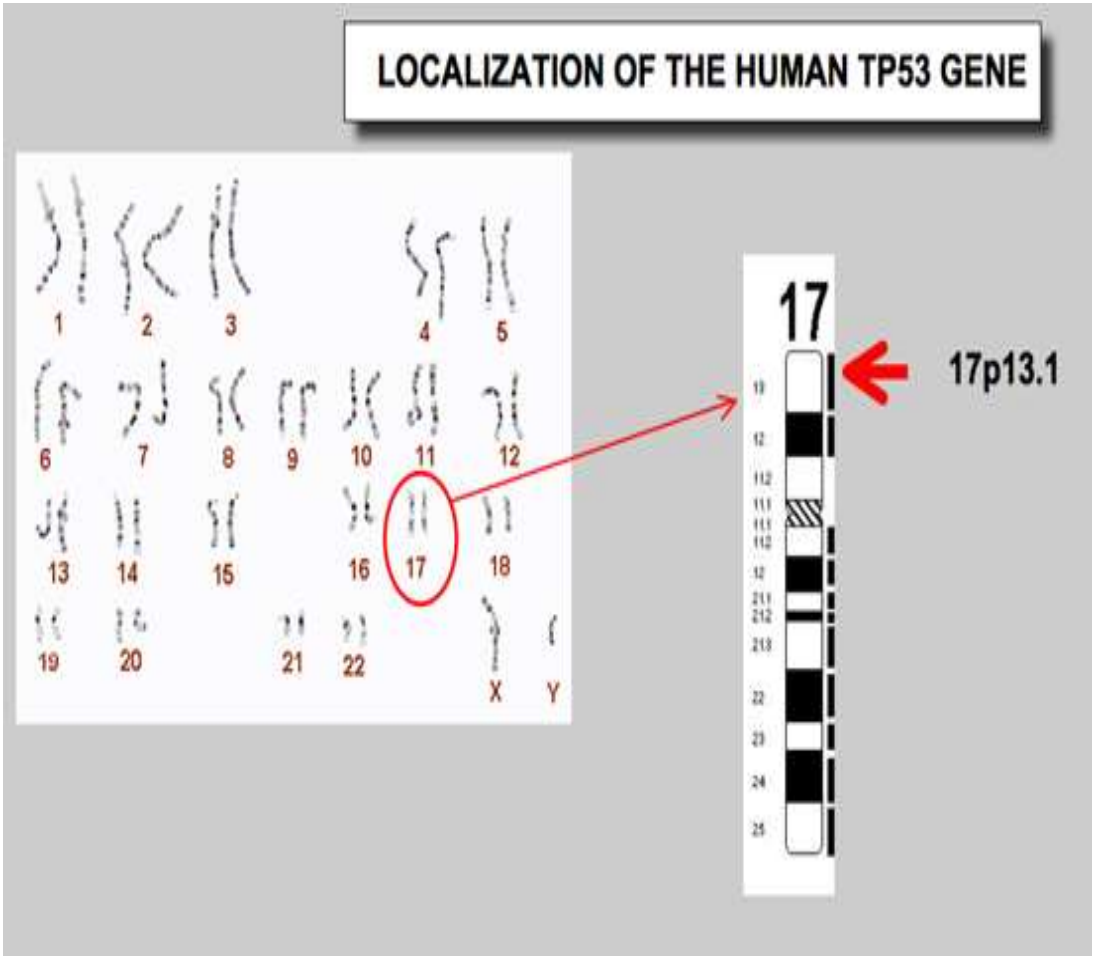
- Later, it was found that animals bearing several types of tumors elicited an immune response specific for p53 (Kress et al. 1979; Melero et al. 1979; Rotter et al. 1980).

- In 1982, Crawford et al. first described antibodies against human p53 protein in 9% of breast cancer patient sera.

No significant clinical correlation was reported, and at that time no information was available concerning mutations of the p53 gene.

p53 gene

The TP53 gene is localized on chromosome 17 (short arm, 17p13), a region that is frequently deleted in human cancer.



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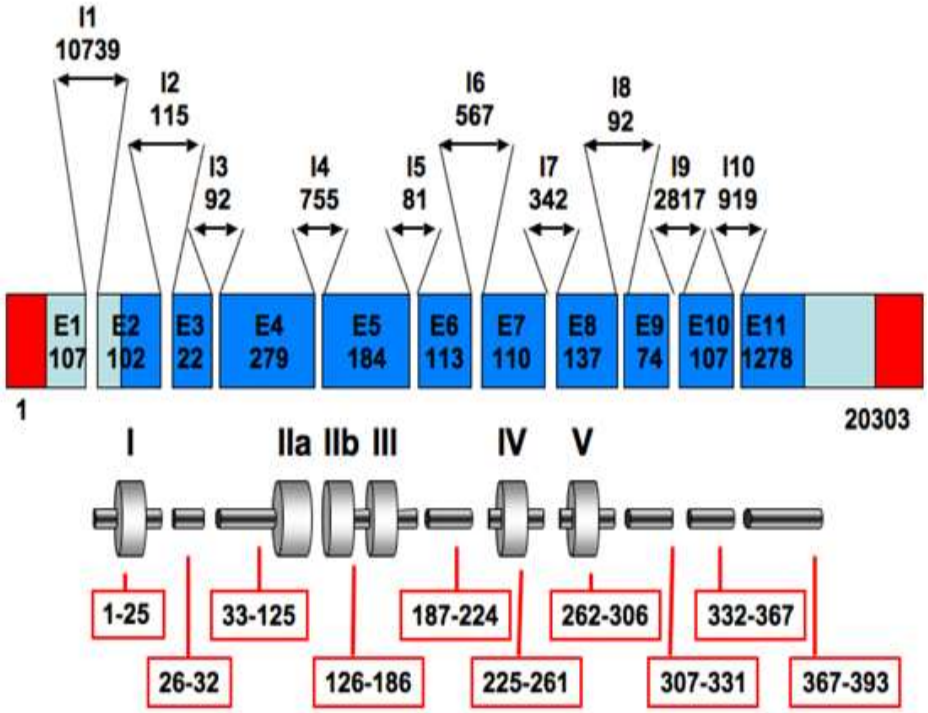
Organization of the human p53 gene



Human p53 is 393 amino acids long and has three domains:

- An N-terminal transcription-activation domain **(TAD)**, which activates transcription factors

- A central DNA-binding core domain **(DBD)**. Contains zinc molecules and arginine amino acid residues.

- C-terminal homo-oligomerisation domain **(OD)**. Tetramerization greatly increases the activity of p53 *in vivo*.

- **11 exons (blue) coding for a 2.2 Kb mRNA. Translation begin in exon 2. Sizes of exons and introns are shown in bp.**

Examples of tumour suppressor genes include:

- RB1 - retinoblastoma susceptibility gene
- WT1 - Wilm's tumour gene
- NF1 - neurofibromatosis type 1 gene
- NF2 - neurofibromatosis type 2 gene
- DCC - involved in colorectal cancer
- BRCA1, BRCA2 - involved in breast cancer

Genetic Mutations That Can Cause Cancer

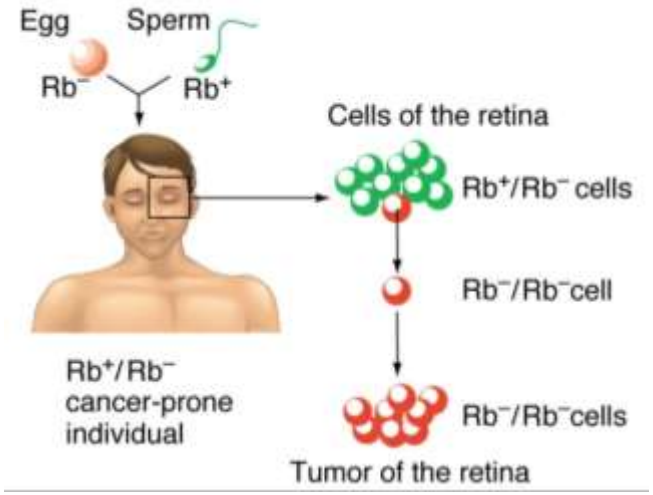
Tumor Suppressor Genes

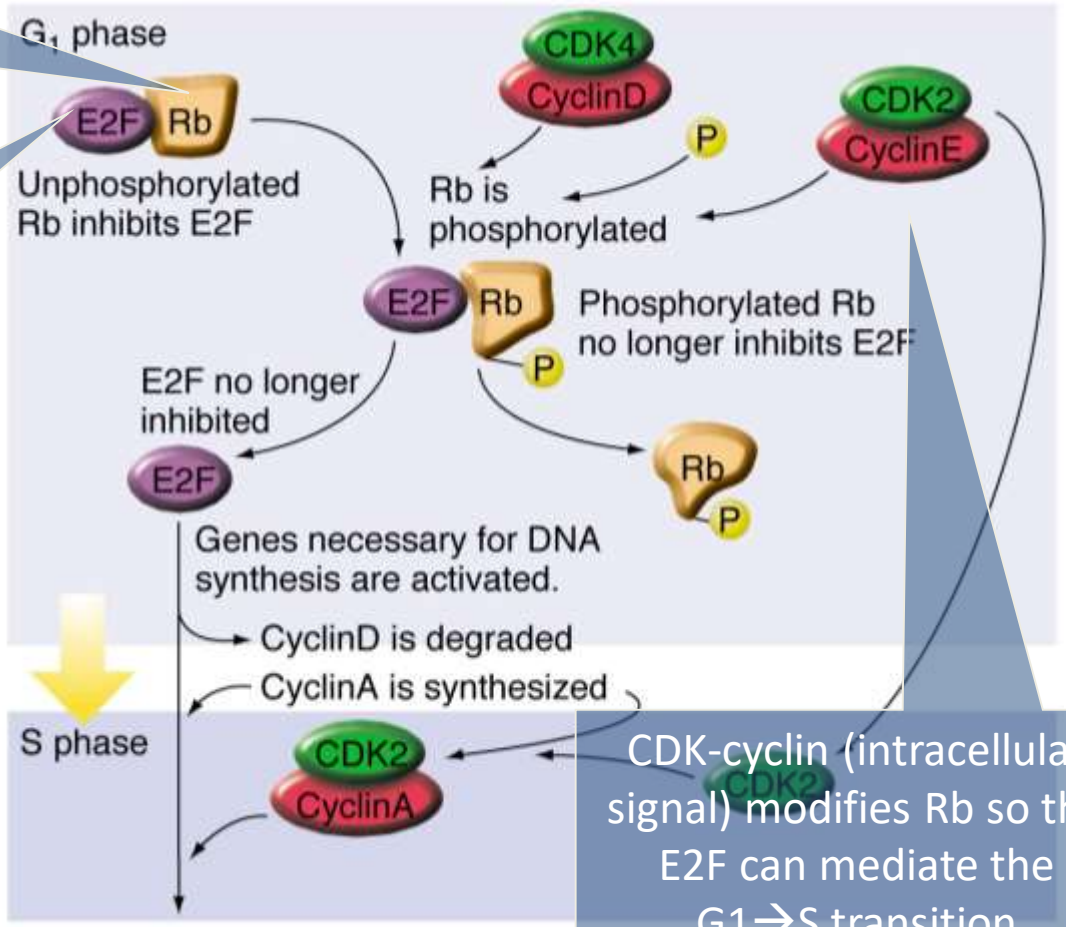
- Genes that inhibit cell division are inactivated.
  - Mutation in a gene that halts the cell cycle in G1 causes retinoblastoma.
  - Mutation in p53, a gene that promotes apoptosis if a cell has damaged DNA, leads to a variety of cancers.
  - Mutation in BRCA1, involved in tumor suppression and DNA repair, leads to inherited breast cancer.

