**Neoplasia III: Tumor suppressor genes**
September 16, 2008
Lecturer: Richard Baer
Readings: Chapter 6, Robbins Basic Pathology, 8th Edition; Kumar *et al*. (2007)

The malignant transformation of a cell from a normal state to a neoplastic state is caused by genetic (as well as epigenetic) lesions in multiple cellular genes. Tumor-associated lesions of these genes can contribute to malignant transformation by loss-of-function (the tumor suppressor genes) or by gain-of-function (proto-oncogenes). To understand the process by which a particular tumor develops, it is necessary to identify the genes that are altered in a causal manner during tumorigenesis and to determine the mechanism by which these alterations promote neoplastic progression. As described by Dr. Lefkowitch's (Neoplasia II), many proto-oncogenes have been uncovered through the use *in vitro* assays of cell transformation or from analysis of chromosome abnormalities that are specifically associated with tumor cells. Today, we will consider the role of tumor suppressor genes in human cancer. Although most human tumors appear to arise sporadically in the absence of an obvious hereditary origin, several familial cancer susceptibility syndromes have been described. Studies of these syndromes have led to the identification of a number of tumor suppressor genes that are implicated in sporadic, as well as heritable, cases of human cancer. To illustrate this point, we will describe several prominent tumor suppressor genes, consider the mechanisms by which inactivation of these genes promotes neoplasia, and discuss how the protein products of proto-oncogenes and tumor suppressor genes can function together in common cellular pathways that are frequently altered in human cancer.

## Multistep nature of cancer development

- Phenotypic progression
  - loss of control over cell growth/death (neoplasm)
  - invasiveness (carcinoma)
  - distal spread (metastatic tumor)
- Genetic progression
  - multiple genetic lesions required for cancer
  - what genes are altered in cancer?

## Cancer genes

- What genes, when altered, promote cancer?
  - tumor suppressor genes and proto-oncogenes
- Some genes are altered in a restricted set of tumor types
  - e.g., the APC tumor suppressor in colorectal carcinoma
- Others are altered in a broad spectrum of tumor types
  - e.g., p53 tumor suppressor and the Ras proto-oncogenes
- The importance of tumor gene “pathways”
  - the p53 and Rb pathways

## Proto-oncogenes vs. tumor suppressor genes

Proto-oncogenes promote cancer when malignantly activated

- An activated proto-oncogene contributes to tumorigenesis by "gain-of-function"
- Thus, an activated proto-oncogene is genetically dominant at the cellular level
  - an activated oncogene can elicit a new phenotype (tumorigenesis) even in the presence of the corresponding wildtype allele

## Proto-oncogenes vs. tumor suppressor genes

Tumor suppressor genes promote cancer when malignantly inactivated

- A tumor suppressor contributes to tumorigenesis by "loss-of-function"
- In most instances, an inactivated tumor suppressor gene is genetically recessive at the cellular level.
  - it will not promote tumorigenesis in diploid cells unless the other (wildtype) allele is also lost or inactivated
  - some exceptions (e.g., dominant-negative p53 mutations)

## Tumor suppressor genes

- in this lecture we will focus on…
  - the retinoblastoma susceptibility (Rb) gene
  - the p53 tumor suppressor gene
- genetic properties
- biochemical functions of their protein products
- the p53 and Rb tumor suppressor "pathways"

## Cancer susceptibility syndromes

- What proportion of human cancers are heritable?
- Hereditary syndromes of cancer susceptibility are usually caused by germline mutations of tumor suppressor genes.
  - familial retinoblastoma: Rb
  - Li-Fraumeni syndrome: p53
  - familial adenomatous polyposis coli: APC
  - hereditary non-adenomatous c.c.: MLH1, MSH2
- Penetrance: fully penetrant mutations segregate as dominant traits in a Mendelian fashion

## Sporadic and Heritable forms of Retinoblastoma

- age of tumor onset
  - sporadic (~60% of cases): ~ 6 years
  - heritable (~40% " " ): ~ 2 years
- number of independent tumors
  - sporadic: single tumor (only one eye is affected)
  - heritable: multiple tumors (both eyes are affected)
- tumor frequencies in children of patients
  - sporadic: 1 in $10^5$
  - heritable: 1 in 2

