

Genes Supresores de Tumor

Dra. María Marcela Barrio
Centro Investigaciones Oncológicas - FUCA

Transformación neoplásica

- ✓ **Resulta de la acumulación de daños genéticos, típicamente involucrando por lo menos 6 mutaciones**
- ✓ **Genes mutados durante la transformación neoplásica:**
 - 1- Mutación de Proto-oncogenes, resultando en un estímulo proliferativo para la célula (c-erb-B)**
 - 2- Inactivación de genes supresores de tumor (p53)**
 - 3- Mutación de genes que regulan la apoptosis (bcl-2)**
 - 4- Mutación de genes que codifican para enzimas de reparación del ADN**

“Gatekeeper” genes

- **The “gatekeeper” gene monitor cell proliferation and death. They include:**
 - **“oncogenes” which are cell growth promoters, where dominant mutations lead to uncontrolled growth, and resistance to apoptosis.**
 - **“tumor suppressor” genes which are negative regulators of cell growth, where recessive mutation can lead to uncontrolled cell growth (e.g., p53).**

“Caretaker” genes

- **The “caretaker” genes include genes that regulate DNA repair and chromosome segregation in mitosis. Mutation of these genes can cause abnormal “gatekeeper” function because of mutation or abnormal gene content. The “caretaker” genes include:**

- **DNA repair genes, whose mutations lead to genetic instability (more mutations) , and**
- **genes that regulate chromosome segregation, whose mutations lead to abnormal chromosome content, or chromosomal instability.**

Tumor suppressor genes

- **First discovered in 1960s by Henry Harris.**
- **Harris fused tumor cells with normal cells and discovered some of the hybrid cells were normal.**
- **Harris hypothesized that the normal cells contained gene products that suppressed uncontrolled cell proliferation.**
- **Some cancers show deletions of specific sites (tumor repressor genes) that normally inhibit cell growth and division.**

e.g., breast cancer, colon cancer, lung cancer

- **Two mutations (one on each allele) are required to inactivate tumor suppressor genes.**

Tumor suppressor genes

Definition:

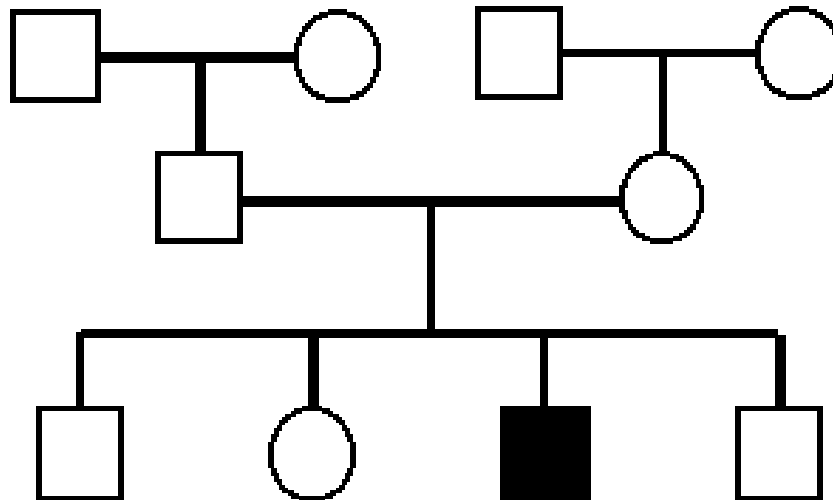
1- its function is lost in the development of the tumor

2- its in vivo inactivation enhances initiation, growth, or progression of a tumor

Sporadic and familial (Mendelian) forms of cancer

Knudson's two-hit hypothesis

Sporadic



Normal
tumor
suppressor
gene

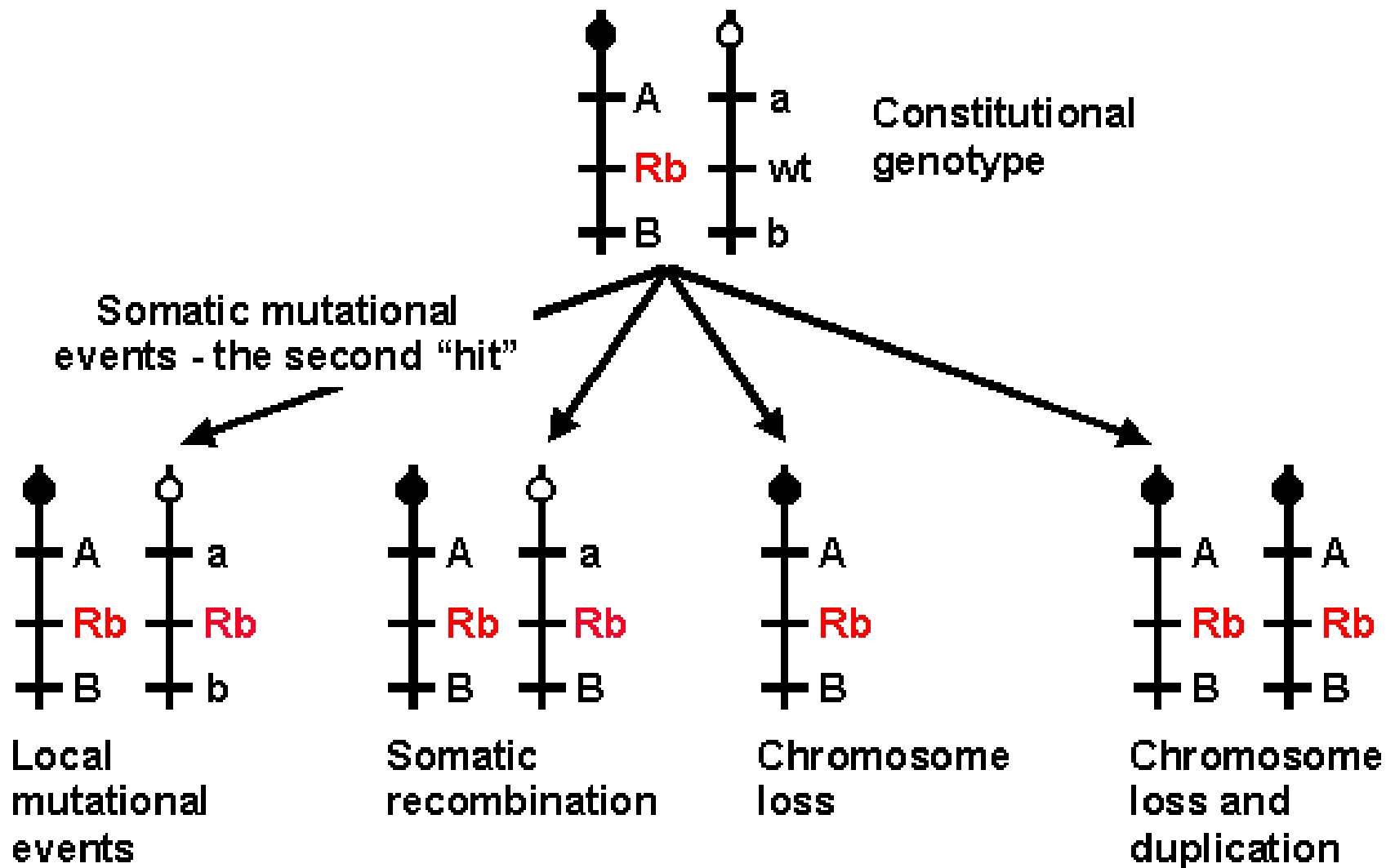
← Somatic
mutation
in one allele

← Somatic
mutation
in other allele

↓
Single tumors,
unilateral,
later-onset

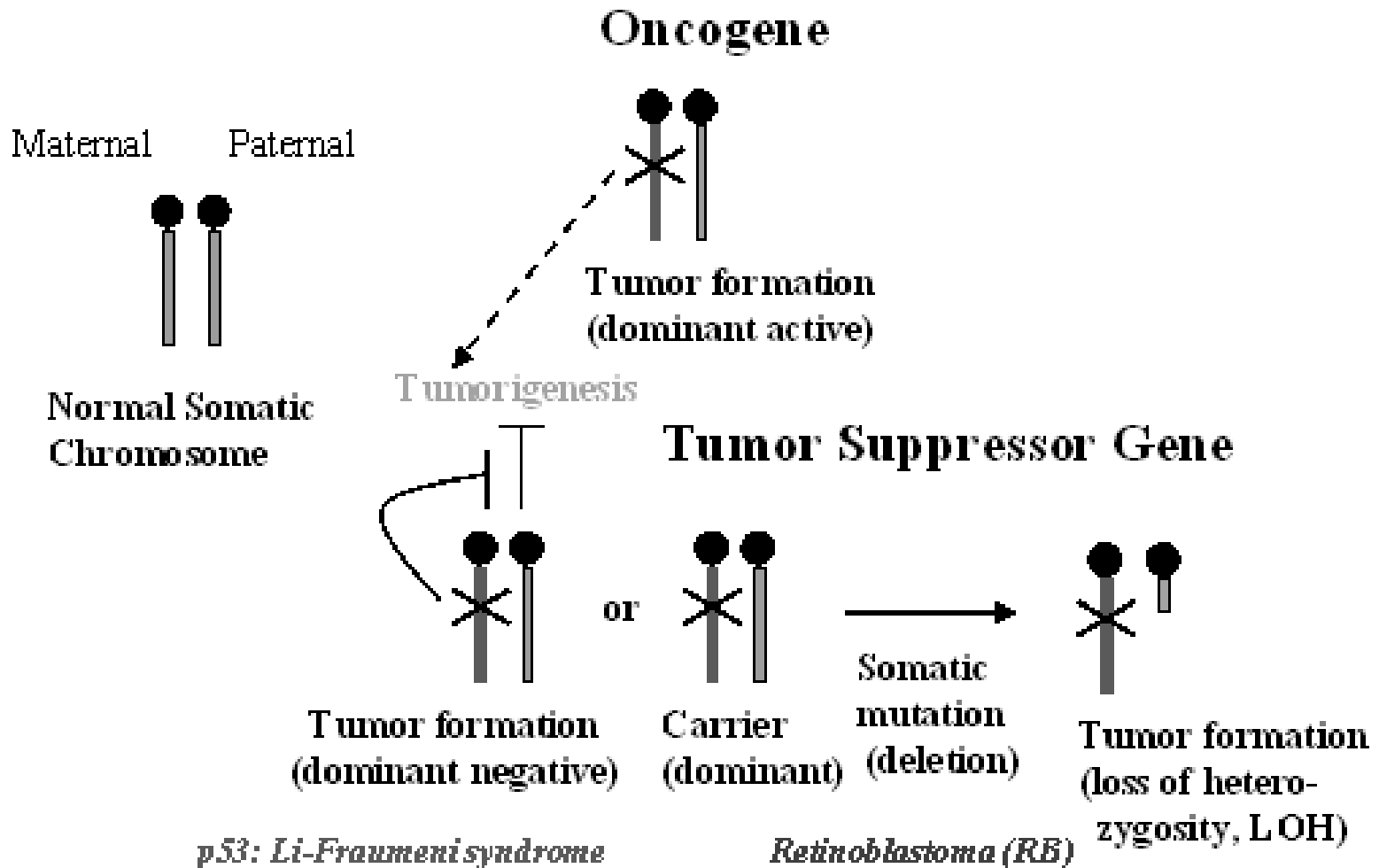
- two mutations (two hits) are required for loss of tumor suppressor function

Chromosomal mechanisms that could lead to loss of function due to loss of heterozygosity



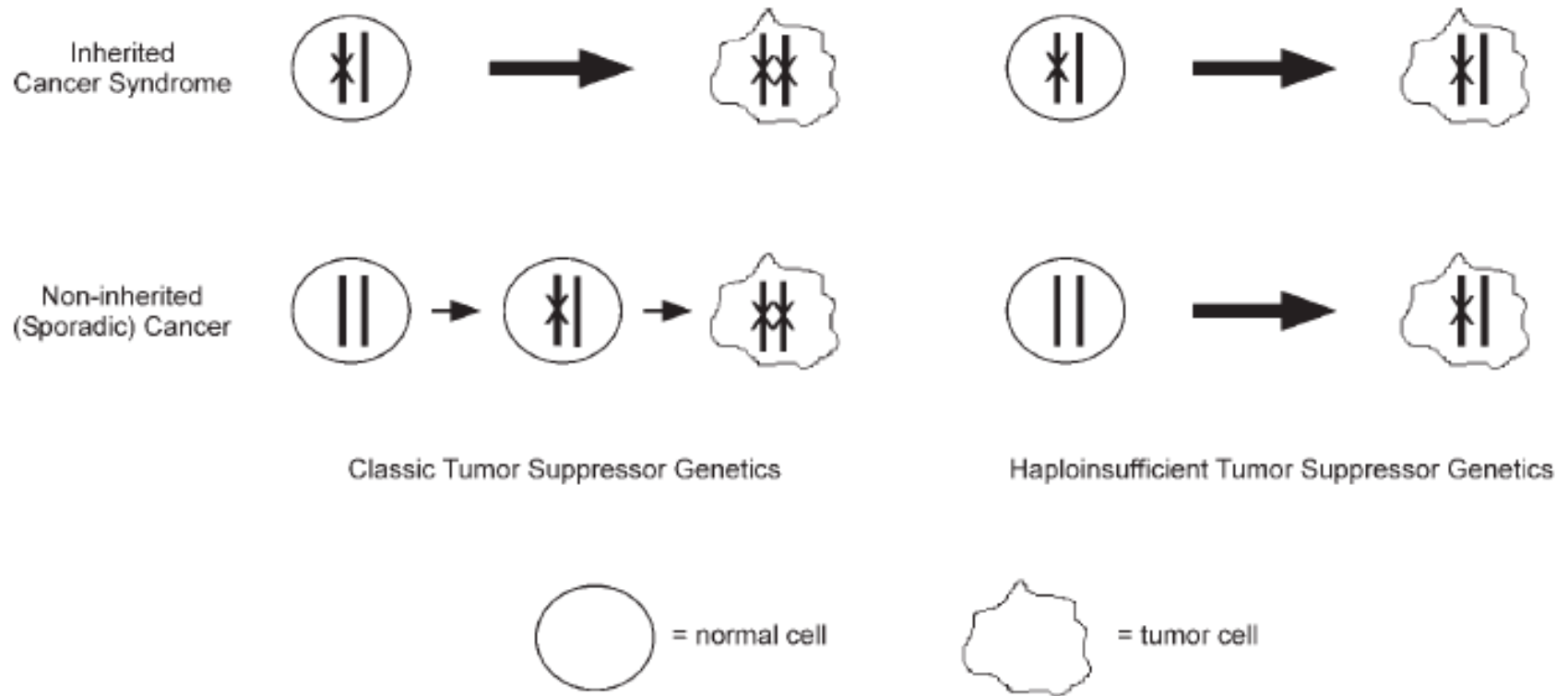
Tumor genotypes in retinoblastoma

Theory of Tumor-prone Autosomal Dominant Mutations (Knudson's "two-hit" theory)