In Normal Cells, the Rb Gene Product Controls the G1→ S Transition

Rb = product of Retinoblastoma gene, inhibits action of E2F until chemically modified

E2F = transcription factor required to activate genes for DNA synthesis





CDK-cyclin (intracellular signal) modifies Rb so the E2F can mediate the G1→S transition

People prone to retinoblastoma have one mutated copy of the Rb gene (Rb-) and one normal copy (Rb^+). Conversion of the Rb^+ copy to Rb^- by mutation leads to uncontrolled growth of retinal cells.

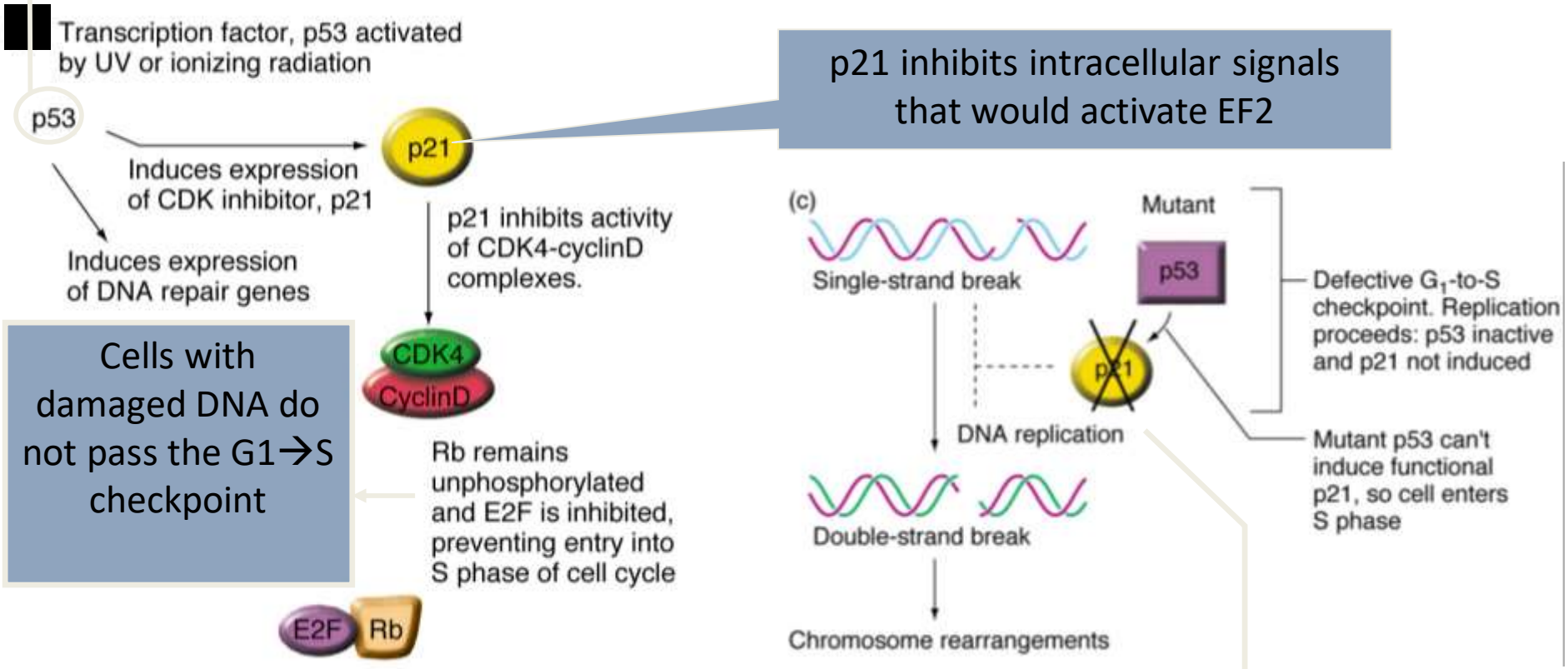
Li-Fraumeni syndrome.

- **THE p53 GENE** like the Rb gene, is a tumor suppressor gene, i.e., its activity stops the formation of tumors.

- If a person inherits only one functional copy of the p53 gene from their parents, they are predisposed to cancer and usually develop several independent tumors in a variety of tissues in early adulthood. This condition is rare, and is known as Li-Fraumeni syndrome.

p53 = transcription factor that causes p21 to be produced

In Normal Cells, the p53 Gene Product Acts at the G1→ S Checkpoint Preventing Entry Into S Phase If DNA Is Damaged



p21 inhibits intracellular signals that would activate EF2

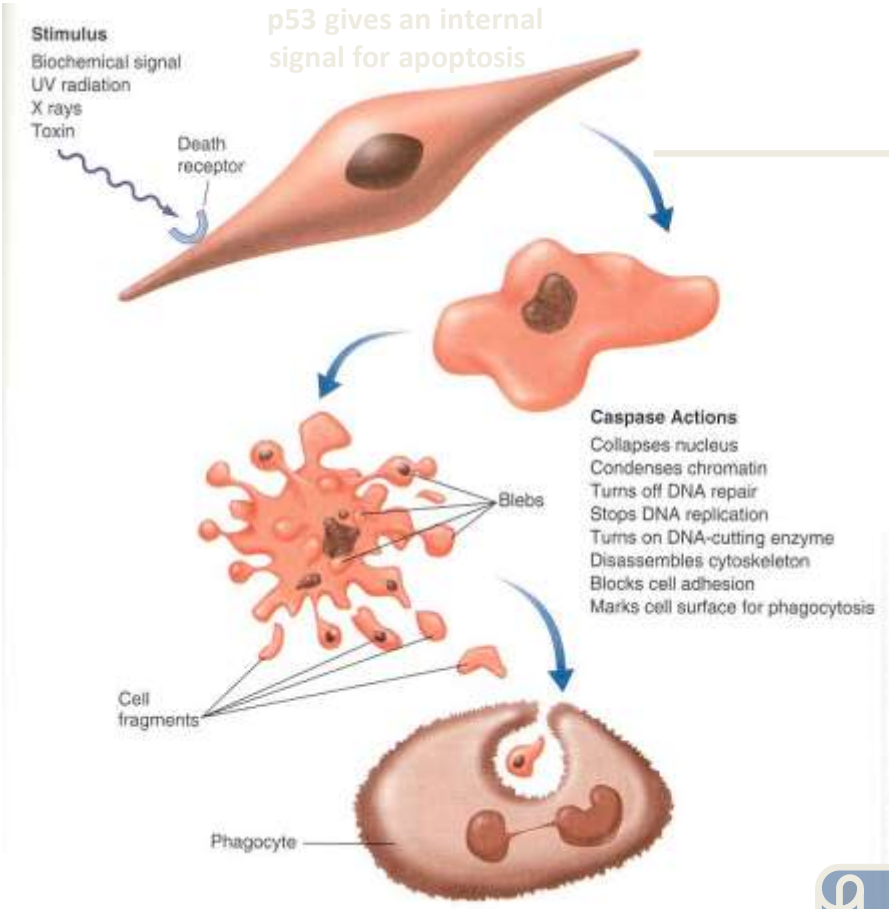
Cells with damaged DNA do not pass the G1→S checkpoint

In cancer cells the mutated p53 gene product no longer stimulates p21 production. Cells will pass the G1→ S checkpoint even when chromosomal damage exists.

P53, Continued

- **THE p53 GENE** like the Rb gene, is a tumor suppressor gene, i.e., its activity stops the formation of tumors. If a person inherits only one functional copy of the p53 gene from their parents, they are predisposed to cancer and usually develop several independent tumors in a variety of tissues in early adulthood. This condition is rare, and is known as Li-Fraumeni syndrome. However, mutations in p53 are found in most tumor types, and so contribute to the complex network of molecular events leading to tumor formation.