➜ patients with heritable retinoblastoma transmit a "Rb susceptibility gene" to their children in a dominant Mendelian fashion

## Knudson's hypothesis (1970s)

- Two (rate-limiting) genetic lesions required…
- Sporadic retinoblastoma:
  - both alterations are acquired somatically
  - incidence: $(10^{-6})(10^{-6})(10^7 \text{ cells}) = 10^{-5}$ tumors/person
  - very rare; involves only one eye
- Heritable retinoblastoma:
  - one alteration is inherited in the germline (i.e., the "Rb susceptibility gene")
  - the second alteration is acquired somatically
  - incidence: $(1)(10^{-6})(10^7 \text{ cells}) = 10$ tumors/person
  - all carriers affected; involves both eyes!!

# Hereditary Retinoblastoma

- incidence: $(1)(10^{-6})(10^{7}\ \text{cells}) = 10\ \text{tumors/person}$

- All mutation carriers are affected
  - Rb susceptibility is a highly penetrant trait
  - tumor susceptibility is transmitted as a dominant trait in family pedigrees (despite the fact that tumor suppression genes function recessively at the cellular level).

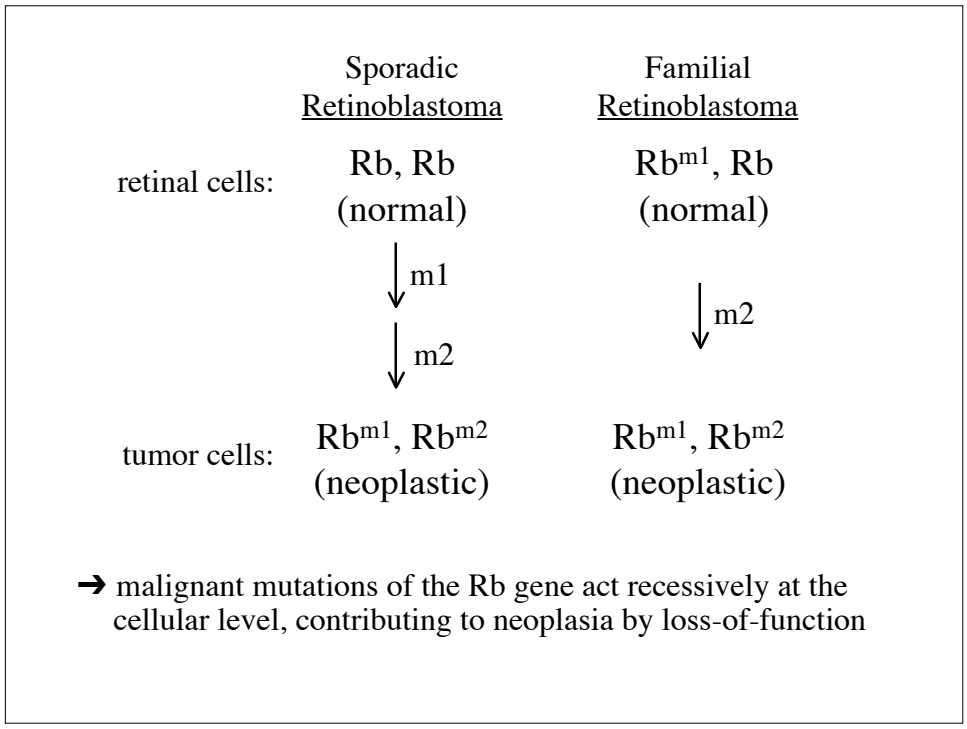
# The Retinoblastoma (Rb) gene

- What are the two rate-limiting genetic alterations?

- Cytogenetic abnormalities of chromosome 13:
  - interstitial deletions of variable length
  - always involve material from chromosome band 13q14
  - sporadic patients: deletions in tumor cells only
  - heritable patients: deletions in both normal & tumor cells

- Is Rb susceptibility due to genetic loss at 13q14?