A mutation in one of two copies (alleles) of an oncogene may be sufficient to cause the cell to lose growth control, both copies of a tumor suppressor gene must be knocked out to induce the same effect

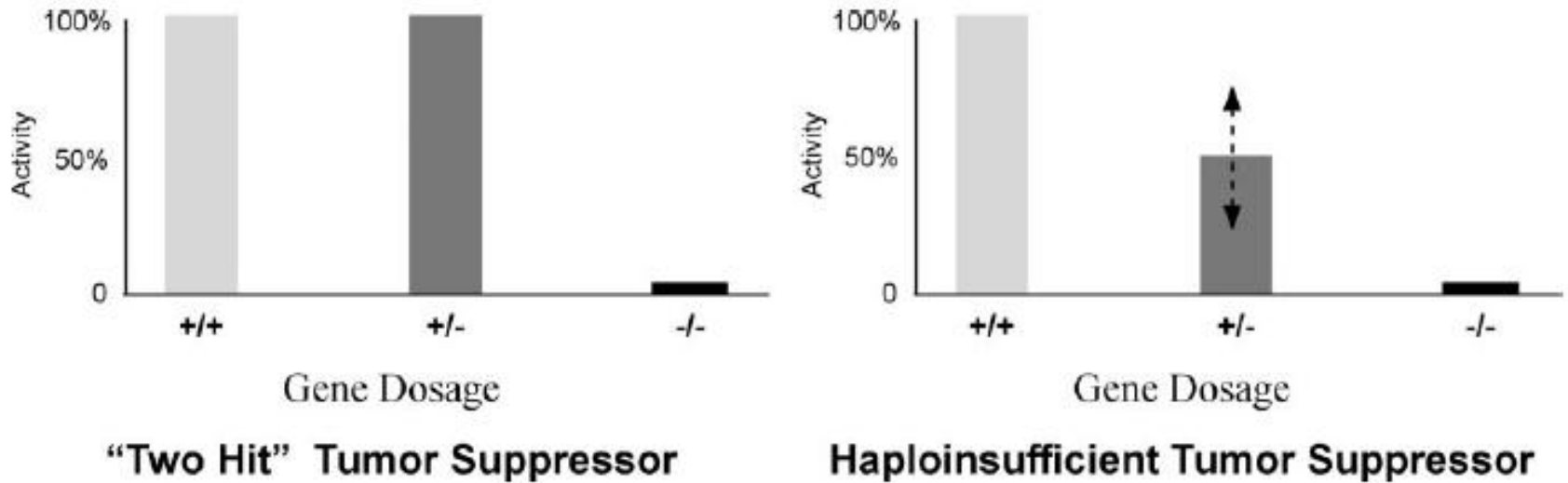
Inactivation of Tumor Suppressor Genes.



Classic tumor suppressor genes are inactivated via 'two hits'. In the case of inherited cancer susceptibility, one of these 'hits' is acquired in the germline with the second 'hit' being acquired somatically during tumor development.

Haploinsufficient tumor suppressor genes are compromised by a single 'hit', obviating the need to sustain 'two hits' during the course of tumor development.

Tumor suppressor activity versus gene dosage



Wild type (+/+) activity represents 100% of diploid gene function and true null (-/-) represents complete loss of functionality.

Haploinsufficient tumor suppressors exhibit a continuum of activity based on gene dosage with even 50% reduction sufficient for phenotypic manifestation, i.e. accelerated tumorigenesis.

While some genes may be less dosage-sensitive than others (in which a true threshold of close to 0% of normal gene product is required in order to detect a phenotype), we predict that most genes will be sensitive to dosage with some threshold that varies on a continuum between 0 and 100%.

Definition of Penetrance:

Complete Penetrance: Every individual who inherits a mutated gene develops the related disease

Ex: Huntington disease



100% Incidence of Huntington Disease



100% Normal Individuals

Incomplete Penetrance: Less than 100% of the carriers of the mutation develop the disease

Ex: BRCA1 Mutation:

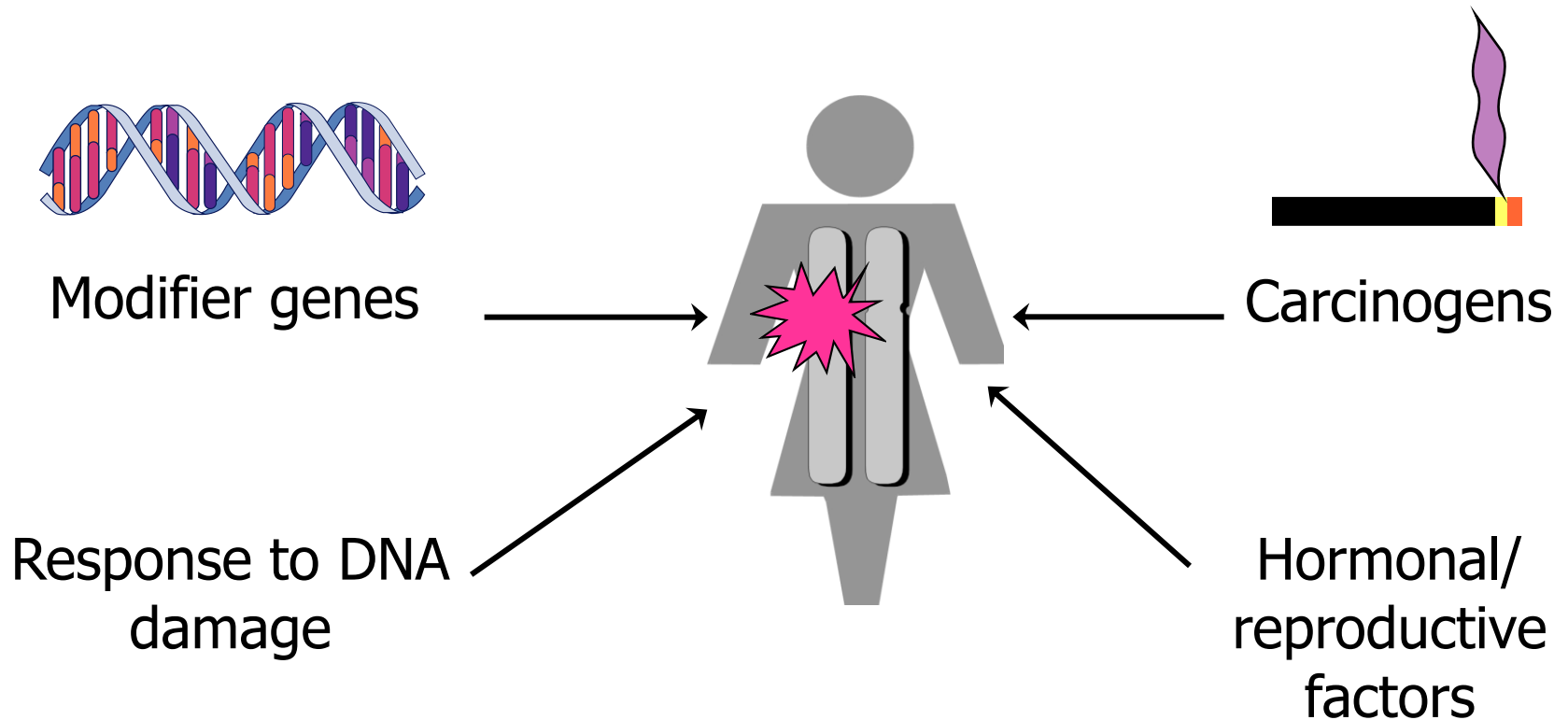


Greater probability of developing breast cancer, but another mutant factor (from environment, other genes) is also needed



May develop sporadic breast cancer

Factors Affecting Penetrance



Not everyone with an altered gene develops cancer

Loss of Heterozygosity

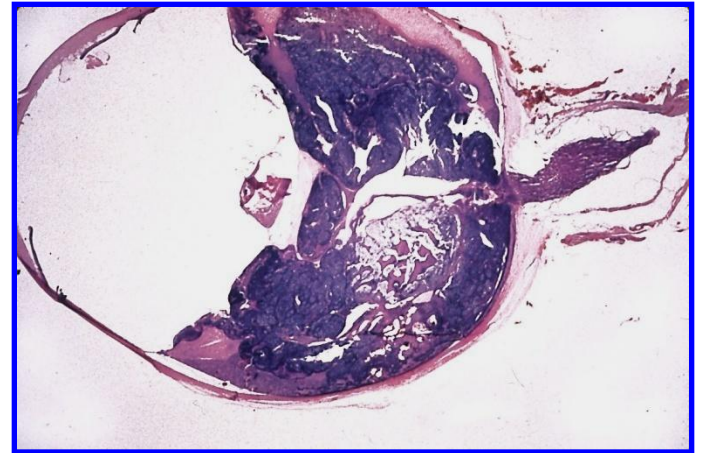
- oncogene activation at one allele --> e.g. activity increase 50 fold --> provide growth advantage --> second allele activation --> activity increase two fold further --> probably provide no further selection advantage. $2 \Rightarrow 51 \Rightarrow 100$ activity change
- tumor suppressor gene inactivation at first allele --> activity decrease half --> possibly provide no growth advantage --> inactivation of second allele --> complete loss of activity --> selection favored. $2 \Rightarrow 1 \Rightarrow 0$ activity change.

Retinoblastoma tumor suppressor gene (Rb)

- ✓ **Mapped to gene chromosome 13 and sequenced.**
- ✓ **180 kb; codes a 4.7 kb mRNA that produces a 928 amino acid nuclear phosphoprotein, pRB.**
- ✓ **pRB is expressed in every tissue that has been examined and regulates the cell cycle.**
- ✓ **Retinoblastoma tumor cells possess point mutations or deletions.**
- ✓ **In hereditary retinoblastoma, second RB mutation often is identical to the inherited one (a possible example of gene conversion).**

Retinoblastoma

- ✓ Rare malignant tumor arising from immature neurons in the retina
- ✓ About 40% of cases are associated with a germline mutation (60% are sporadic)
- ✓ 5-10% of germline cases are inherited germline mutations
- ✓ 20-30% are NEW germline mutations



Inherited germline Rb

- ✓ **Affected child inherits one defective Rb allele, together with one normal gene**
- ✓ **If the remaining normal Rb allele is inactivated by deletion or mutation, the loss of its suppressor function leads to development of a retinoblastoma**
- ✓ **Thus, susceptibility to Retinoblastoma is inherited in a dominant fashion**
- ✓ **At the cell level TSG is recessive to show the phenotype**

Sporadic retinoblastoma

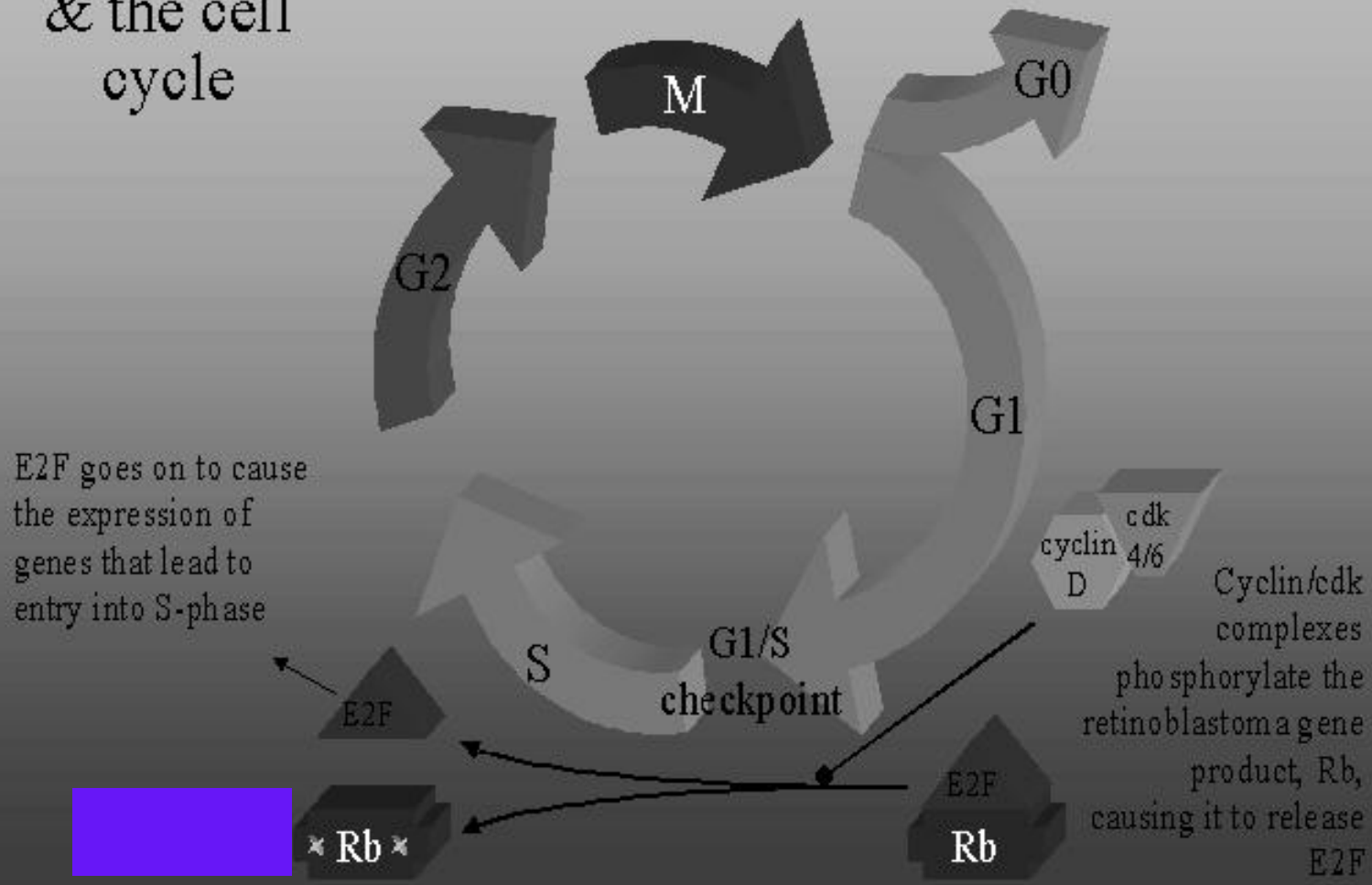
- ✓ **Child is homozygous for two normal Rb Alleles**
- ✓ **Both alleles must be inactivated in a retinal cell for disease to develop**
- ✓ **Sporadic disease is rare (1/30,000)**