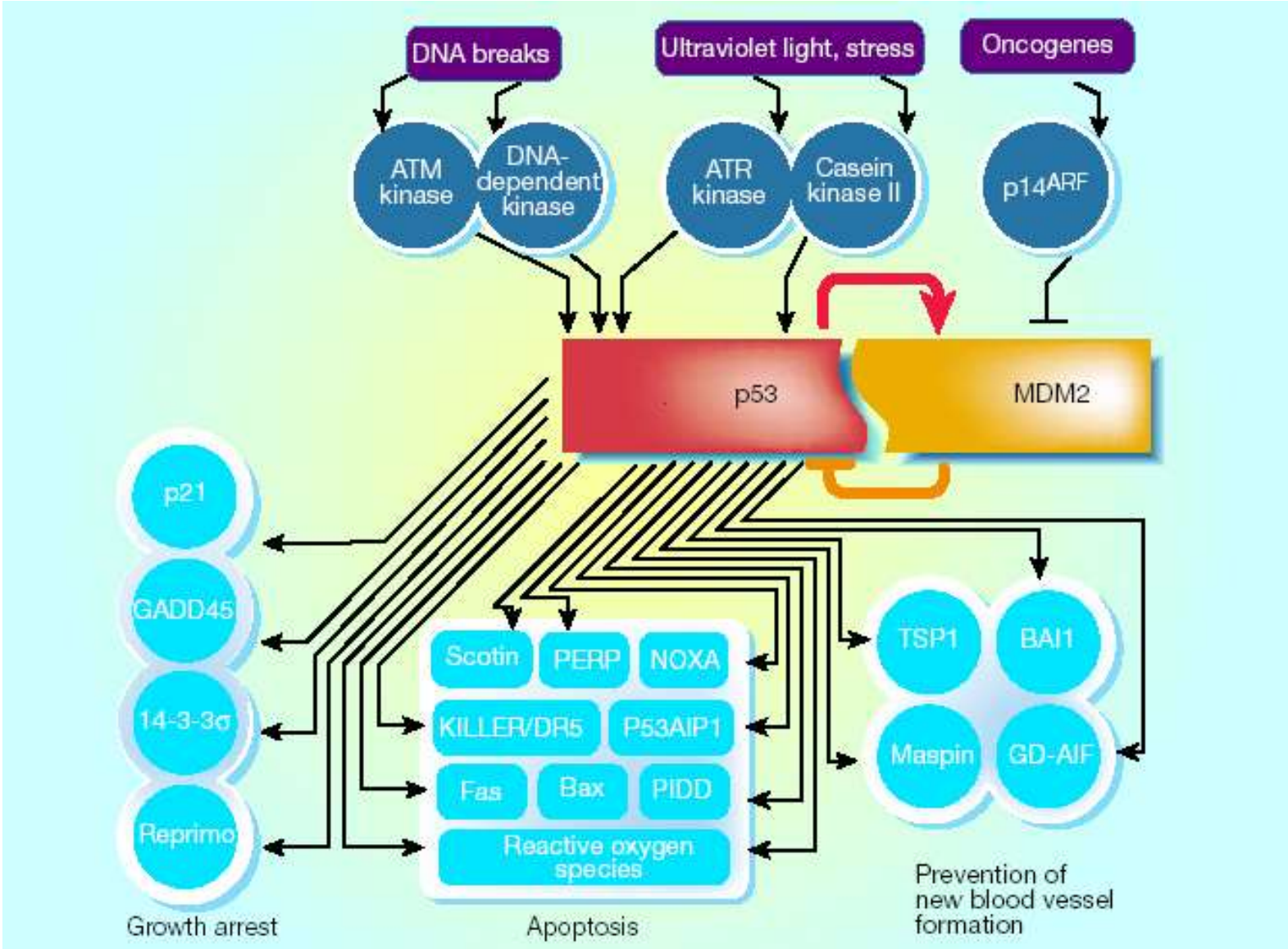
In Normal Cells, the p53 Gene Product Stimulates Apoptosis If DNA Damage Cannot Be Repaired



In cancer cells, a mutated p53 gene product no longer initiates self-destruction. Cells with damaged DNA can divide and more DNA damage can be accumulated.

p53 is the most frequently mutated of all known cancer-causing genes, contributing to many types of cancer.

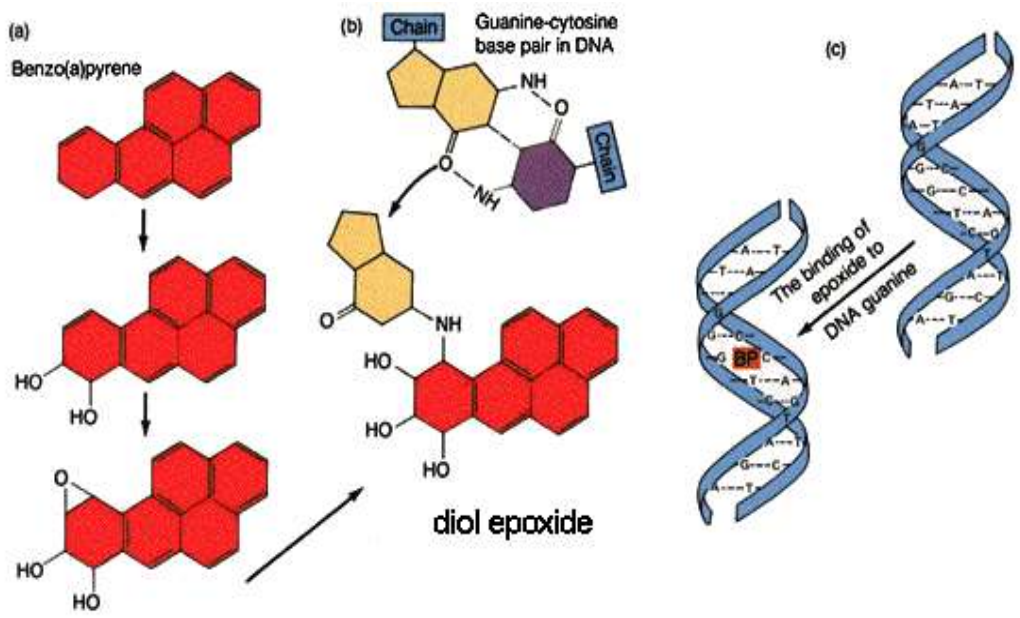
p53 network

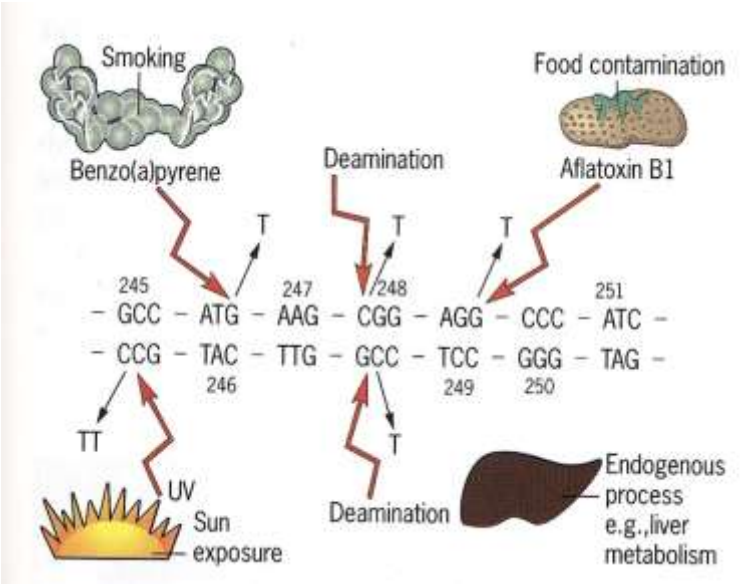


Vogelstein, B., Lane, D. P., and Levine, A. J. (2000). Surfing the p53 network. Nature 408: 307-310.

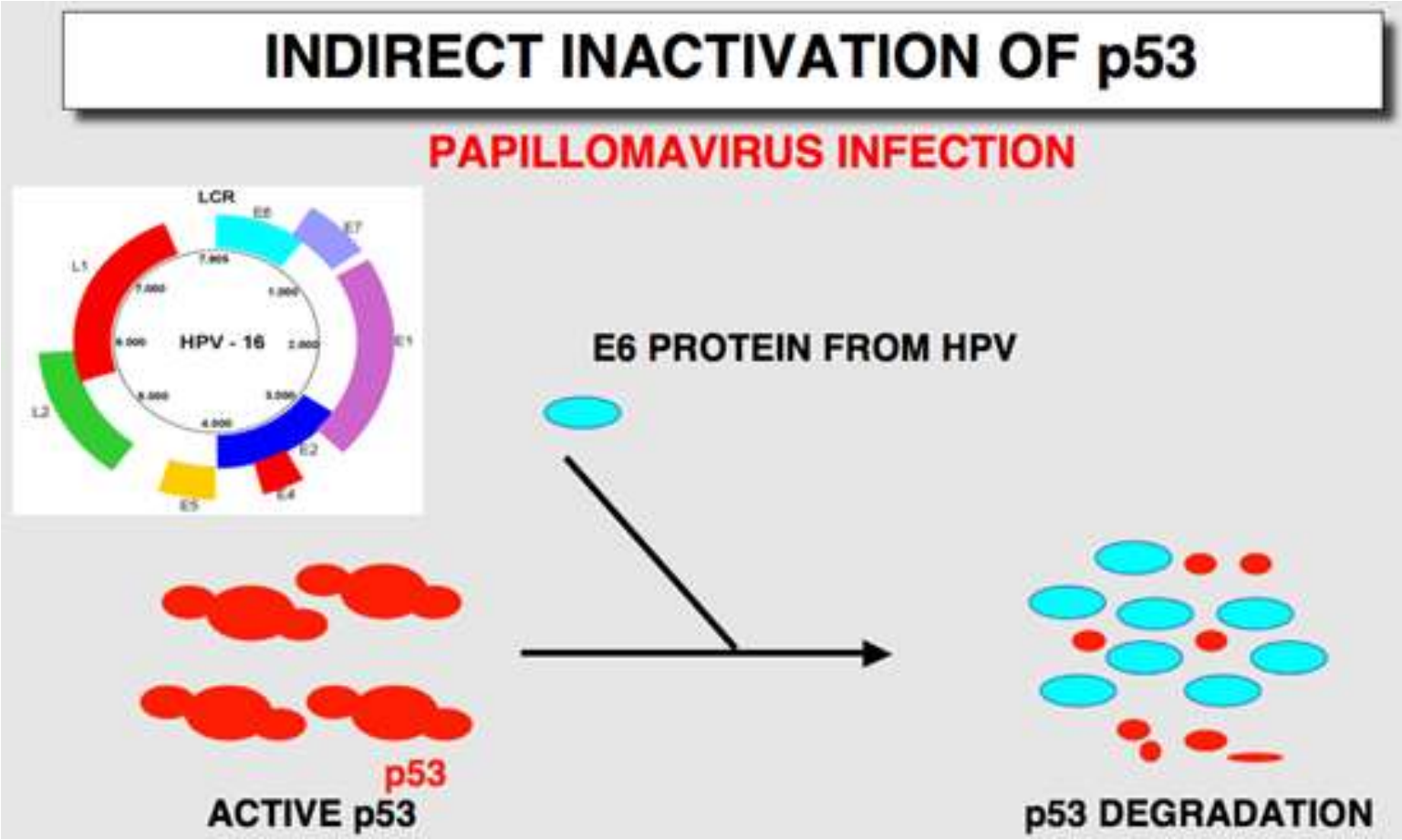
Carcinogens can Damage the p53 Gene

Benzo(a)pyrene, a chemical produced by internal combusion engines and thus common in the environment, is not itself mutagenic.
In the mammalian liver, benzo(a)pyrene is metabolized to **diol epoxide**, which binds covalently to guanine bases, preventing proper base pairing with cytosine bases.
Bulky Addition Products such as diol epoxide or **Aflatoxin B_1** may result in **depurination mutagenesis** and are known to be **carcinogens**.