➜ If so, then the two mutations required for retinoblastoma might represent inactivation of both alleles of a single gene at 13q14




➜ malignant mutations of the Rb gene act recessively at the cellular level, contributing to neoplasia by loss-of-function

# The Retinoblastoma gene (Rb)

- 1988: isolation of the Rb gene on 13q14

Familial retinoblastoma

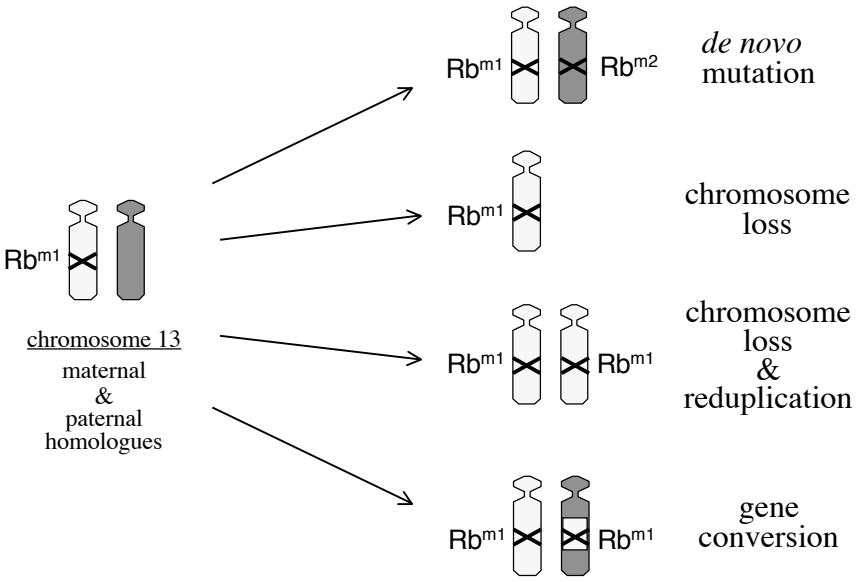
- one Rb gene lesion in germline of familial patients
- other (normal) Rb allele lost or inactivated in tumors

Sporadic retinoblastoma

- both alleles of Rb are normal in germline
- both Rb alleles lost or inactivated in tumors

➜ Genetic lesions in the same gene are responsible for both the familial and sporadic forms of retinoblastoma!!

## Inactivation of the second Rb allele (in somatic cells)




## The penetrance of germline Rb mutations

- Almost all carriers will develop retinoblastoma
  - high penetrance
    - (mutation rate)(target cells) = $(10^{-6})(10^{7}$ cells) = 10
    - retinoblastoma susceptibility is transmitted as a dominant Mendelian trait
- Some carriers will also develop osteosarcoma
  - low penetrance
    - (mutation rate)(target cells) < 1
    - incomplete penetrance of osteosarcoma susceptibility

## Tumor Suppressors: the p53 gene

- p53 encodes a transcription factor
- the p53 gene is altered in many human tumors (usually by missense mutation).
- *in vitro* cell transformation by mutant p53 genes.

➜ Is p53 a proto-oncogene?

- murine erythroleukemias induced by Friend Leukemia Virus: a natural knockout of the p53 gene by proviral insertion!
- suppression of cell transformation by the wild-type p53 gene.

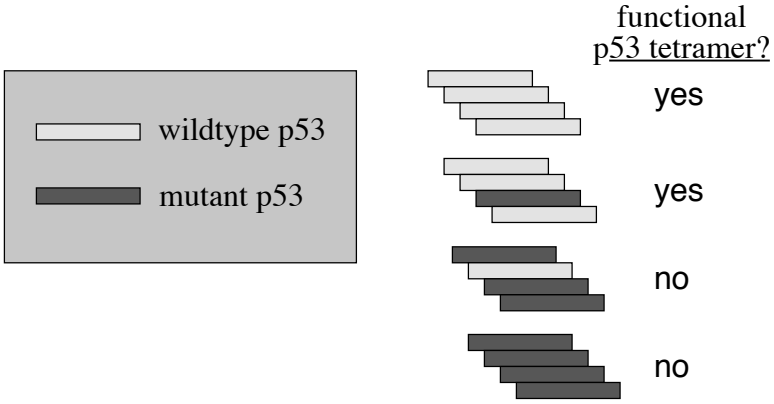
➜ Is p53 a tumor suppressor gene?