Retinoblastoma

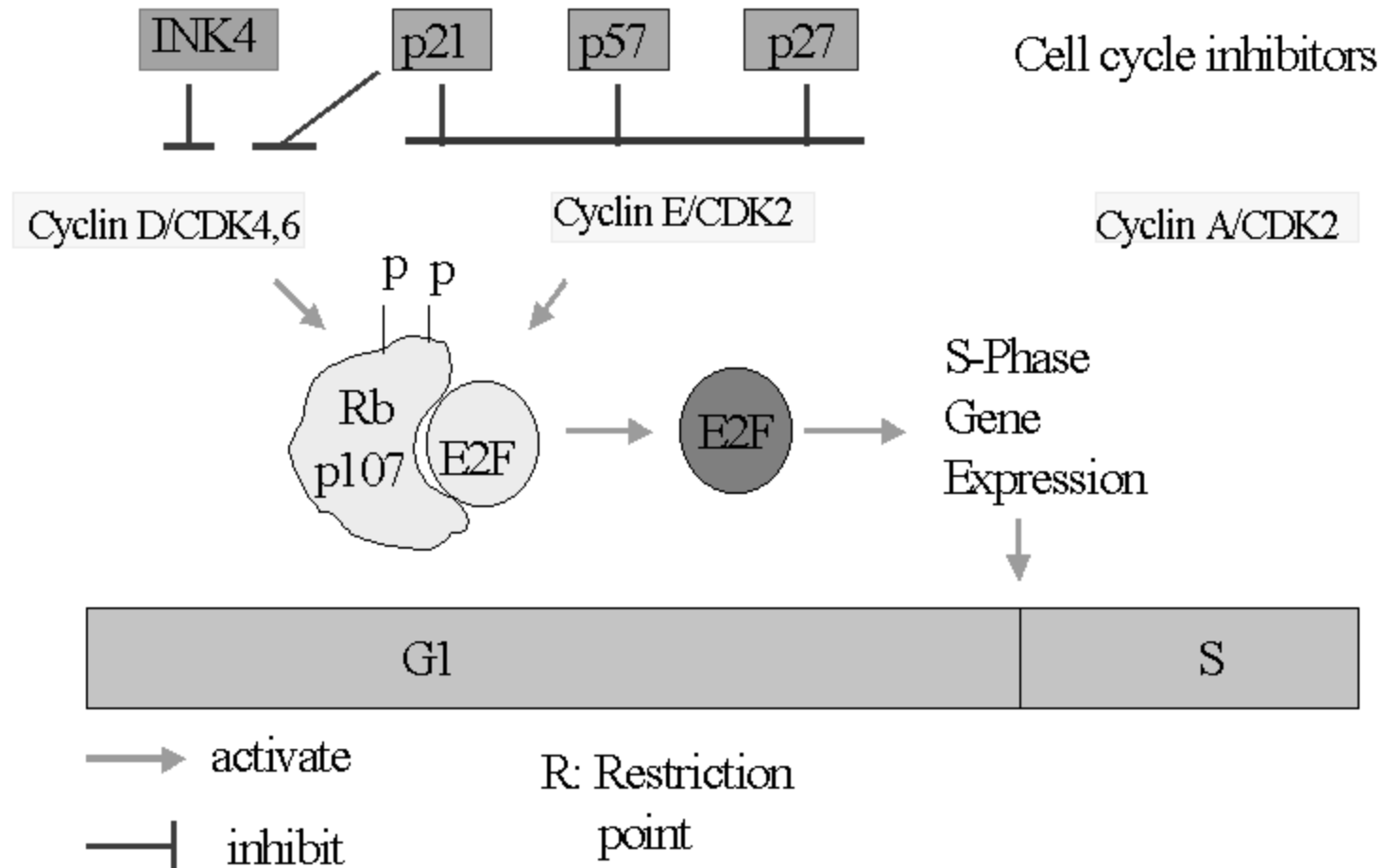
- ❖ **pRb; tumor suppressor**
- ❖ **deleted in retinoblastoma**
 - plays a pivotal role in mammalian cell cycle**
 - represses transcription - E2F**
 - regulated by phosphorylation**
- ❖ **-underphosphorylate: growth suppressive**
- ❖ **-hyperphosphorylated: growth promoting**

Oncogenes & the cell cycle

The G1/S checkpoint is controlled by phosphorylation of Rb



S-Phase Entry



E2F regulates genes required for S-phase



Target genes:

Genes encoding proteins involved in DNA synthesis

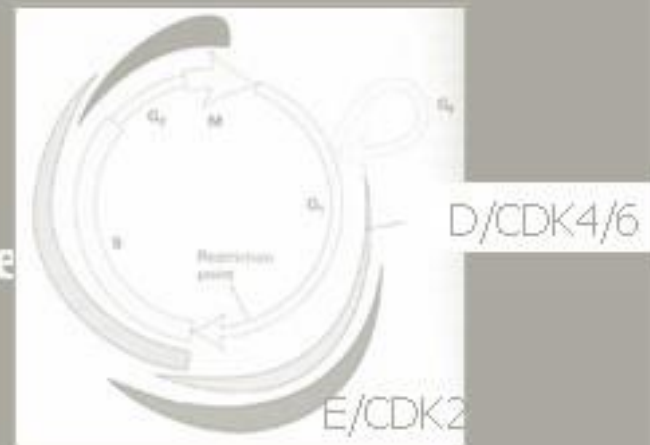
- DNA polymerase α
- thymidine kinase
- thymidylate synthase
- ribonucleotide reductase

DNA repair proteins

-RAD51

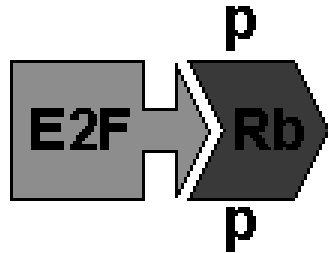
Cell cycle regulators

- Cdk2 and cyclins A and E.



Function of the Rb protein

Growth suppression

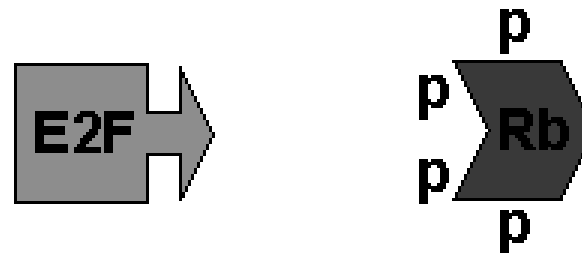


- E2F is a transcription factor that mediates growth-dependent activation of genes required to make the transition into and through S phase

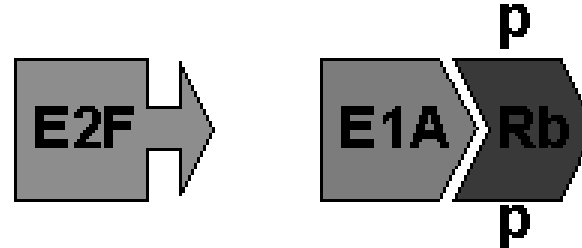
- Rb binds and inactivates E2F under conditions of growth suppression

- There are several ways to alleviate growth suppression resulting in controlled or uncontrolled cell growth

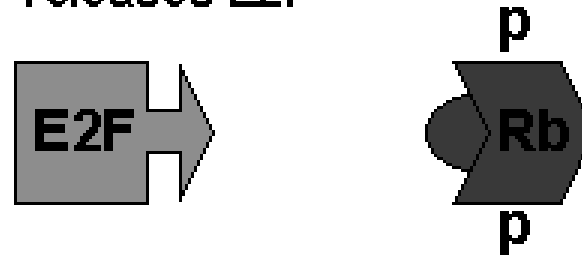
Relief of growth suppression



G1 phase phosphorylation releases E2F



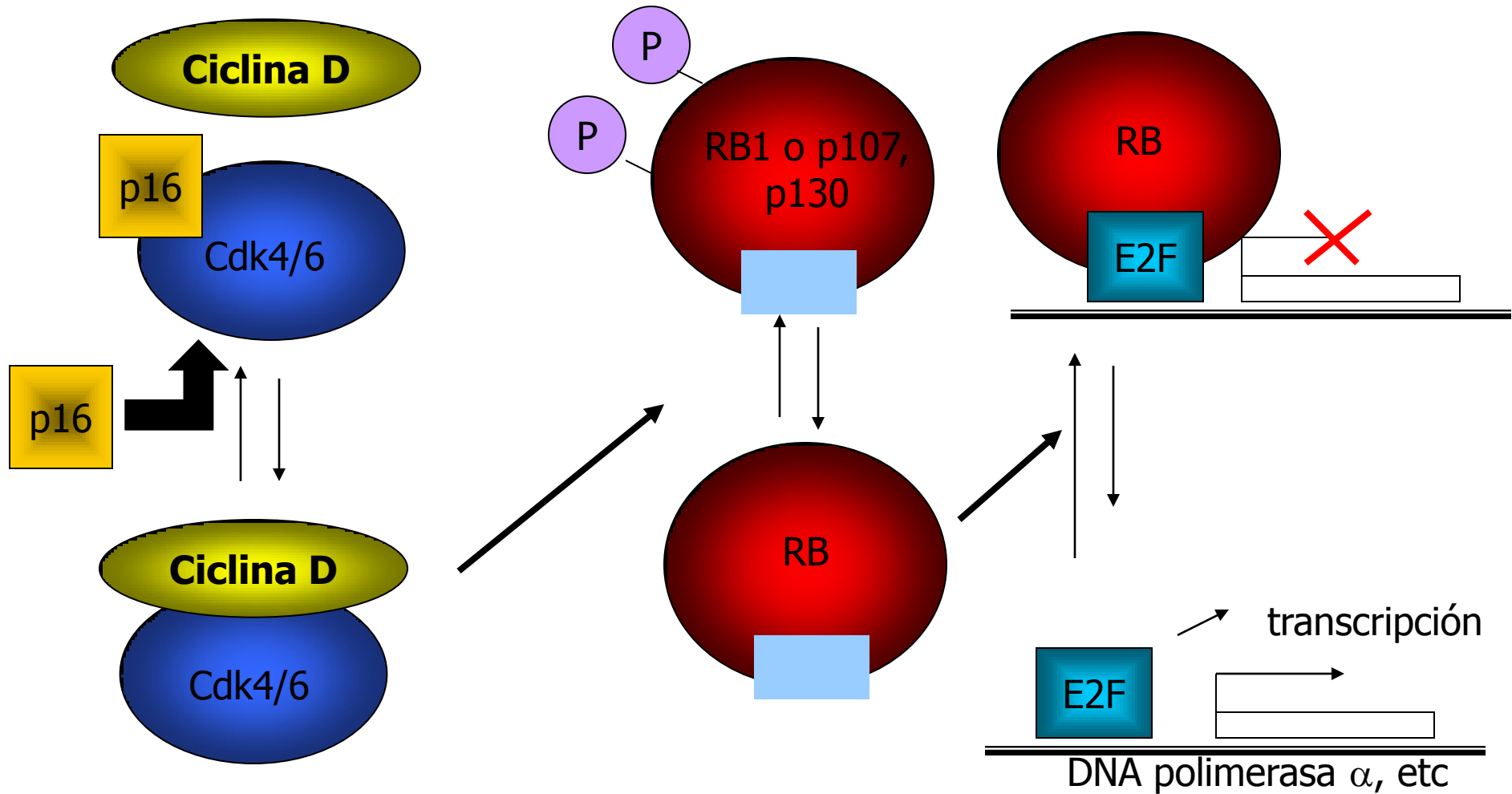
Adenovirus E1A oncoprotein binding releases E2F



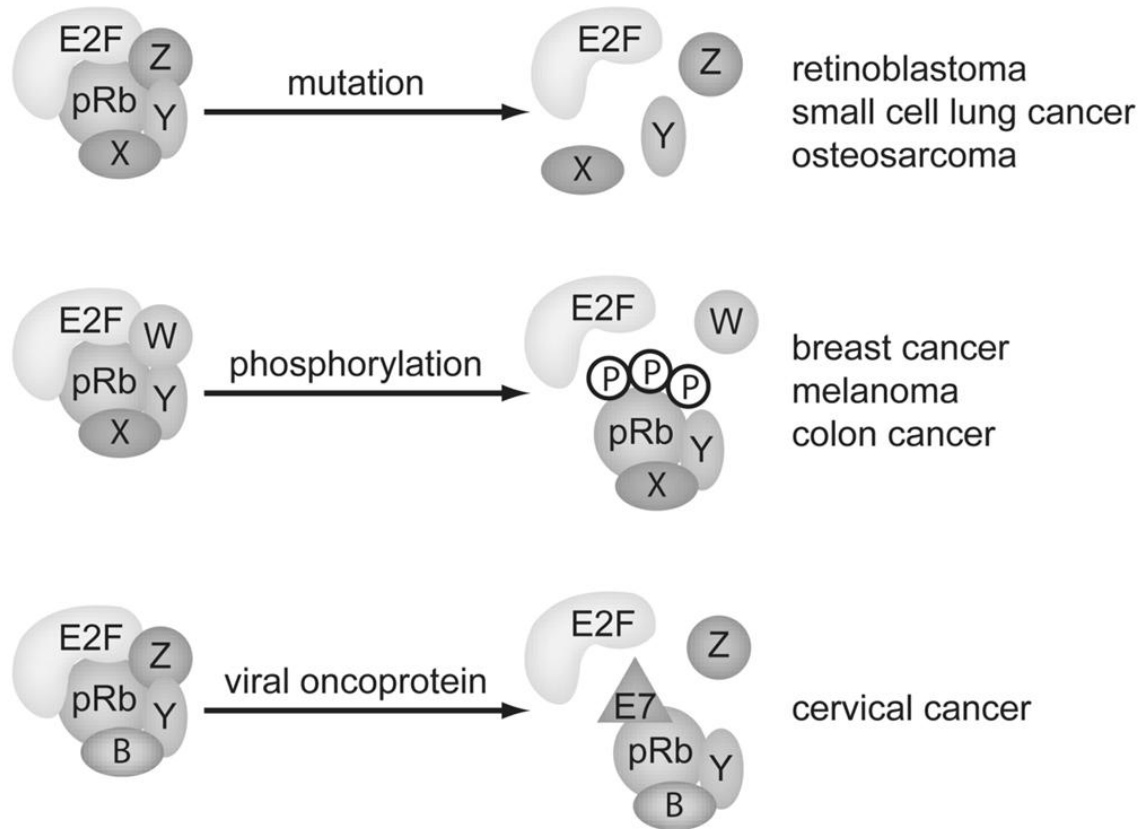
Gene mutation affecting binding pocket releases E2F

Inhibidores de Cdk4 y Cdk6 (INK4)

- p15, p16, p18 y p19 → bloquean actividad de las quinasas dependientes de ciclina D produciendo el arresto del ciclo celular en G1.