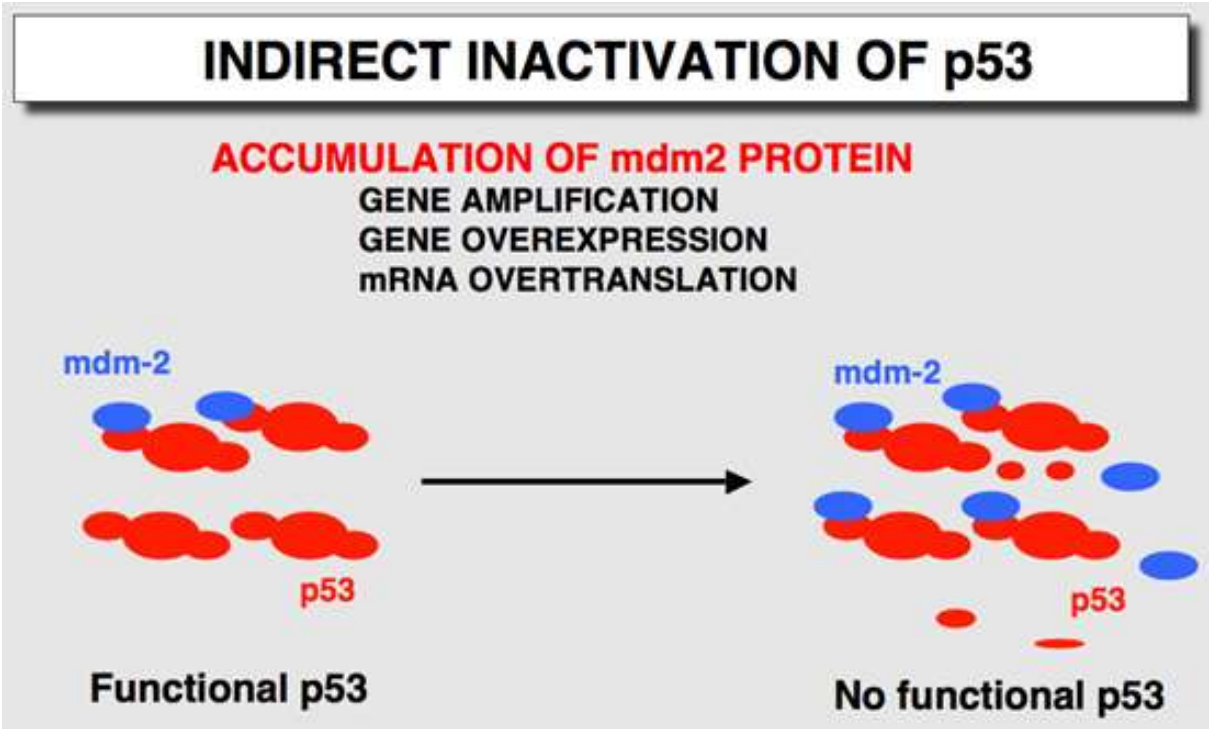
Another factor that influences p53 mutation is Caused by exposure to Ultra Violet Radiation



- The E6 viral protein expressed by HPV specifically binds to the p53 protein and induces its degradation (Scheffner et al., 1990).

- p53 mutations in cervical cancer is VERY RARE (Crook et al., 1992).

- p53 inactivation by a viral protein has not been formally demonstrated in other human cancers associated with viral infection, such as HCC (associated with HBV)

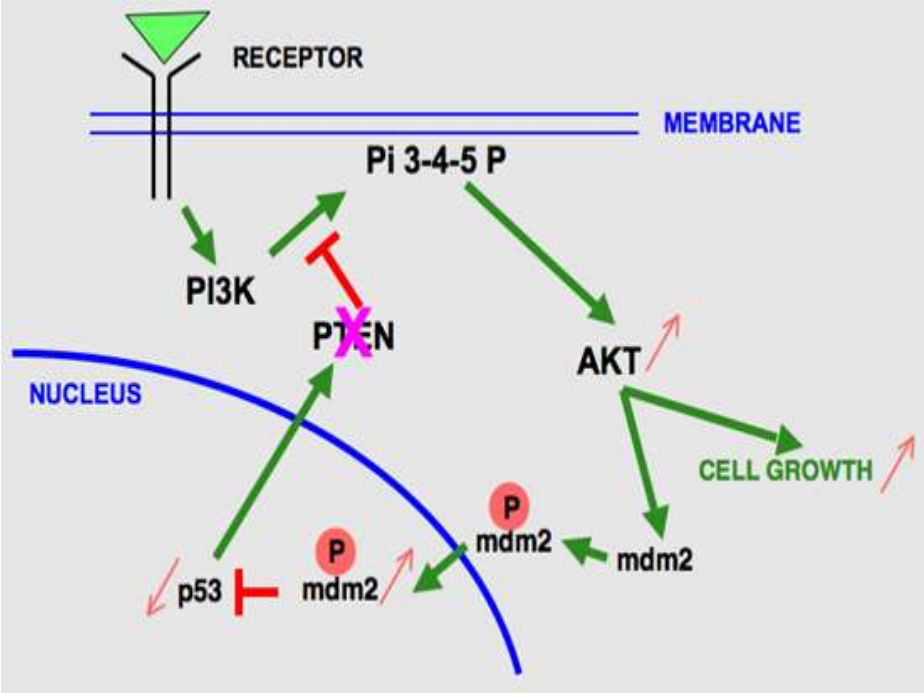


The mdm2 protein regulates the stability of the p53 protein by ubiquitination and transport towards the proteasome (Iwakuma and Lozano, 2003; Moll and Petrenko, 2003).

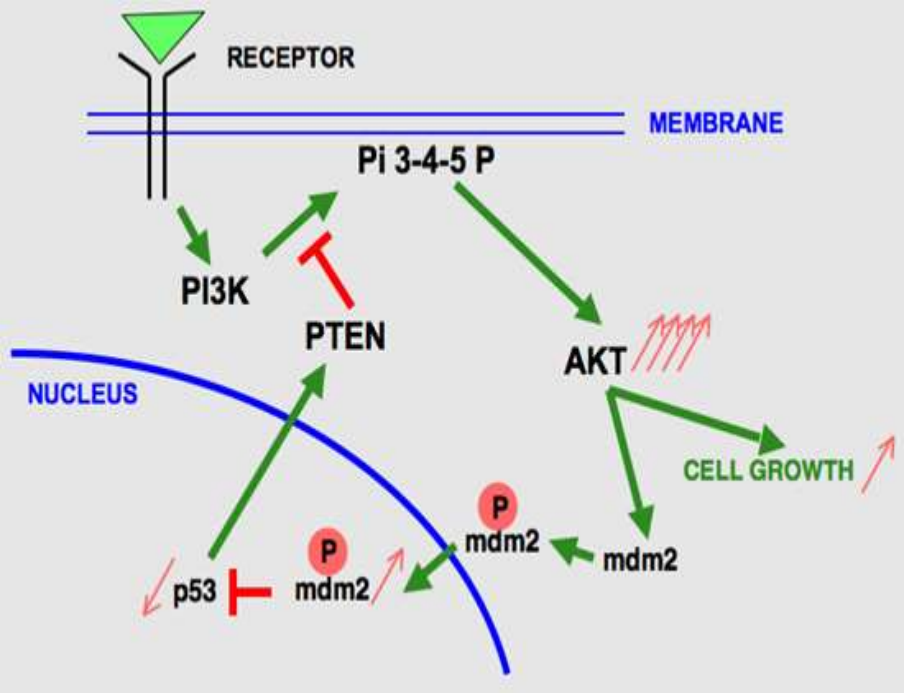
Abnormal accumulation of the mdm2 protein is observed in many tumours, especially sarcomas (Onel and Cordon-Cardo, 2004).
These tumours would be expected to no longer express p53, the opposite situation is generally observed, with a large number of tumours overexpressing both p53 and mdm2.

Suggesting that this situation could be due to an oncogenic activity of mdm2 independent of p53.