## Dominant-negative mutations of a tumor suppressor gene

- dominant-positive mutation (e.g., missense mutations in the *Ras* proto-oncogenes).
- recessive-negative mutation (e.g., Rb loss).
- dominant-negative mutation (e.g., many p53 missense mutations).
- note: dominant-negative mutations result in functional inactivation of the protein products of both alleles (including the normal allele).

## Dominant-negative mutations of p53

- how do dominant-negative mutations of p53 work?
- p53 normally functions as a homo-tetramer
- consider p53 function in a cell with one wildtype and one mutant p53 allele:

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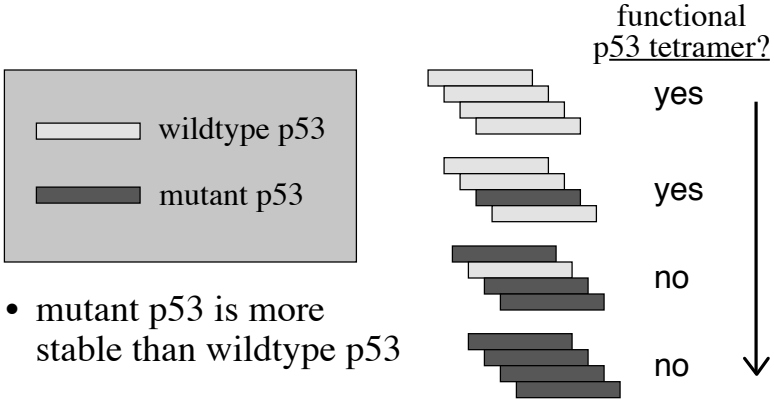



- mutant p53 is more stable than wildtype p53

## p53 mutations in hereditary and sporadic cancer

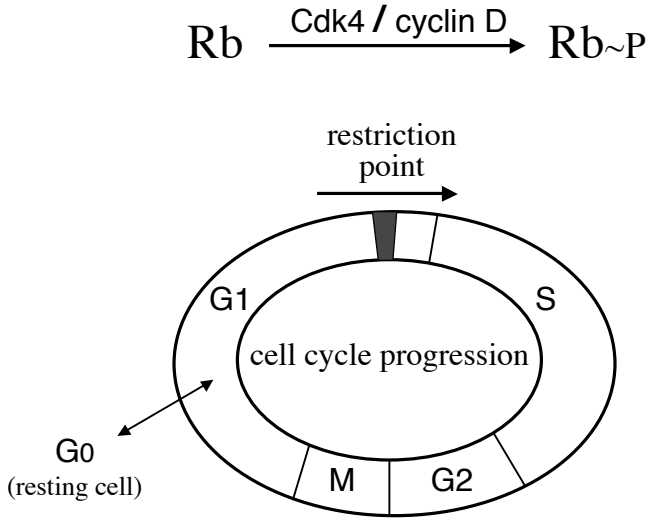
- Li-Fraumeni Syndrome (LFS)
  - caused by germline mutations of p53
  - LFS carriers develop many different forms of cancer
- sporadic cancer
  - often caused by somatic mutations of p53
  - very common in human cancer
  - found in many different forms of cancer

## Tumor suppressor proteins

- proteins encoded by Rb and p53
- the normal functions of these proteins
- mechanisms of tumor suppression
- the Rb and p53 tumor suppressor pathways

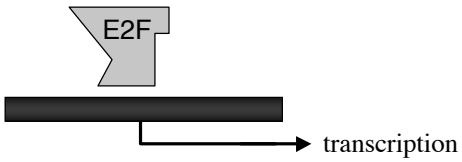
## Phosphorylation of the Rb protein

- The phosphorylation state of Rb changes during normal cell cycle progression.
  - Rb is hypophosphorylated in:
    - G0 (resting cells)
    - early G1 (cycling cells)
  - Rb is hyperphosphorylated in:
    - S phase
    - G2 phase
  - Rb is phosphorylated before the G1/S transition…
    - by an enzymatic complex: CDK4 / cyclin D



## The restriction point (in late G1)

- the major control point of cell cycle progression
- G1/S transition is mediated by the E2F family of transcription factors
- E2F binds the promoters of genes required for cell cycle progression (G1/S transition and S phase).