Mechanisms of pRb inactivation in human cancer



-Inactivation by deregulated pRb phosphorylation is common in a distinct subset of human cancers, but Rb1 mutation is rarely observed in this subset.

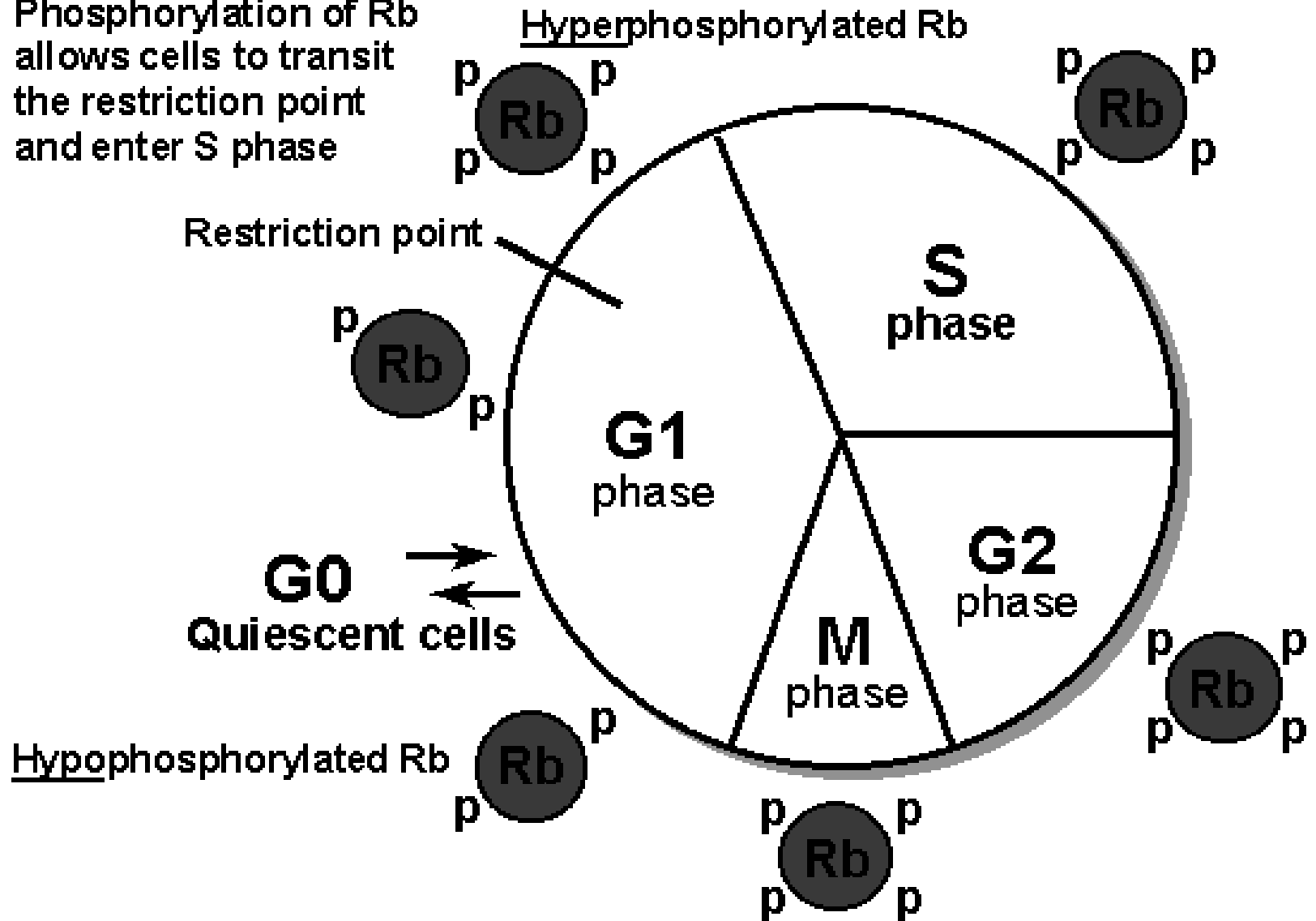
- The model proposes that each mechanism of inactivation has different molecular and functional consequences.

-Deregulated pocket protein phosphorylation is proposed to abrogate some protein interactions but not others.

- Different cell types may select for different mechanisms of pRb inactivation because the molecular consequences are more favorable for Carcinogenesis.

Cell-cycle dependent phosphorylation of Rb

Phosphorylation of Rb allows cells to transit the restriction point and enter S phase



Tumor suppressor genes

<u>Gene (locus)</u>	<u>Function</u>	<u>Disorders in which gene is affected</u>	
		<u>Familial</u>	<u>Sporadic</u>
DCC (18q)	cell surface interactions	unknown	colorectal cancer
WT1 (11p)	transcription	Wilm's tumor	lung cancer
Rb1 (13q)	transcription	retinoblastoma	small-cell lung carcinoma
p53 (17p)	transcription	Li-Fraumeni syndrome	breast, colon, & lung cancer

Genes supresores en tumores humanos

GEN	Localización	Cánceres asociados
RB	13p14	Retinoblastoma, osteosarcoma, leucemias linfoides pulmón, mama ovario, próstata, vejiga.
p53	17p13	Li-Fraumeni síndrome, mama, próstata, pulmón, colon, vejiga, hígado, linfomas/leucemias, cerebro, adrenal.
WT1	11p13-15	Tumor de Wilm's (cáncer renal infantil).
NF-1	17q11.2	Neurofibromatosis, neurofibromas.
NF-2	22q	Meningioma, mama, colon.
VHL	3p26	Células renales. Hemangioblastoma de cerebro.
APC	5q21	Colon (FAP)
MSH-2	2p16	Colon (HNPCC, proximal colon), endometrial, estómago.
MLH-1	3p21.3-23	Páncreas, vejiga, ovario, mama.
PMS 1	2q31-33	
PMS 2	7p22	
MTS1(p16) MTS2(p15)	9p21	Familial melanoma, glioblastoma, T-ALL, mesotelioma. NSCLC, vejiga.
BRCA-1	17q21	Ca. de mama temprano, ovario, próstata, colon.
BRCA-2	13q	Ca. de mama temprano (Ca.de mama masc.) CIO-FUCA

E2F regulates genes required for S-phase



Target genes:

Genes encoding proteins involved in DNA synthesis

- DNA polymerase α
- thymidine kinase
- thymidylate synthase
- ribonucleotide reductase

DNA repair proteins

-RAD51

Cell cycle regulators

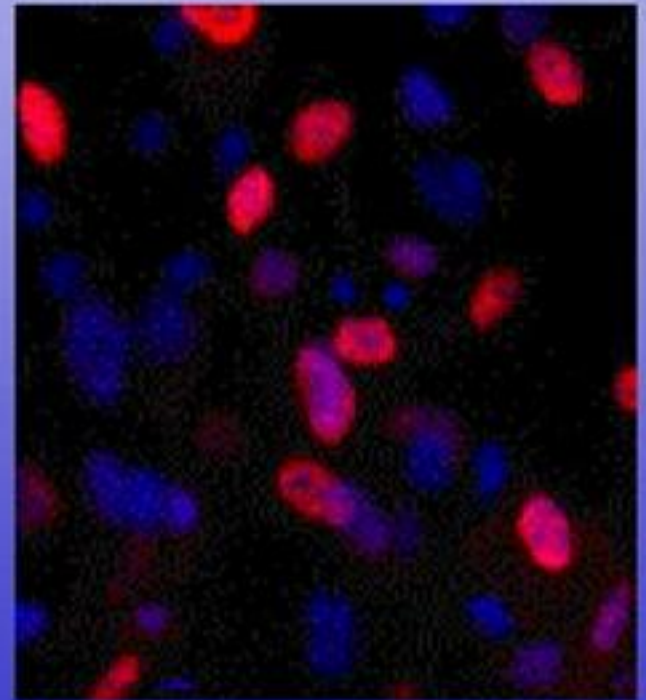
- Cdk2 and cyclins A and E.



P53 tumor suppressor gene

P53 as tumor antigen and oncogene

- P53 was initially identified as a tumor specific nuclear antigen of molecular weight 53kDa
- isolation of the gene from tumor cells yielded an *oncogene*: when it was transfected into normal cells it could transform them (together with ras)
- a variety of different sequences were identified raising the question of which sequence represented the wild-type



P53 as tumor suppressor gene

- When the p53 gene was isolated from the genomic DNA of normal cells, it was discovered that the tumor derived gene was mutated - still consistent with the characteristics of an oncogene
- when the wild-type p53 gene was transfected into tumor cells it stopped their growth, a key characteristic of a *tumor suppressor gene* - wild-type dominant over mutant
- genetic analysis of p53's genomic region 17p suffered loss of heterozygosity as a follow up to mutation

P53 central to cancer

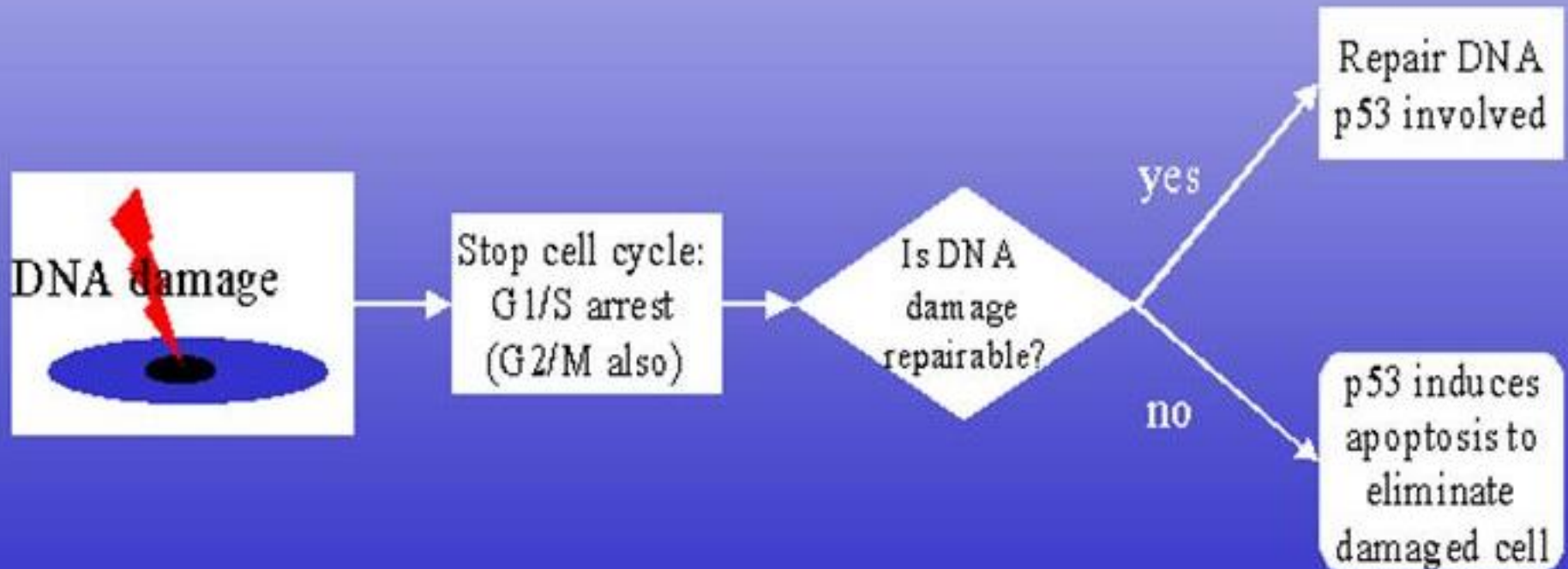
- **p53**: the most significant cancer gene has properties of both an oncogene and a tumor suppressor gene
- it is mutated in about half of all human cancers
- A note on viral tumor antigens:
 - DNA tumor viruses (Adenovirus, SV40, polyoma virus) all encode proteins that interact with cellular proteins in the viruses' bid to take over the cell's metabolism
 - in all viruses one protein binds and inactivates p53, further evidence that it is central to the biology of cancer (the other popular cellular protein target is Rb)