PTEN mutations



AKT Alterations



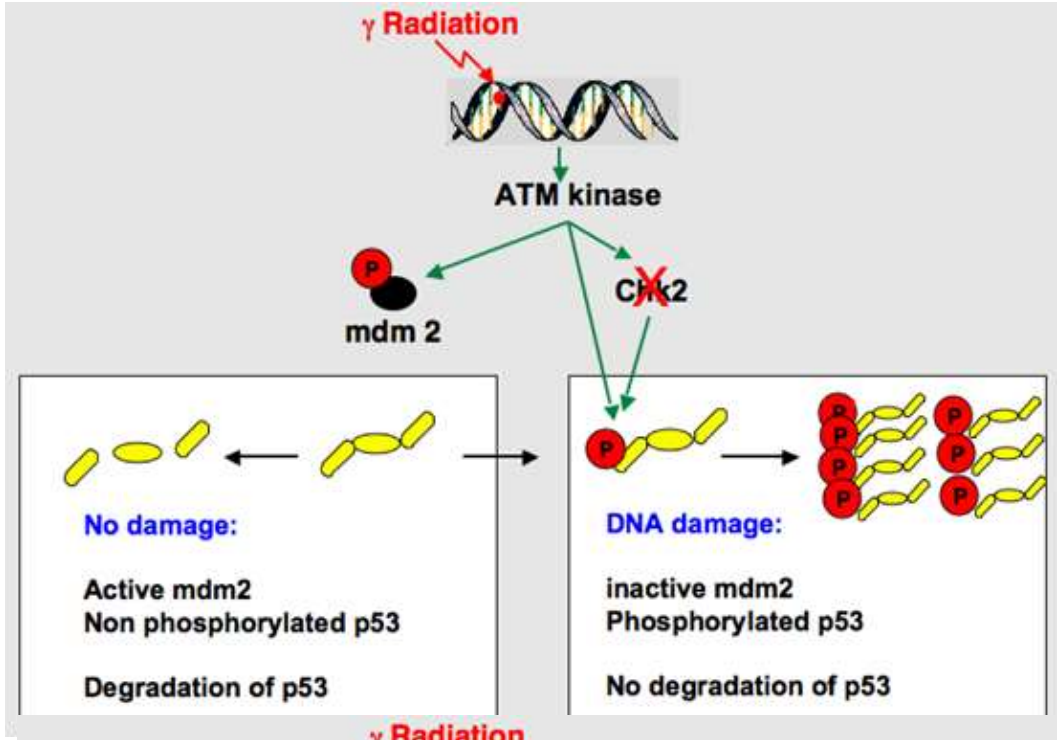
AKT kinase phosphorylates mdm2 protein and induces its migration into the nucleus where it binds and ubiquinates p53. Upon growth factor activation, mdm2 activation through AKT activation ensure proper cell growth.

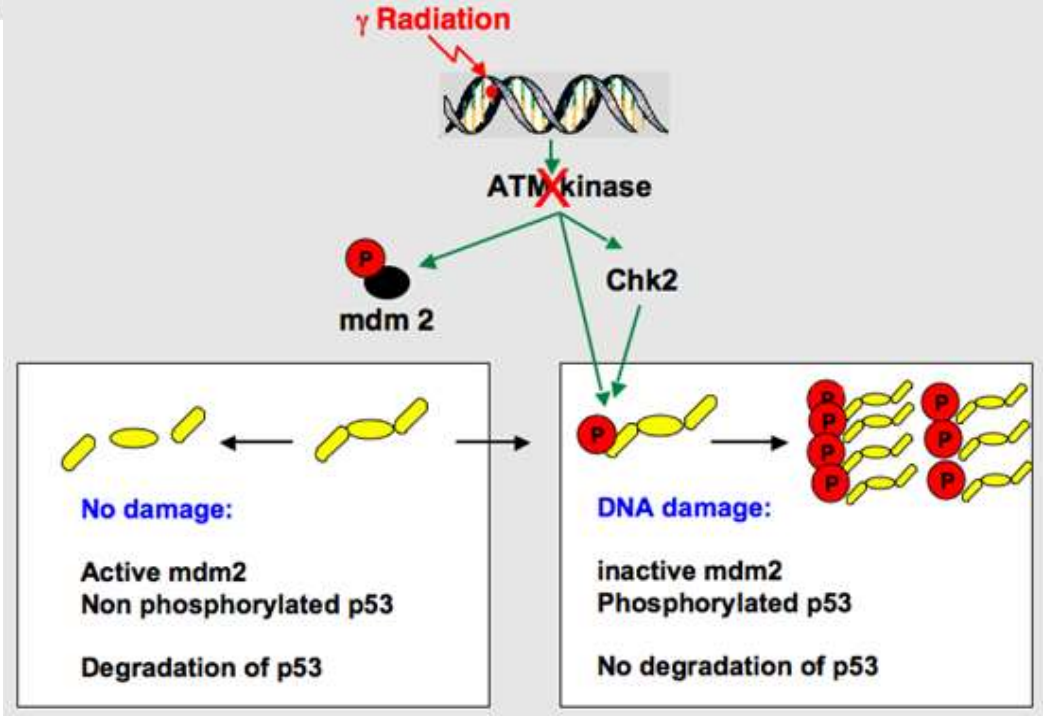
PTEN, a p53 regulated gene, down regulate the AKT pathway.

PTEN deletion leads to an increase of AKT activity, an increase of nuclear mdm2 and impairs p53 response

Although no mutation of AKT has been found in human cancer, constitutive activation of its kinase activity has been observed via deregulation of the upstream pathway

Upstream signaling





DNA damage induced by gamma radiation INDUCED activated ATM phosphorylates p53 on Ser15, CHK2 on Thr68, and murine double minute 2 (*mdm2*) on Ser395.

Activated CHK2 phosphorylates p53 on Ser20. Together, these phosphorylations interfere with p53 binding to MDM2, leading to stabilization and activation of p53.

Mutations of ATM in T-cell leukaemia impair the p53 response after gamma radiation

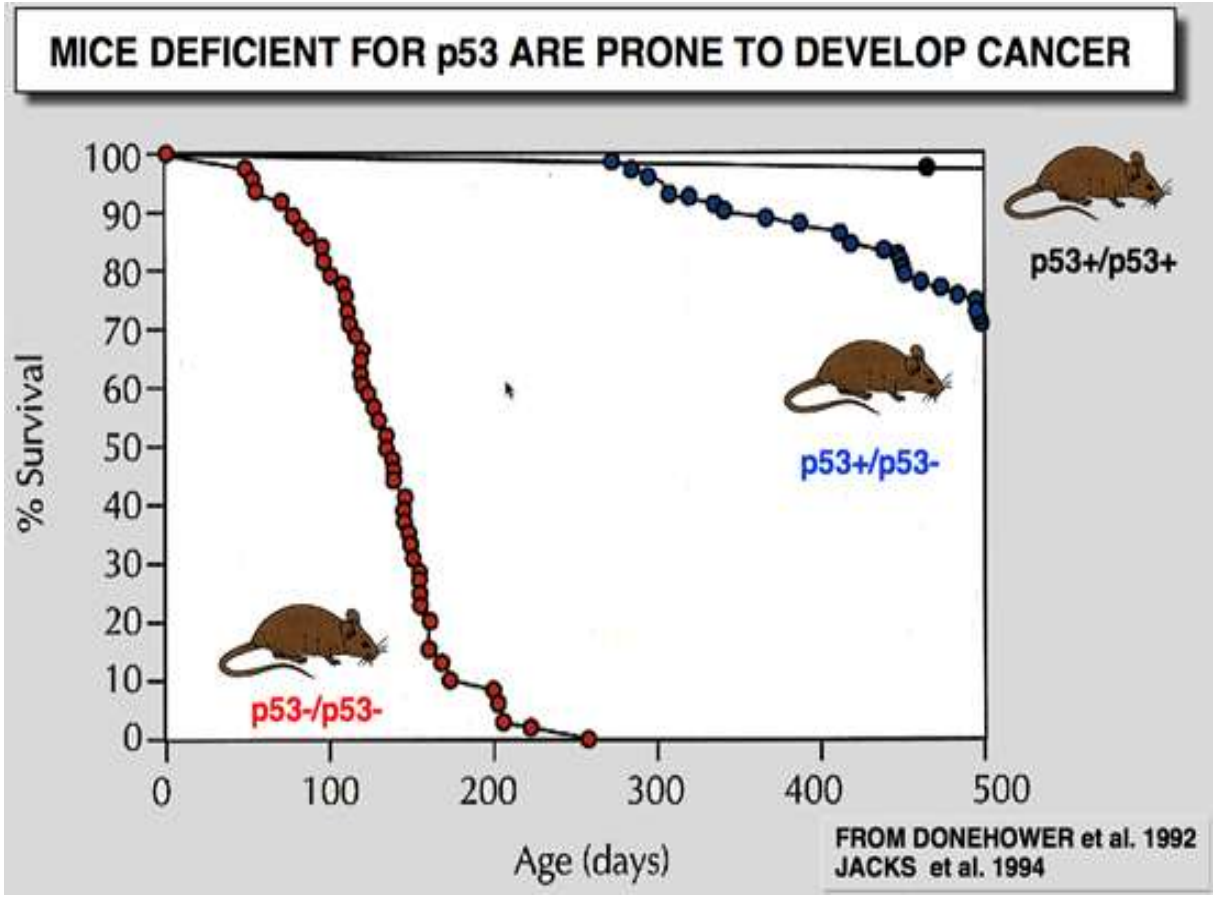
Mutations of CHK2 are found in Li-Fraumeni like families.

P53 mutations

- Mutations that deactivate p53 in cancer usually occur in the DBD.
- Most of these mutations destroy the ability of the protein to bind to its target DNA sequences, and prevent transcriptional activation of these genes.
- Mutations in the DBD are recessive loss-of-function mutations.
- Molecules of p53 with mutations in the homo-oligomerisation domain (OD) dimerise with wild-type p53, and prevent them from activating transcription. Therefore OD mutations have a dominant negative effect on the function of p53.

knockout model (p53 KO)

Endogenous p53 gene is either totally or partially deleted either in whole body or in specific tissue.



A null mutation was introduced into the gene by homologous recombination in murine embryonic stem cells.

Mice homozygous for the null allele appear normal but are prone to the spontaneous development of a variety of neoplasms by 6 months of age.