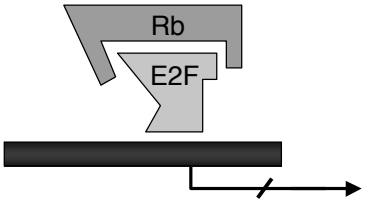

promoter of
S-phase genes

## Some S phase genes regulated by E2F:

| S phase gene | Function |
|---|---|
| • thymidine kinase | nucleotide synthesis |
| • DHFR (dihydrofolate reductase) | " " |
| • DNA polymerase α | DNA synthesis |
| • ORC1 | " " |
| • histone H2A | chromosome assembly |
| • cyclin E | cell cycle progression |
| • cyclin A | " " " |

## Resting cells and early G1 phase cells

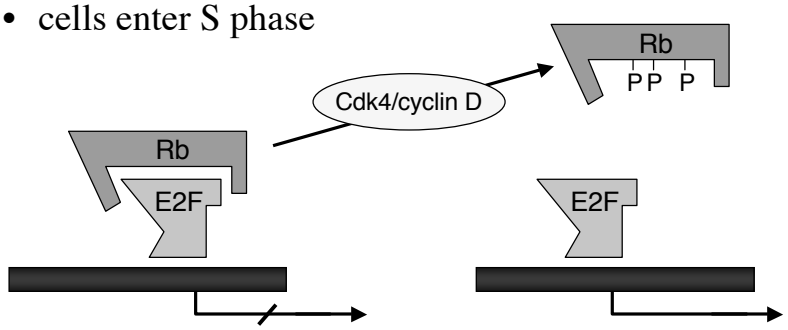
- hypophosphorylated Rb binds promoter-bound E2F
- Rb inactivates transcription by E2F
- S phase genes are repressed
- G1/S transition is blocked




S-phase genes
repressed

## restriction point

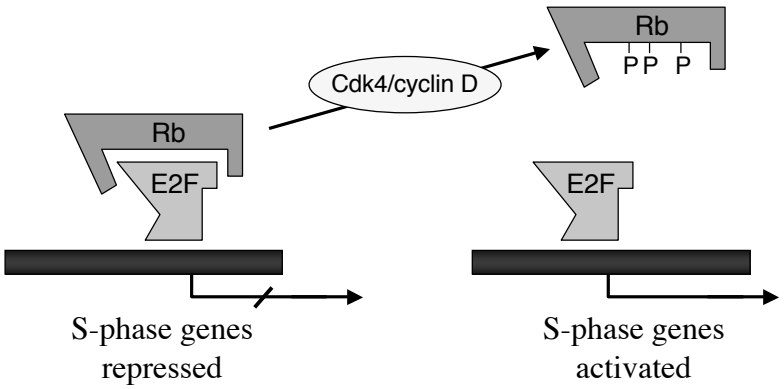
- CDK4/cyclin D phosphorylates Rb in the “pocket”
- hyperphosphorylated Rb dissociates from E2F
- E2F activates transcription of S phase genes
- cells enter S phase