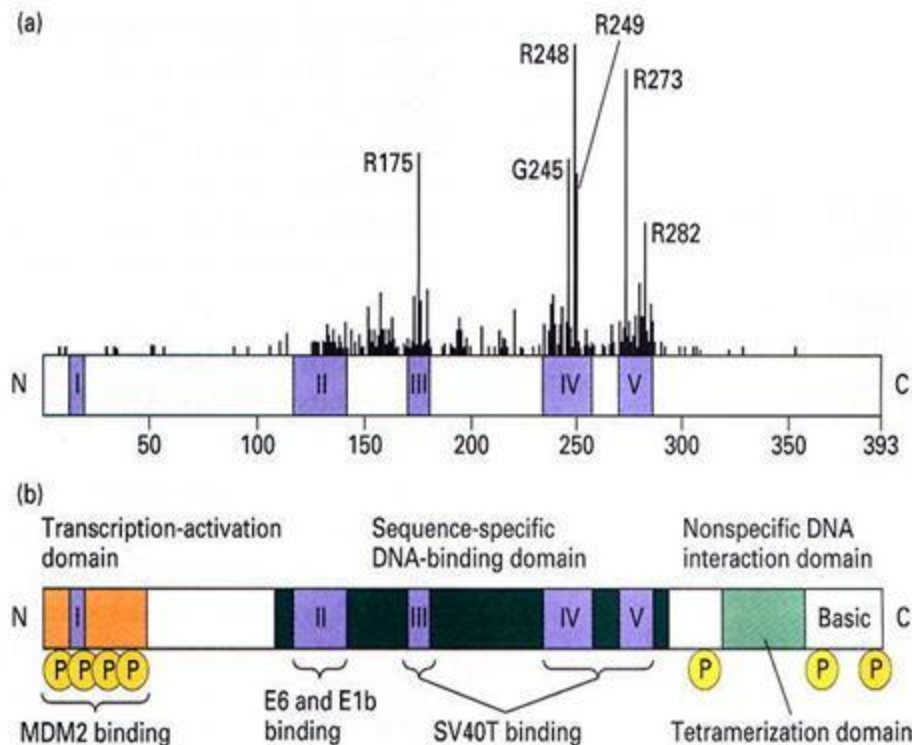
p53 tumor suppressor gene

- **53 kDa nuclear phosphoprotein**
- **the most commonly altered gene identified in human cancers to date**
- **functions as a tumor suppressor when normal and a dominant oncogene when mutant**
- **many mutations result in a prolonged protein half-life and/or overexpression**

Functions of p53: simple

- One way of thinking about p53 is that it is a “guardian of the genome”: it protects the cells DNA from damage



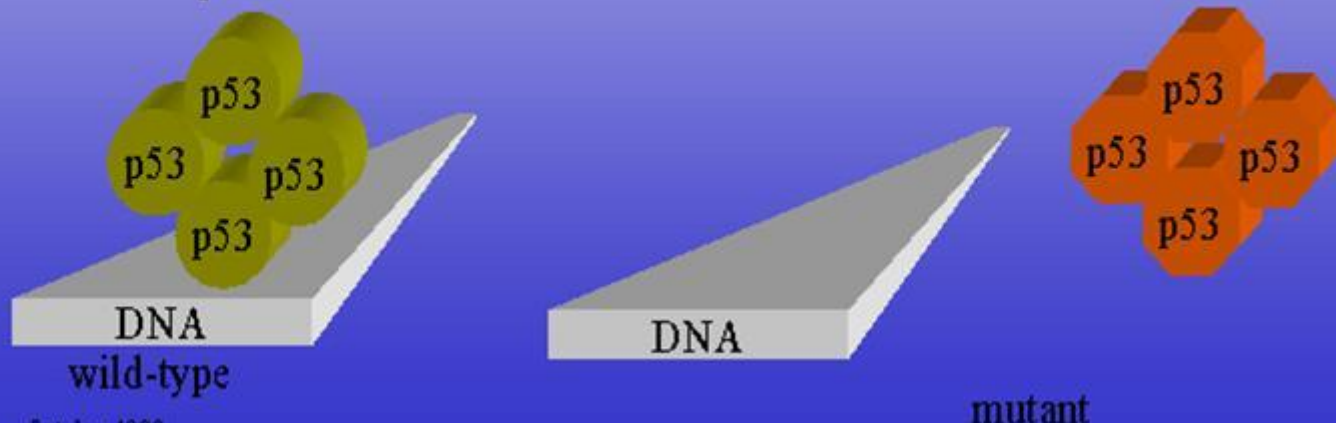


▲ FIGURE 24-21 The human p53 protein. (a) Mutations in human tumors that inactivate the function of p53 protein. Hatched boxes represent sequences highly conserved in evolution. Vertical lines represent the frequency at which mutations are found at each residue in various human tumors. These mutations are clustered in conserved regions II–V. (b) Structural organization of the p53 protein. Phosphorylation by various kinases at the sites

indicated by **P** stabilize p53. MDM2 protein binds at the indicated site and represses transcription activation by p53 as part of the normal control of p53 function. The activity of p53 also is inhibited by binding of viral proteins such as E6 from human papillomavirus and E1b from adenovirus. [Adapted from C. C. Harris, 1993, *Science* **262**:1980; and L. Ko and C. Prives, 1996, *Genes & Develop.* **10**:1054.]

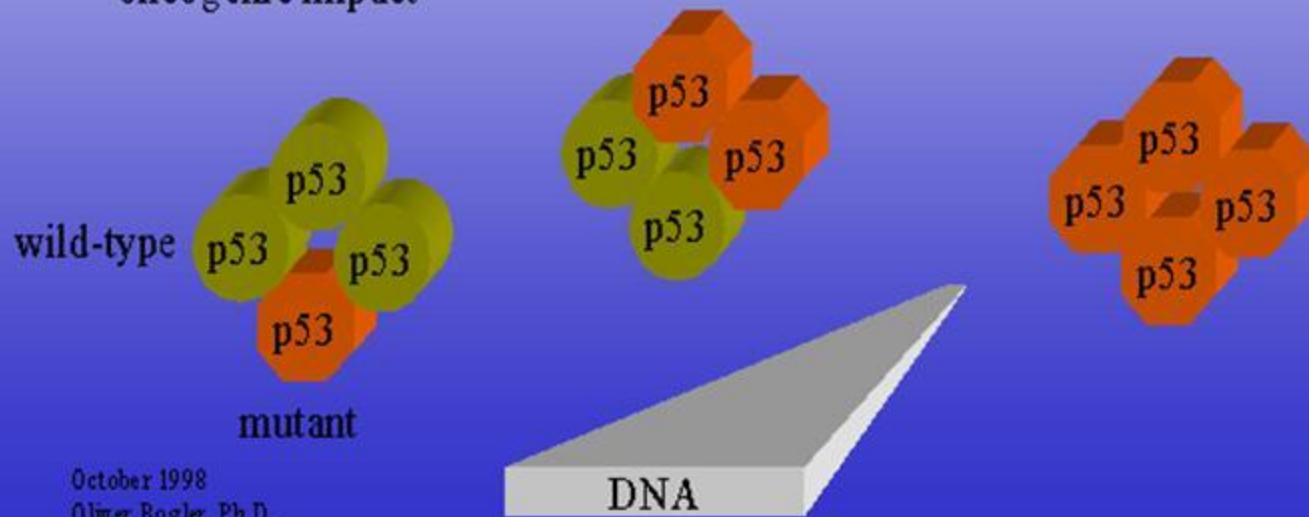
P53 mutations: loss of function

- 80% point mutations, 20% truncations
- almost all mutants have an abnormal structure, leading to loss of DNA binding capability and so loss of normal function
- loss of wild-type p53 function leads to loss of cell cycle arrest in response to DNA damage ie tumor suppression
- loss of p53 function leads to increase in mutation rate



P53 mutations: dominant negative

- P53 acts as a tetramer
- heterotetramers containing mutant p53 are non functional, and so the presence of mutant p53 can prevent wild-type from functioning properly
- explains why introduction of mutant p53 into normal cells has oncogenic impact

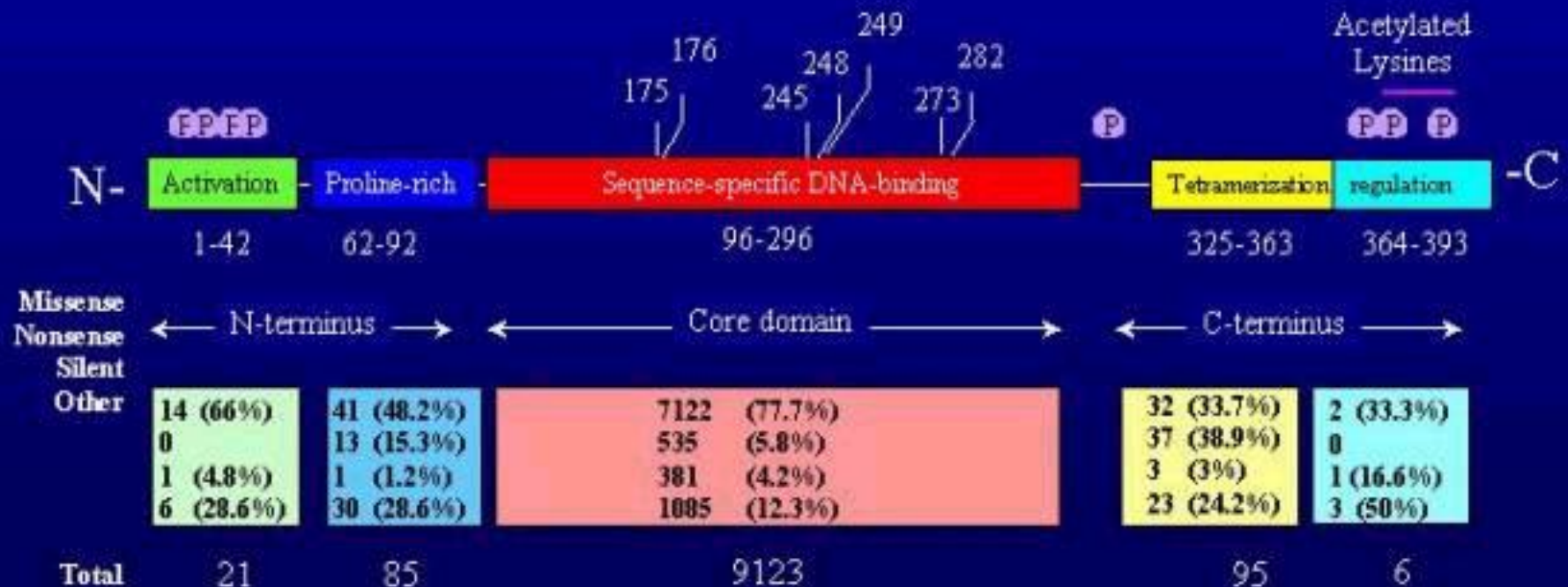


p53 tumor suppressor gene

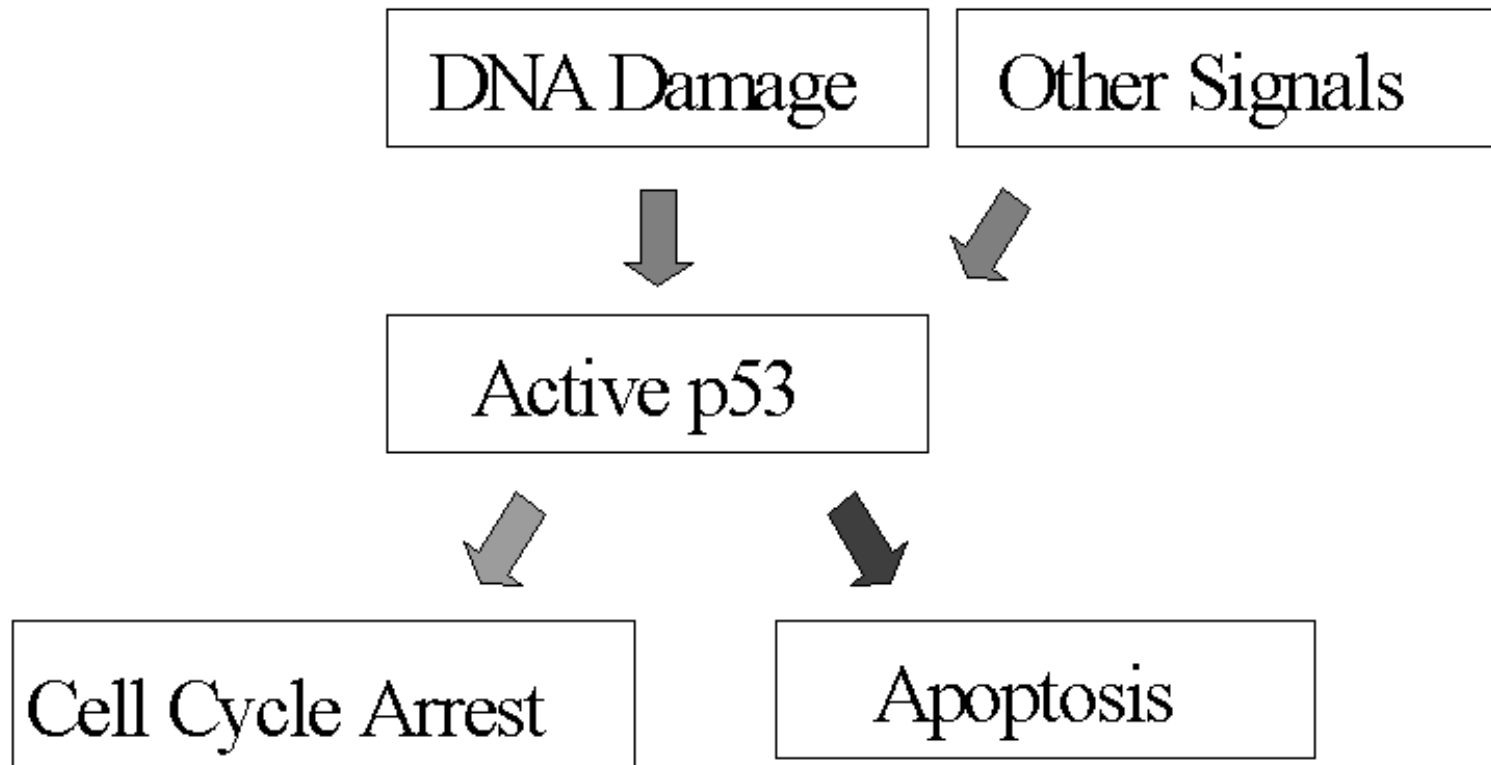
- ✓ **Mutations in p53 are implicated in ~50% of human cancers, including cancers of the:

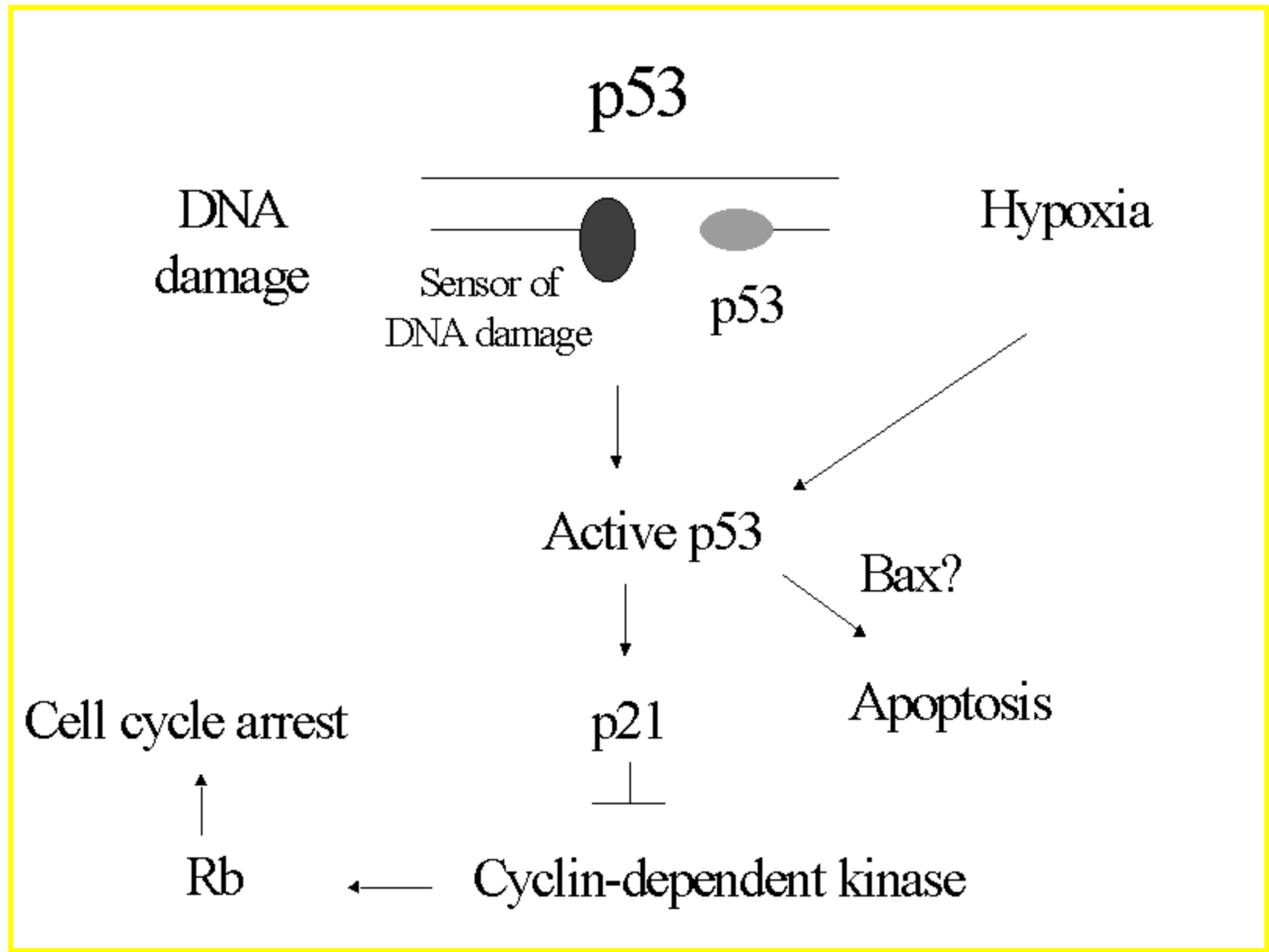
breast, brain, liver, lung, colorectal, bladder, and blood**
- ✓ **Development of tumors requires mutations on two p53 alleles.**
- ✓ **Codes a 393 amino acid protein involved in transcription, cell cycle control, DNA repair, and apoptosis (programmed cell death).**
- ✓ **p53 binds to several genes, including WAF1, and interacts with at least 17 cellular and viral proteins.**
- ✓ **Transgenic mice with deletions of both p53 alleles are viable, but 100% develop cancer by ten months of age.**

Mutations in the p53 coding sequence

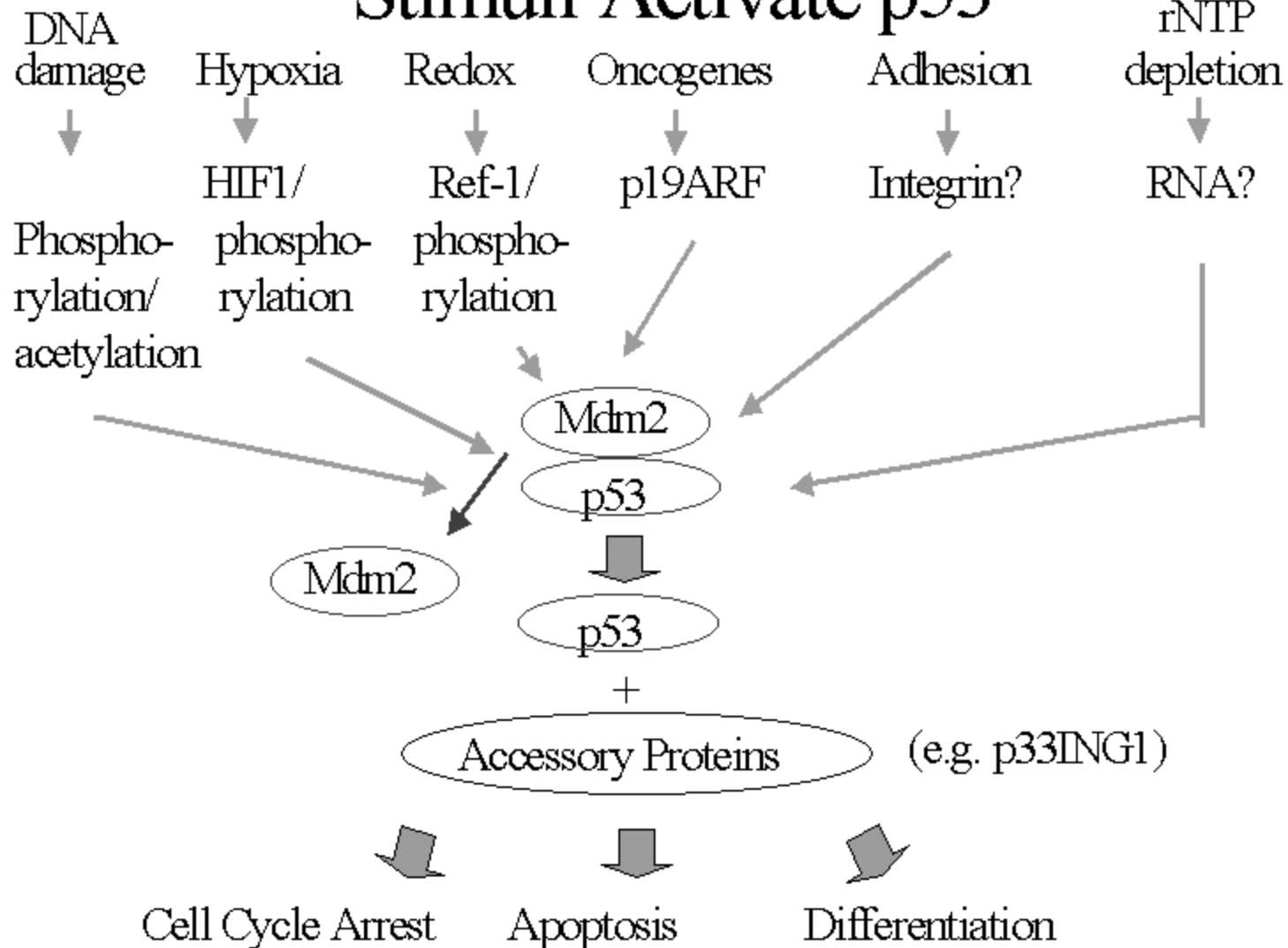


p53 Signaling



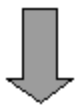


Stimuli Activate p53



Functions of Activated p53

p53



Direct
protein-
protein
interaction?



Caspase, Fas?



Apoptosis

p53



Transcriptional
Repression



p53



Transcriptional Activation



Bax
IGFBP3



MDM2



p53



p21
GADD45



Cell Cycle
Arrest

Algunos genes target de p53...

- P21, GADD45: Se une a CDK2 → inhibe fosforilación del RB → arresto del ciclo celular
- BAX, PUMA, NOXA: mitocondria → liberan citocromo c → activan caspasas – apoptosis
P53AIP1
- MDM2: ejerce loop feedback negativo autoregulatorio de p53 por ubiquitinación y degradación en le proteasoma de p53
- P53R2: Induce el arresto en el checkpoint G2/M y participa en la reparación al daño del DNA
- Existen 4000 genes con sitios potenciales de regulación por p53
- También p53 controla la transcripción de múltiples miRNA (miR-34, miR-192/215, miR-107) y de otros que actúan como supresores de tumor. Como resultante p53 puede controlar la progresión tumoral, invasión y metastasis, entre otras funciones.

Control de la estabilidad de p53 y modificaciones post-traduccionales en p53

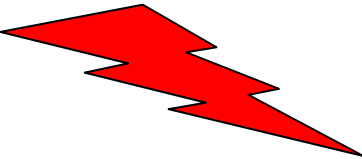
- MDM2 ubiquitina el extremo COOH-ter para enviar a p53 al proteasoma para su degradación
- MDM2 además impide la unión al DNA para la transcripción de genes target al unirse al dominio de trasactivación NH₂-ter
- MDM2 asistiría en el reclutamiento físico de p53 ubiquitinada hacia el proteasoma, contribuyendo a su degradación

La inducción y activación de p53 en respuesta al stress está sujeta a regulación por Modificaciones post-traduccionales:

- acetilación
- sumoilación
- fosforilación
- o-glicosilación

DNA damage response

Sensor de daño al DNA
γHistona H2AX fosforilada
Nuclear foci cerca de lesiones DNA