(Donehower LA *et.al.,* 1992, *Nature*)

mouse p53m

p53

$p53^m$

deletion of exon 1 to 6

Viable
No susceptibility to spontaneous tumours (enhance tumor resistance)
Median lifespan lower than for wt mouse (96 wks versus 118 wks)
Early aging phenotypes (reduced longevity, osteoporosis, organ atrophy)

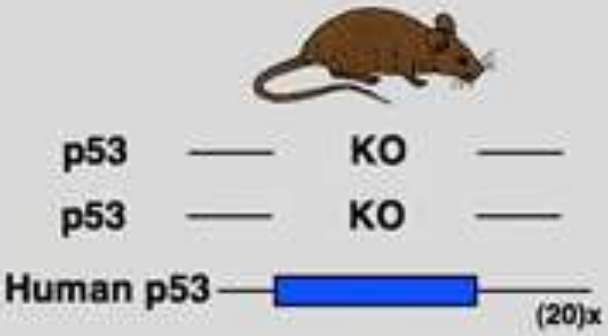
Note: $p53^m$: a putative 24 kDa C-ter protein can be expressed by this mutant (not detected so far)

MEF are more resistant to transformation than wt MEF; enhanced p53 stability and transactivation activity

Mice with a deletion mutation in the first six exons of the p53 gene that express a truncated RNA capable of encoding a carboxy-terminal p53 fragment.

P53m exon 1 to 6 deletion /Aging phenotype

Swap p53



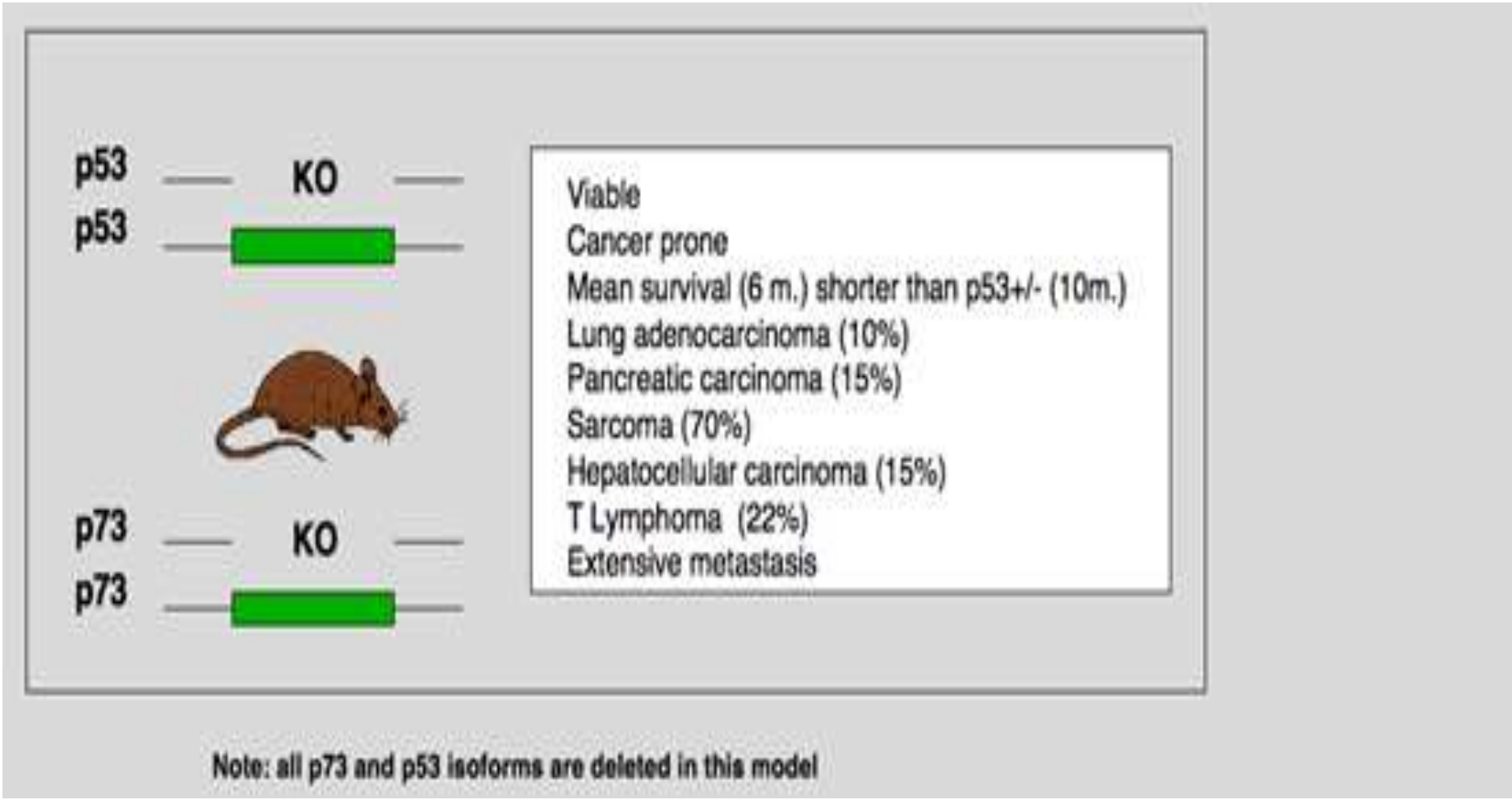
Viable
Phenotypically normal
Shorter life span than normal mice
Tumor prone (lymphomas)
Impaired apoptosis induction after DNA damage despite p53 accumulation

Mice carrying the human p53 transgene did not show early onset of tumors as typically seen for p53-null mice.

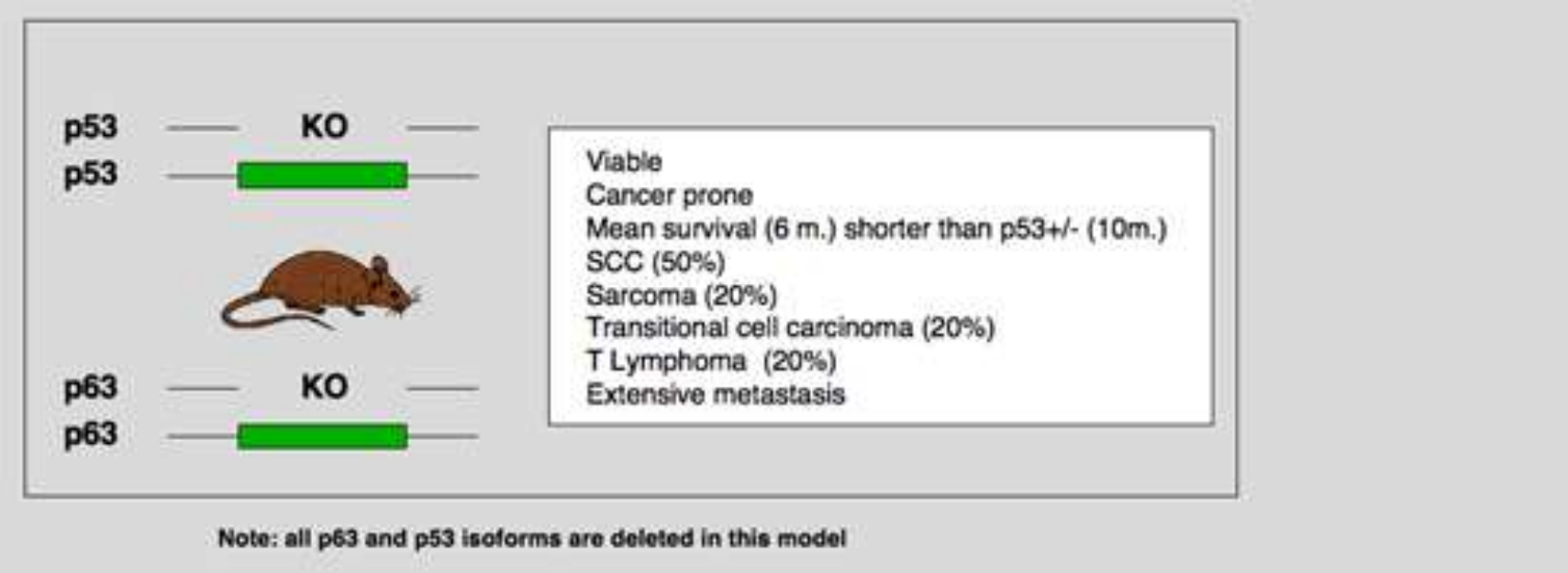
In contrast, human p53 in the p53-null background did not prevent accelerated tumor development after genotoxic or oncogenic stress.

Such behavior of human p53 expressed at physiologic levels in transgenic cells could be explained by unexpectedly high binding with Mdm2. By using Nutlin-3a, an inhibitor of the interaction between Mdm2 and p53, we were able to partially reconstitute p53 transactivation and apoptosis in transgenic cells.

Our findings indicate that the interaction between p53 and Mdm2 controls p53 transcriptional activity in homeostatic tissues and regulates DNA damage- and oncogene-induced, but not spontaneous, tumorigenesis.