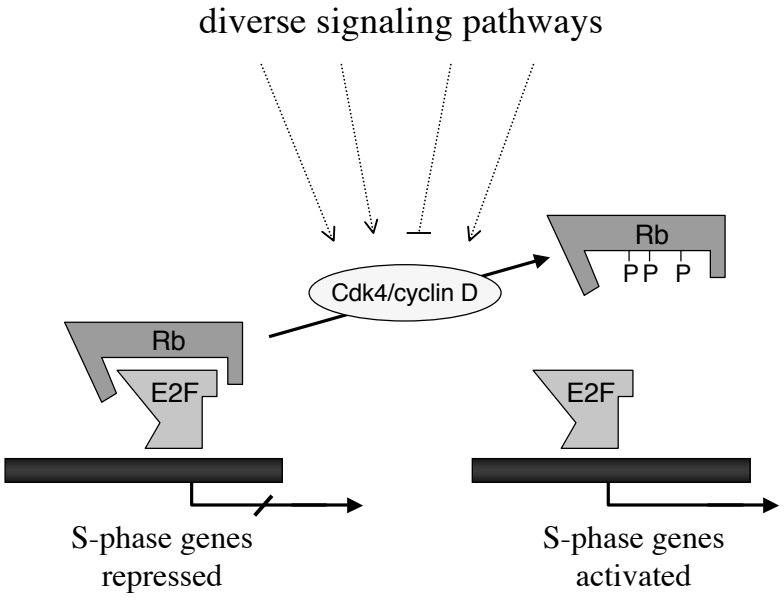



S-phase genes
repressed

S-phase genes
activated

- In normal cells, phosphorylation of Rb by the CDK4/cyclin D kinase is a highly regulated
- Focal point for the major signal transduction pathways that control normal cell growth



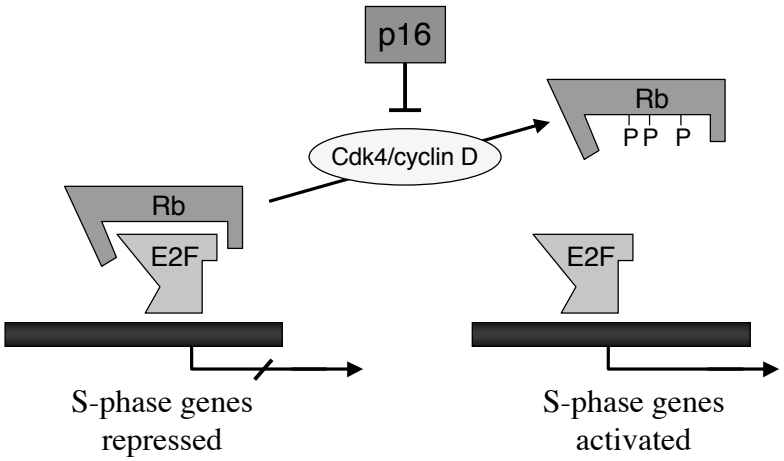
## The function of Rb

- hypophosphorylated Rb serves to restrain the proliferation of normal cells.
- regulated phosphorylation of Rb allows normal cells to proliferate at the correct time and place.
- therefore, imagine the consequences of losing normal Rb function…
  - deregulation of E2F (and the G1/S transition)!
- how might Rb become inactivated in cancer?

## Inactivation of Rb function in tumors (leaving E2F unregulated)

- Direct inactivation:
  - Rb gene deletion (occurs in retinoblastoma)
  - point mutations in the Rb pocket (in retinoblastoma)
  - occupancy of the Rb pocket by early proteins of DNA tumor viruses
    - human papilloma virus (HPV), an etiological agent in human cervical carcinomas
    - HPV encodes two proteins required for tumorigenesis
    - E7 binds the pocket of hypophosphorylated Rb
    - Deregulation of E2F (and the G1/S transition)

# The Rb tumor suppressor pathway




## Indirect inactivation of Rb function in tumors

- overexpression of cyclin D1
  - breast cancer, B cell lymphoma
- loss of p16, an inhibitor of Cdk4
  - many human cancers
- inherited point mutation in Cdk4 that renders it insensitive to inhibition by p16
  - familial melanoma

» Inactivation of the Rb pathway occurs in most, if not all, human tumors!