Activación check point ciclo celular

Arresto ciclo celular

Reclutamiento factores
Tempranos de reparación

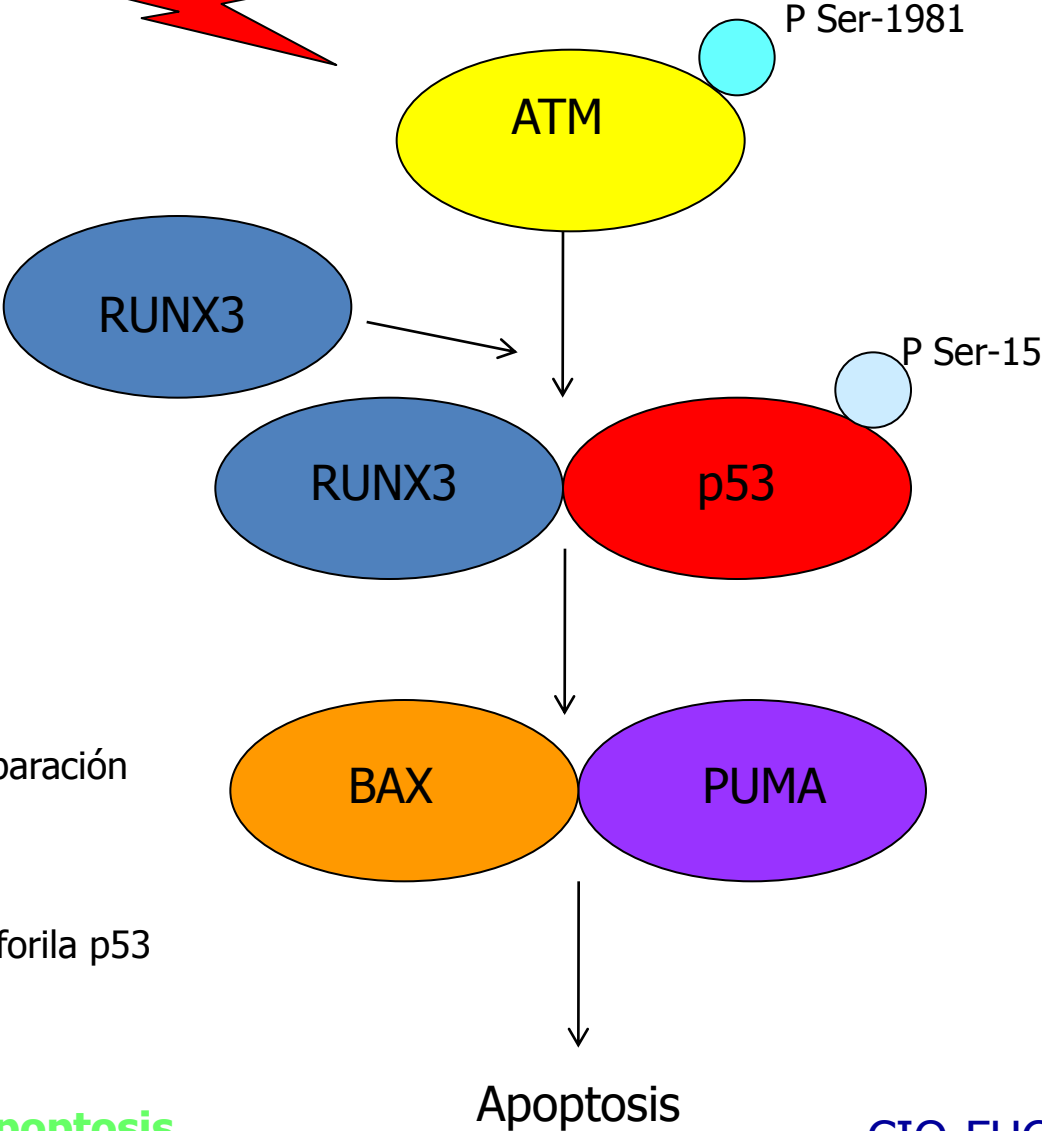
NFBD1 inhiben fosforilación de p53

Bloquea apoptosis

Tardío en la reparación

NFBD1 t ATM fosforila p53

Activa apoptosis



P53 como target terapéutico

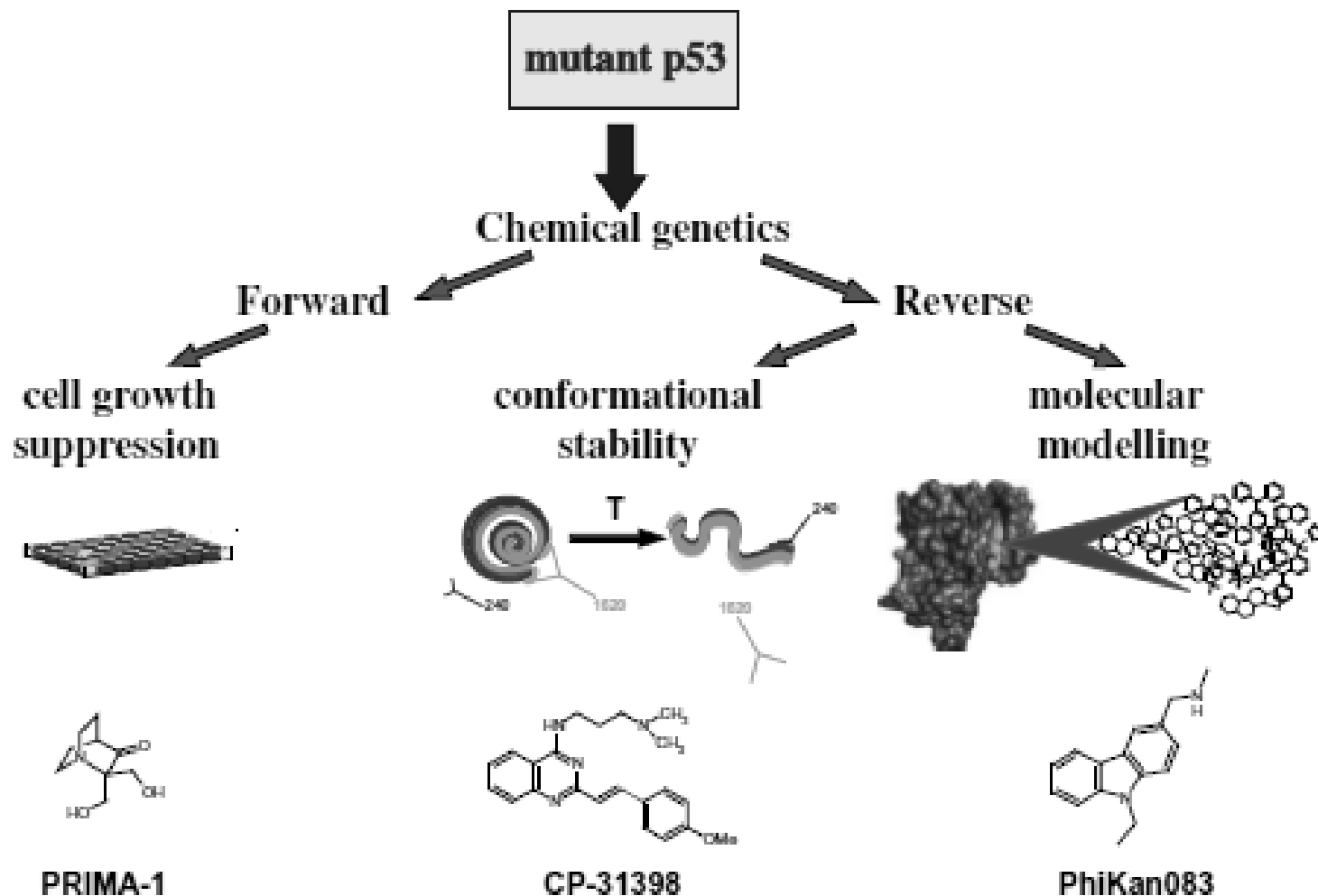
-P53 mutada debe ser reactivada y no inhibida como otros targets mutados (oncogenes)

-Múltiples mutaciones lo convierten en un target estructural heterogéneo

- CP-31398 re-pliega p53 mutante dándole estabilidad conformacional activa, restaurando el fenotipo wild type
- PhiKan083 se diseñó por moldeado molecular para unirse a residuos críticos que confieren estabilidad conformacional activa a p53 mutada
- PRIMA-1 es un compuesto que restaura la conformación wild type de p53 mutante e inhibe el crecimiento de muchas líneas tumorales en forma dependiente de p53. (in vitro e in vivo)

Se están realizando ensayos clínicos con PRIMA-1 en ptes con ca. próstata y cánceres hematológicos- Se vio respuesta clínica en ptes con p53 mutado

P53 como target terapéutico



BRCA 1 Y BRCA 2

CANCER DE MAMA Y OVARIO

Breast Cancer Tumor Suppressors

- ✓ Breast cancer affects 1 in 10 women and represents 31% of cancers in women (~215,990 women diagnosed each year, 2004 estimate).
- ✓ ~5% of breast cancers are hereditary; age of onset for hereditary breast cancer is earlier than other forms (mutations at 2 alleles).
- ✓ A small proportion of breast cancer is heritable. Two genes are associated with predisposition to developing breast cancer.
 - BRCA1 on chromosome 17
 - BRCA2 on chromosome 13
- ✓ Normal function of both is in repair of ds DNA breaks.

BRCA 1

- ✓ **A tumor suppressor gene for breast and ovarian cancer**
- ✓ **Localized on chromosome 17q21**
- ✓ **Approximately 5-15% of breast Ca is inherited - 85% is sporadic**
- ✓ **BRCA1 is responsible for approximately HALF of the INHERITED breast cancers**

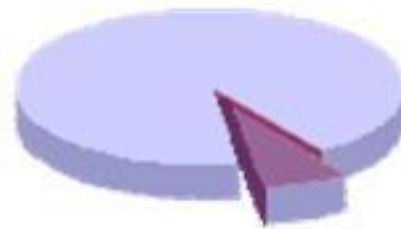
BRCA 2

- ✓ **Chromosome 13q12-13**
- ✓ **Germline mutations in BRCA2 gene increase risk of breast (male and female) and ovarian cancer**

CANCER DE MAMA HERED.

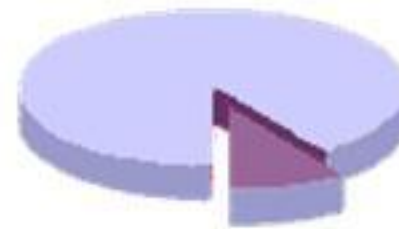
- **ANÁLISIS: 237 FAMILIAS**
- **BRCA 1: 52 %**
- **BRCA 2: 32 %**
- **INDETERM.: 16 %**
- **Familias con:**
- **Ca. mama y ov.: BRCA1 >> BRCA2**
- **Ca. mama fem.y masc. BRCA2>>BRCA1**

CÁNCER DE MAMA Y OVARIO



Breast Cancer

181,600 total new cases/year

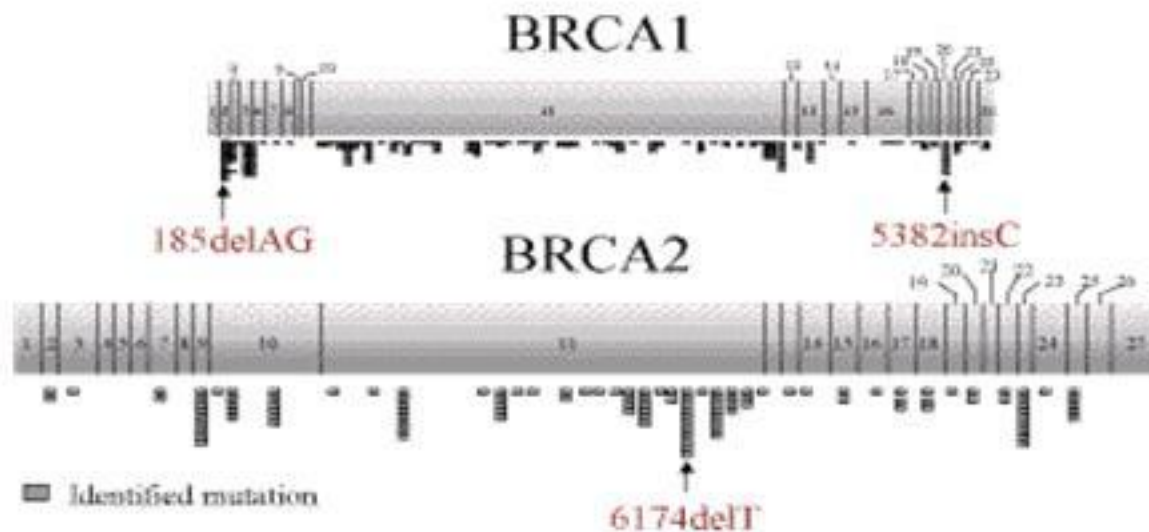


Ovarian Cancer

26,800 total new cases/year

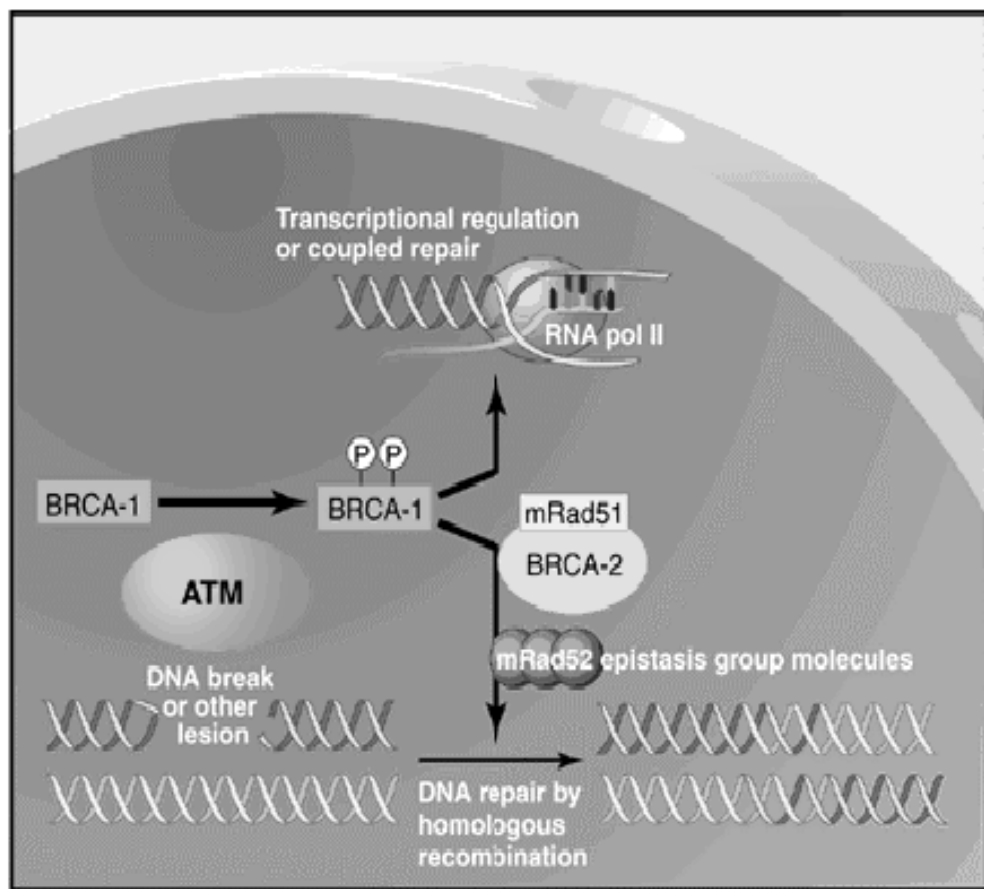
Claus EB et al. *Cancer* 77:2318, 1996
American Cancer Society, 1997

■ Sporadic
■ Hereditary



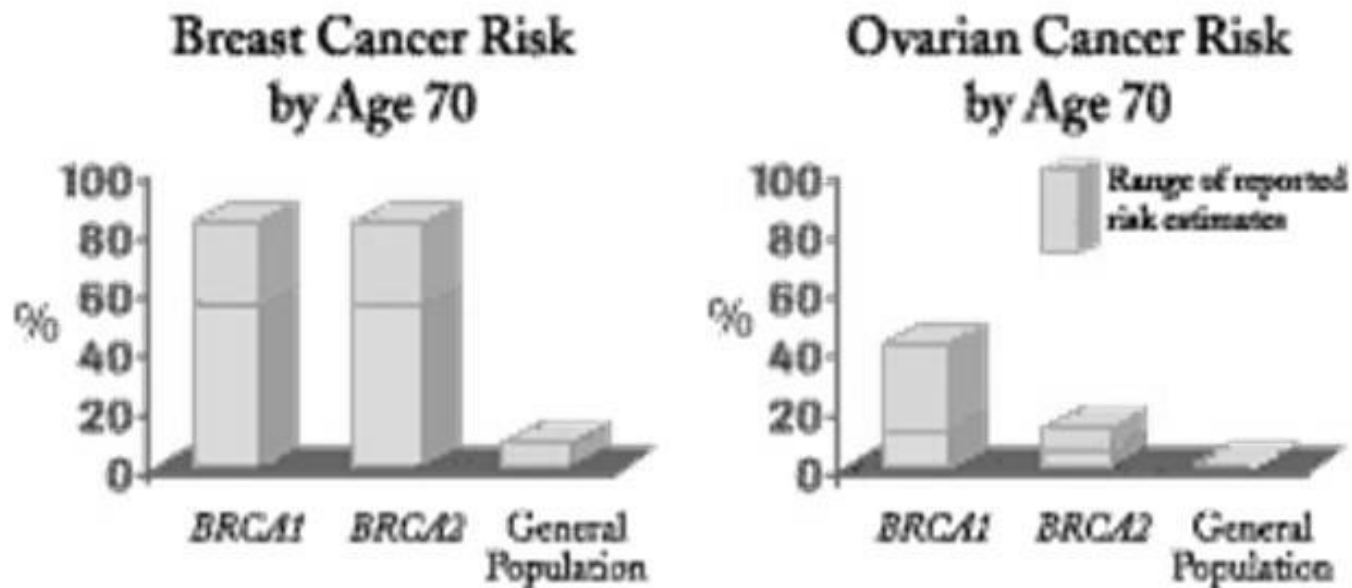
Breast Cancer genes and DNA Repair

Repairing damaged DNA.
BRCA1 is phosphorylated by the ATM protein kinase in response to DNA damage induced by γ radiation. Phosphorylated BRCA1 activates DNA repair through homologous recombination, in cooperation with BRCA2, mRad51, and other molecules related to members of the yeast RAD52 epistasis group. Phosphorylated BRCA1 may also regulate transcription and transcription-coupled DNA repair.