P73- has high homology with the tumour suppressor p53

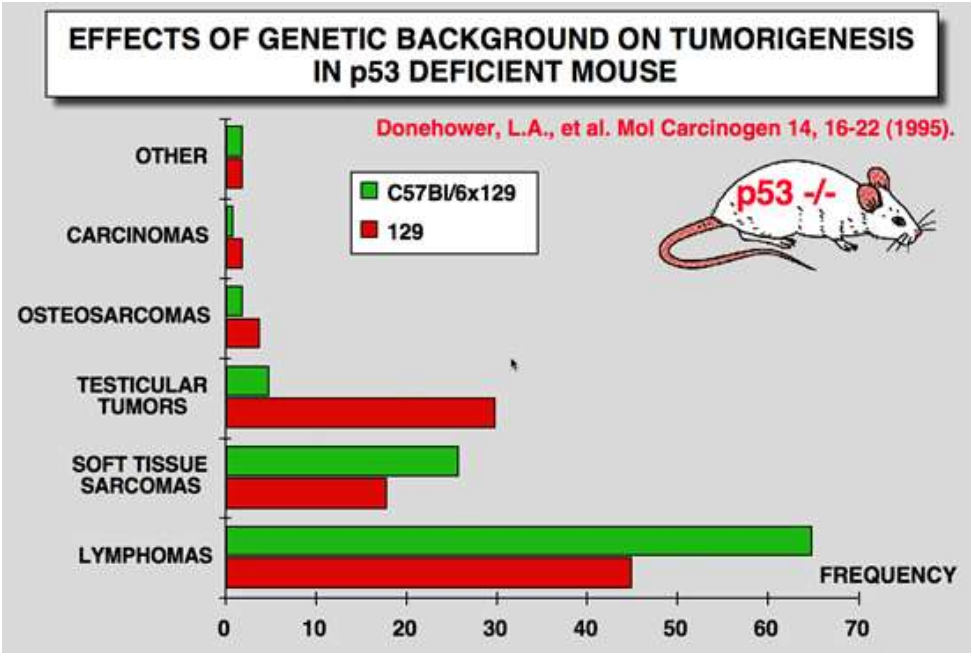


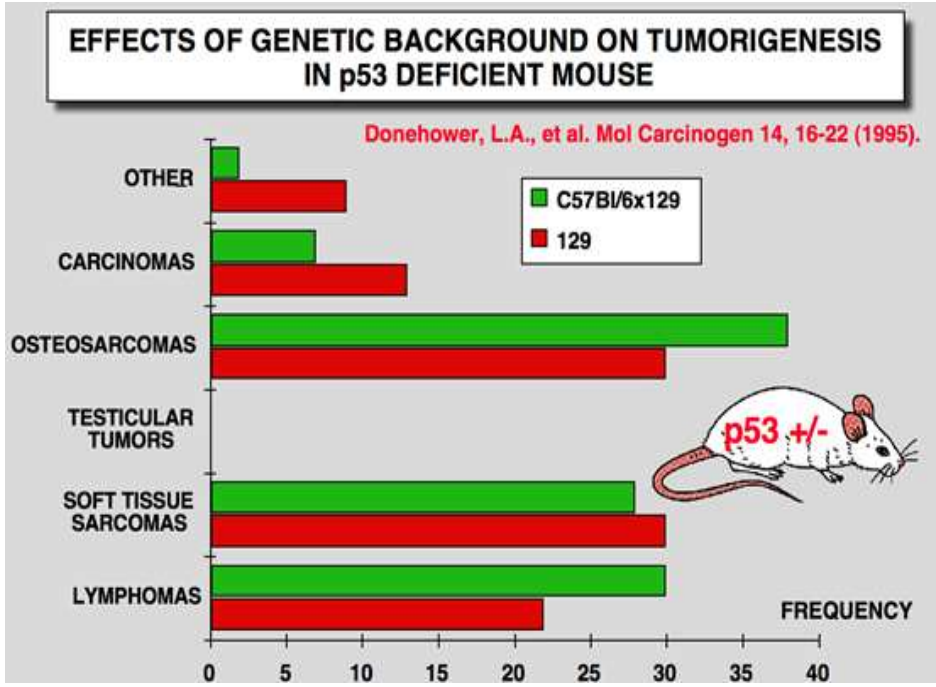
p63(+/-);p73(+/-) mice develop spontaneous tumors.

Loss of p63 and p73 can also cooperate with loss of p53 in tumor development.

Mice heterozygous for mutations in both p53 and p63 or p53 and p73 displayed higher tumor burden and metastasis compared to p53(+/-) mice. T

p53 Knockout mice phenotype: Effects of genetic background





p53+/- and p53-/- 129/Sv mice show accelerated tumorigenesis rates compared with their p53-deficient counterparts of mixed C57BL/6 x 129/Sv genetic background.

The heterozygous mice may be a useful model for Li-Fraumeni syndrome, a human inherited cancer predisposition

p53 Knockout mice phenotype: developmental abnormalities

A SUBSET OF p53-DEFICIENT EMBRYOS EXHIBIT DEVELOPMENTAL ABNORMALITIES

Sah, V.P., et al. Nat Genet 10, 175-180 (1995).
Armstrong, J.F., Kaufman, M.H., Harrison, D.J. & Clarke, A.R. Current Biology 5, 931-936 (1995).

NEURAL TUBE MALFORMATIONS IN FEMALE
-> EXENCEPHALY
-> REDUCTION OF FEMALES AT BIRTH

OCCULAR ABNORMALITIES

DENTAL ABNORMALITIES
-> FUSION OF THE UPPER INCISORS

A SUBSET OF p53-DEFICIENT MOUSE EXHIBIT THE TESTICULAR GIANT CELL DEGENERATIVE SYNDROME

Rotter, V., et al. Proc Natl Acad Sci USA 90, 9075-9079 (1993).

p53: A CELLULAR PROOF READER ?

p53 reduces the amount of defectives embryo/foetuses after exposure to drugs or radiation

Brash DE (1996) Cellular proofreading. Nature Med. 2: 525-526.

WILD TYPE p53

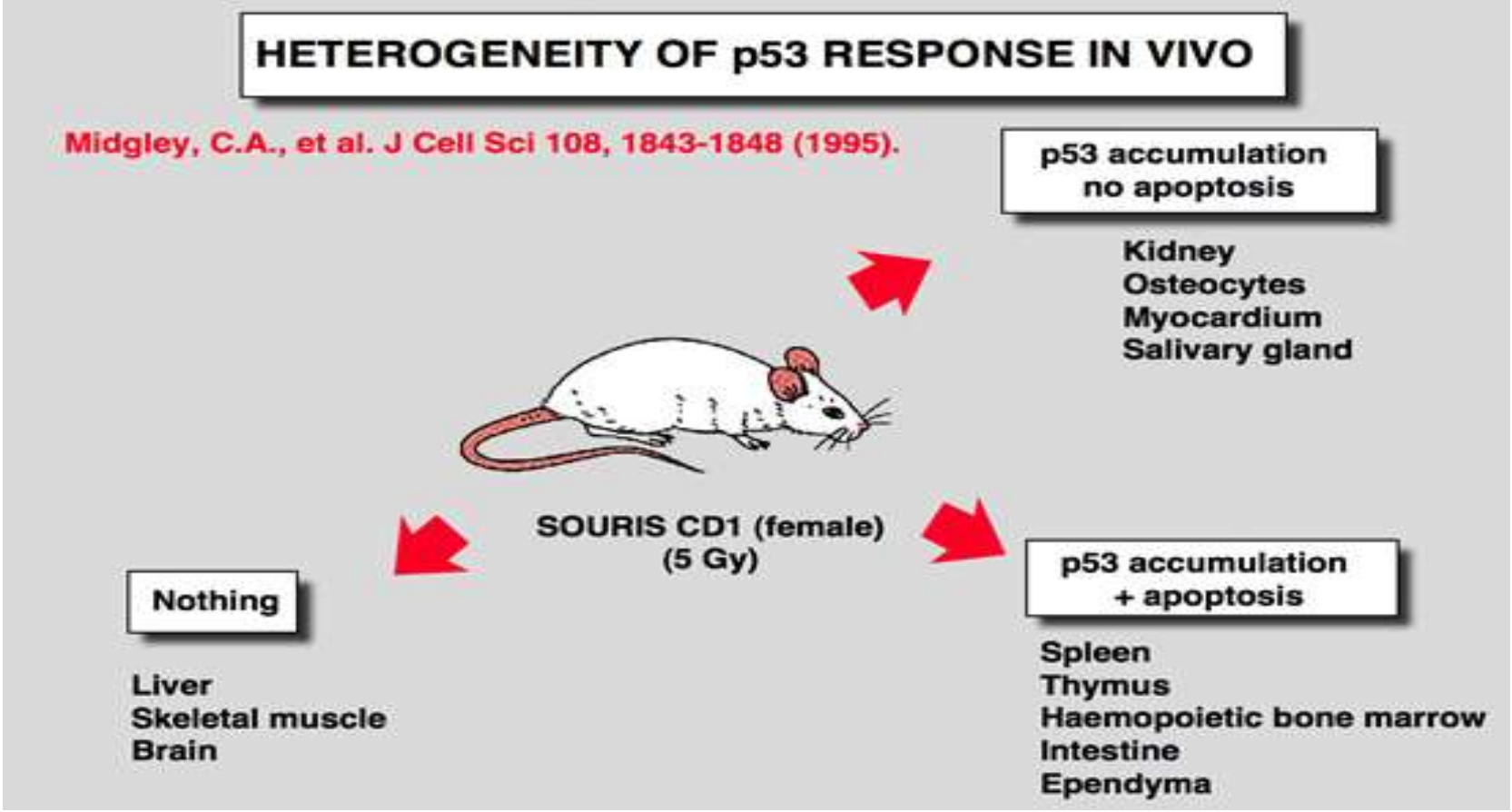
Low incidence of anomalies
High incidence of death
Apoptosis

Low level of embryotoxicity
Low level of teratogenicity
Low level of resorptions

NULL p53

High incidence of anomalies
Low incidence of death
No apoptosis

High level of embryotoxicity
High level of teratogenicity
High level of resorptions

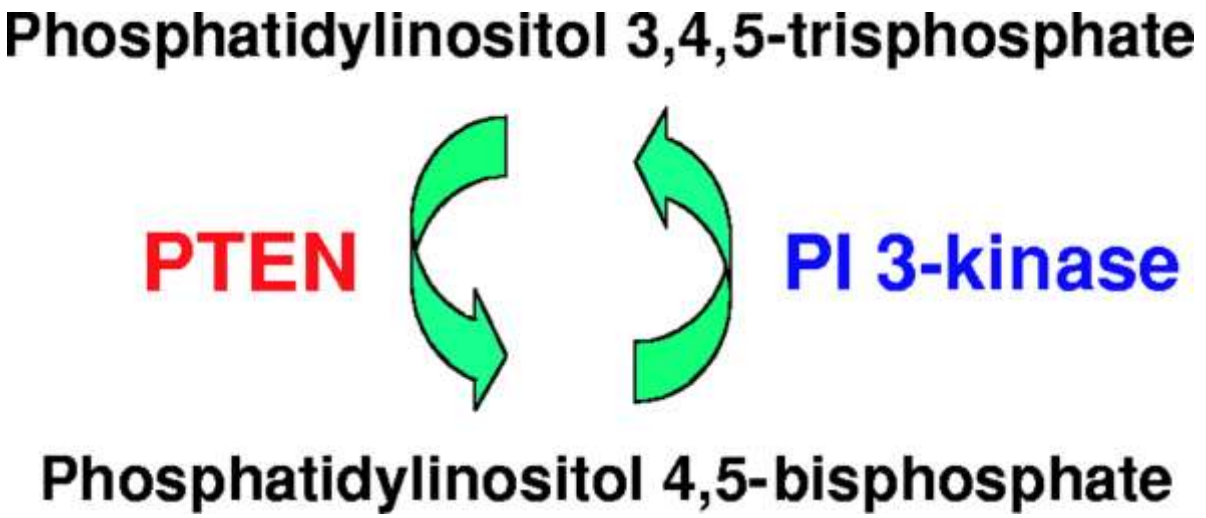


The accumulation of p53 protein following whole body irradiation of adult mice was studied using a new polyclonal antibody to mouse p53. While dramatic accumulation of the protein was apparent in splenocytes, thymocytes and osteocytes no p53 protein accumulation was detected in the hepatocytes of the irradiated mouse. Thus, the upstream initiating signals that control the induction of p53 are controlled in a tissue specific manner. While massive apoptosis accompanies p53 induction in thymocytes and splenocytes it is not seen in the osteocytes. Thus the downstream consequences of p53 induction are also tightly controlled. These results have profound significance for an understanding of the role of the p53 tumour suppression pathway in different tissues.