## Normal functions of the p53 protein

- p53 polypeptides are very unstable in normal cells (1/2 life of ~30 minutes)
- the cellular response to genotoxic stress
  - DNA damage by UV light, ionizing radiation, chemical carcinogens, errors in replication, etc.)
  - induction of certain signal transduction pathways
- post-translational modifications of p53 polypeptides:
  - especially, phosphorylation and acetylation
  - stabilize p53 (1/2 life of ~150 min.), leading to higher steady-state levels
  - increase the transcriptional activity of p53

## consequences of p53 activation

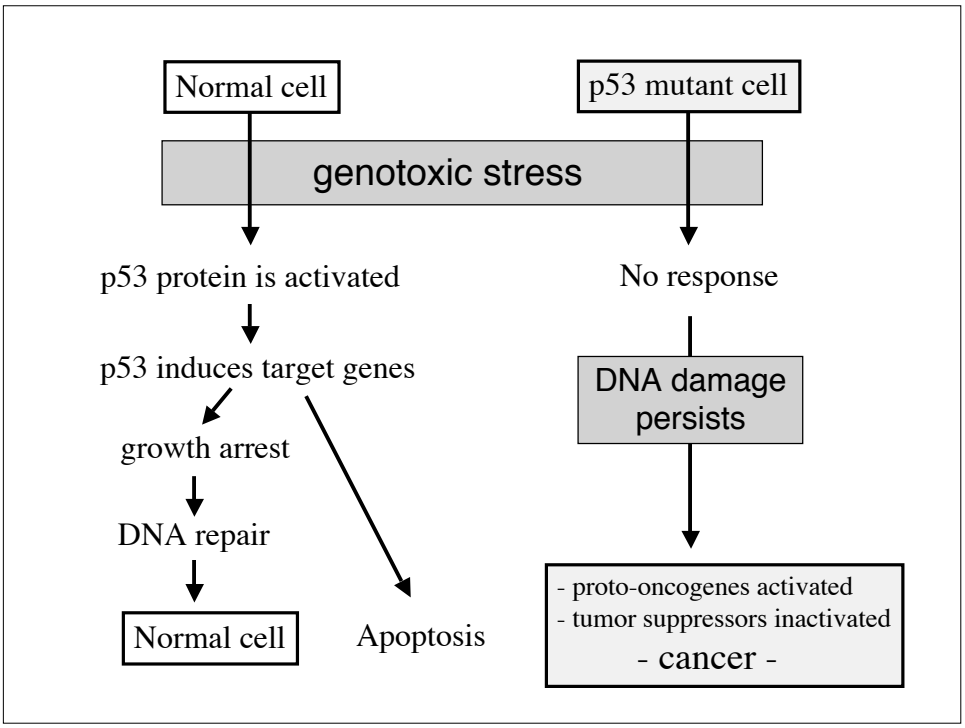
- The transcriptional activity of p53 induces a cellular response, the nature of which is dependent on various factors, including the cell type.
  - p53 induces G1 arrest (and DNA repair) in:
    - normal fibroblasts
    - certain epithelial cells
  - p53 induces apoptosis in:
    - thymocytes

## p53 induction of cell cycle arrest or apoptosis

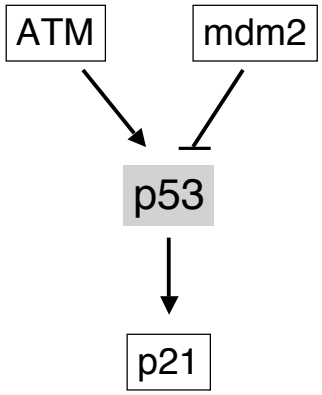
- in either case, replication of damaged DNA ceases
- prevents accumulation of oncogenic mutations
- In essence, p53 suppresses tumor formation by maintaining the integrity of the genetic material in cells subjected to genotoxic stress.

## Transcriptional targets of p53

- p21 CDK inhibitor
  - ➜ G1 and G2 arrest in fibroblasts
- 14-3-3σ
  - ➜ G2 arrest in epithelial cells
- PUMA
  - ➜ promotes apoptosis in thymocytes, fibroblasts, neurons
- p53R2 nuclear ribonucleotide reductase
  - ➜ required for DNA repair
- p48 subunit of the XPA complex
  - ➜ required for nucleotide excision repair
- etc…




# The p53 tumor suppressor pathway




» Inactivation of the p53 pathway occurs in most, if not all, human tumors!