Major enzymatic function of PTEN.

PTEN is a tumor suppressor gene

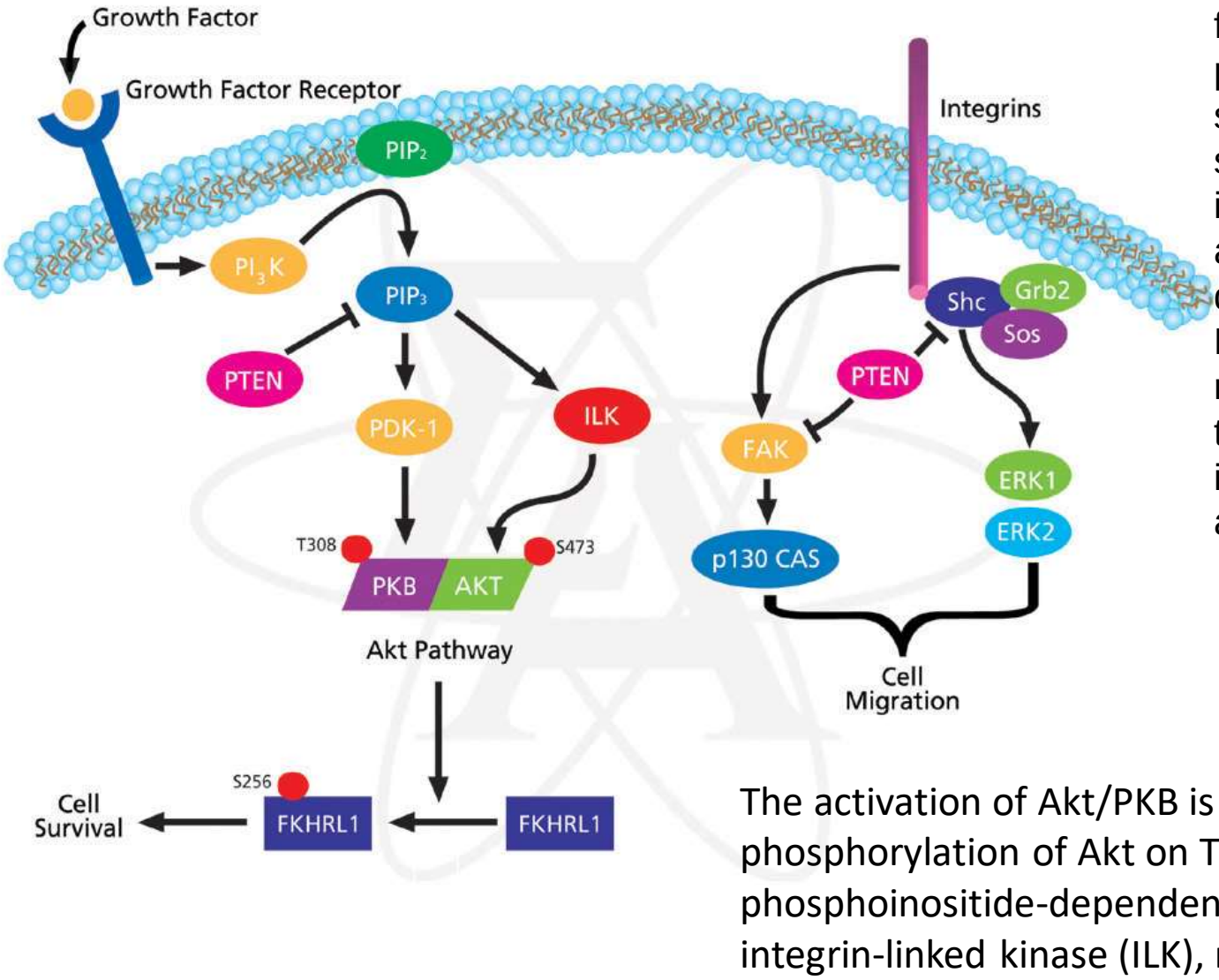
PTEN opposes action of PI3K by dephosphorylation



Yamada K M , Araki M J Cell Sci 2001;114:2375-2382

PTEN Pathway

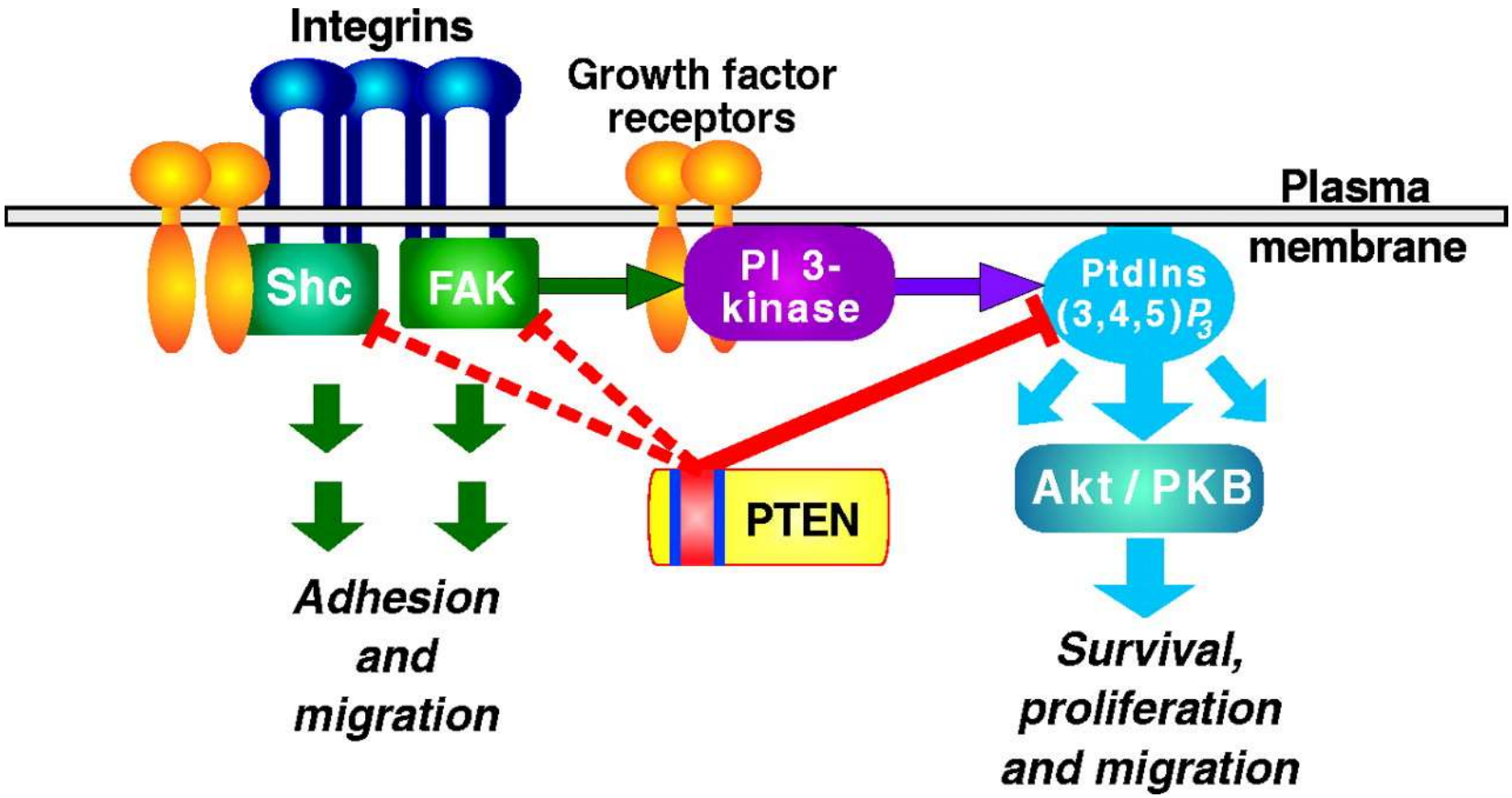
SIGMA-ALDRICH



PTEN also inhibits growth factor (GF)-induced Shc phosphorylation and suppresses the MAP kinase signaling pathway. PTEN interacts directly with FAK and is able to dephosphorylate activated FAK. PTEN-induced down-regulation of $p130^{CAS}$ through FAK results in inhibition of cell migration and spreading.

The activation of Akt/PKB is regulated by the phosphorylation of Akt on Thr^{308} and Ser^{473} by phosphoinositide-dependent kinase (PDK) and integrin-linked kinase (ILK), respectively.

Reported sites of action of PTEN.



Yamada K M , Araki M J Cell Sci 2001;114:2375-2382

Thanks for your Attention

Acknowledgement

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- Thanks are due to all the original contributors and entities whose pictures were used in the creation of this presentation.