Science (1999) 285: 747-750;
286: 1100-1102, 1162-1166

MUTACIONES EN BRCA 1 Y BRCA 2 INCREMENTAN EL RIESGO DE CONTRAER CÁNCER DE MAMA Y OVARIO



The New England Journal of Medicine

Copyright © 2001 by the Massachusetts Medical Society

VOLUME 345

JULY 19, 2001

NUMBER 3



BREAST CANCER AFTER PROPHYLACTIC BILATERAL MASTECTOMY IN WOMEN WITH A *BRCA1* OR *BRCA2* MUTATION

HANNE MEIJERS-HEIJBOER, M.D., BERT VAN GEEL, M.D., PH.D., WIM L.J. VAN PUTTEN, M.Sc.,
SONJA C. HENZEN-LOGMANS, M.D., PH.D., CAROLINE SEYNAEVE, M.D., PH.D.,
MARIAN B.E. MENKE-PLUYMERS, M.D., PH.D., CARINA C.M. BARTELS, M.D., LEON C. VERHOOG, M.D.,
ANS M.W. VAN DEN OUWELAND, PH.D., MARTINUS F. NIERMEIJER, M.D., PH.D.,
CECILE T.M. BREKELMANS, M.D., PH.D., AND JAN G.M. KLIJN, M.D., PH.D.

Conclusión: en mujeres con mutación *BRCA1* o *BRCA2*, la mastectomía total profiláctica bilateral reduce la incidencia de cáncer de mama luego de 3 años de follow-up.

The New England Journal of Medicine

Copyright © 2001 by the Massachusetts Medical Society

VOLUME 345

JULY 26, 2001

NUMBER 4



PARITY, ORAL CONTRACEPTIVES, AND THE RISK OF OVARIAN CANCER AMONG CARRIERS AND NONCARRIERS OF A *BRCA1* OR *BRCA2* MUTATION

BARUCH MODAN, M.D., PATRICIA HARTGE, Sc.D., GALIT HIRSH-YECHEZKEL, M.Sc., ANGELA CHETRIT, M.Sc.,
FLORA LUBIN, M.Sc., UZI BELLER, M.D., GILAD BEN-BARUCH, M.D., AMIRAM FISHMAN, M.D., JOSEPH MENCZER, M.D.,
JEFFERY P. STRUEWING, M.D., MARGARET A. TUCKER, M.D., AND SHOLOM WACHOLDER, Ph.D.,
FOR THE NATIONAL ISRAEL OVARIAN CANCER STUDY GROUP*

El riesgo de cáncer de ovario en portadoras de mutaciones *BRCA1* o *BRCA2*, disminuye con cada nacimiento pero no con duración aumentada de anticonceptivos orales. Es prematuro recomendar el uso de anticonceptivos orales para quimioprevención de cáncer de ovario en portadoras de tales mutaciones

The New England Journal of Medicine

Copyright © 2001 by the Massachusetts Medical Society

VOLUME 344

FEBRUARY 22, 2001

NUMBER 8



GENE-EXPRESSION PROFILES IN HEREDITARY BREAST CANCER

INGRID HEDENFALK, M.S., DAVID DUGGAN, PH.D., YIDONG CHEN, PH.D., MICHAEL RADMACHER, PH.D.,
MICHAEL BITTNER, PH.D., RICHARD SIMON, D.Sc., PAUL MELTZER, M.D., PH.D., BARRY GUSTERSON, M.D., PH.D.,
MANEL ESTELLER, M.D., PH.D., OLLI-P. KALLIONIEMI, M.D., PH.D., BENJAMIN WILFOND, M.D., ÅKE BORG, PH.D.,
AND JEFFREY TRENT, PH.D.

En cáncer de mama con mutaciones en BRCA1 o BRCA2 se expresan grupos de genes diferentes

TIPOS DE BRCA- ANÁLISIS

- 1- Total: para determinar mutación por primera vez se secuencia todo el gen.
- 2- Sitio Único: para pacientes con parientes cuya mutación BRCA1/BRCA2 es conocida.
- 3- Multisitios: para individuos Ashkenazi.
Detecta la presencia de mutaciones en tres sitios diferentes.
185 del AG, 5382 insC y 6174 del T

Tumor suppressor genes

Gene	Contribution to Hereditary Breast Cancer
<i>BRCA1</i>	20%–40%
<i>BRCA2</i>	10%–30%
<i>TP53</i>	<1%
<i>PTEN</i>	<1%
<i>CHK2</i>	<1%
Undiscovered genes	30%–70%

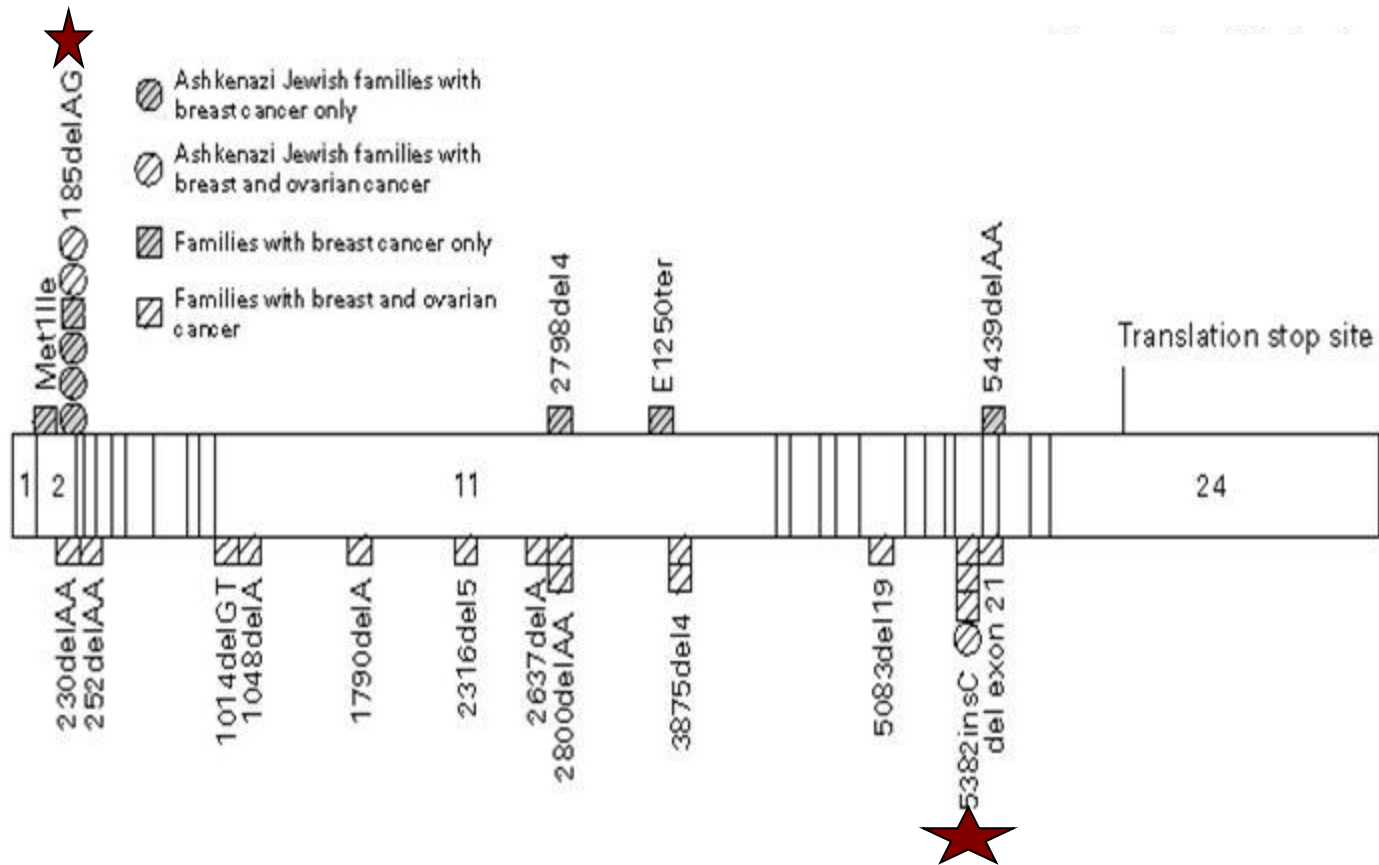


Figure 1. Location and Tumor Specificity of the 27 *BRCA1* Mutations Identified in Families with Breast Cancer.
 Exon 1 and the coding region of *BRCA1* are depicted, with exons 1, 2, 11, and 24 included for reference. The translation start site is located at the mutation Met11le.

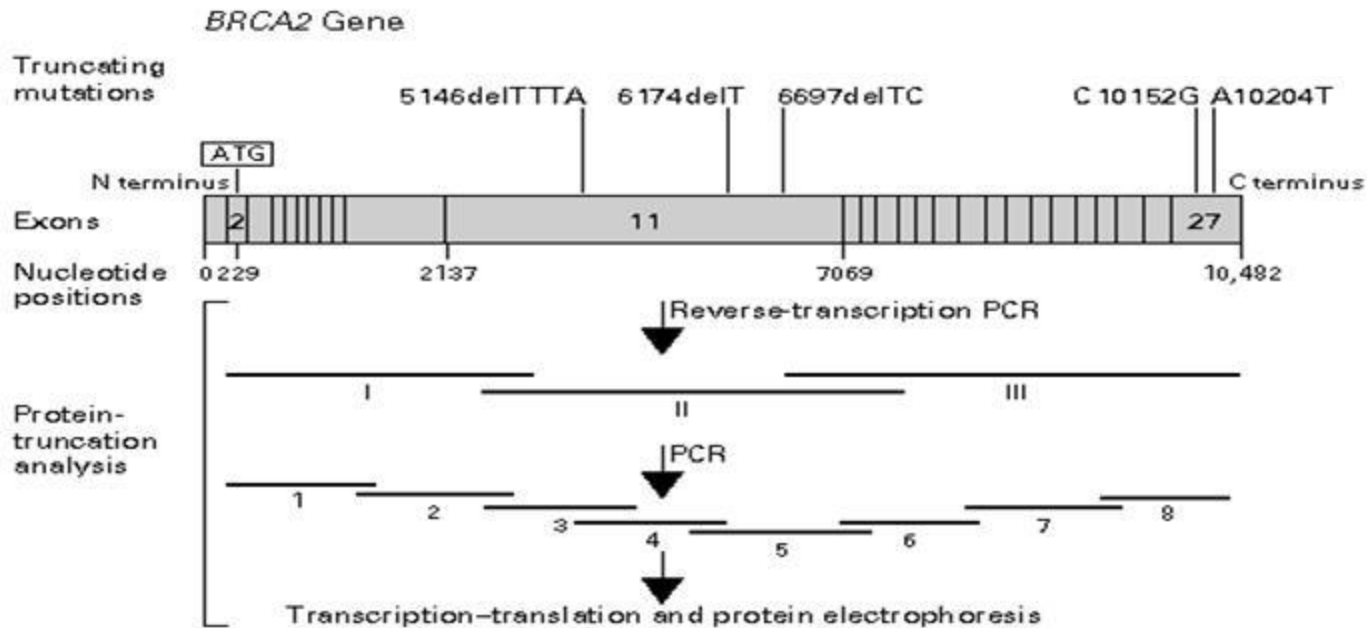


Figure 1. Identification and Location of *BRCA2* Mutations That Truncate the Polypeptide Chain. The *BRCA2* gene is encoded by 27 exons, the second of which contains the initiator codon (ATG) at nucleotide position 229. The coding sequence spans 10,254 nucleotides, encoding 3418 amino acids. Each truncating mutation results in the insertion of a premature stop codon in the sequence. Detecting these mutations by a protein-truncation assay involves making a complementary DNA copy of the *BRCA2* transcript, performing two rounds of nested PCR amplification, and synthesizing messenger RNA and protein *in vitro* from the PCR-generated templates. Abnormally shortened protein products are created when there is a truncating mutation in one of the two *BRCA2* alleles; these products can be detected by electrophoresis.

ESTRUCTURA Y FUNCION DE BRCA 1

- **Fosfoproteína nuclear de 220 kDa en células normales.**
- **Los niveles de mRNA de BRCA1 disminuyen con la transición CIS a carcinoma invasor.**
- **Como BRCA1 y RAD51, BRCA2 relocaliza a sitios de replicación en fase S luego de exposición a OH-urea ó luz UV.**
- **INTERVIENE EN LA REPARACION DEL DNA**

Functions of Non-mutant BRCA1 Protein

- **Suppressor of tumors**
 - How we know: when normal, it constrains cell division, but when mutated, inappropriate cell division results
- **Facilitator of DNA repair**
 - How we know:
 - BRCA1 has been found at replication forks just after a cell has been exposed to UV radiation
 - BRCA1 contains 2 BRCT domains—found in proteins involved in DNA repair
 - BRCA1 is phosphorylated after DNA is damaged and BRCA1 is relocated from cytosol into nucleus
- **Factor in cell degradation**
 - How we know:
 - BRCA1 contains a RING finger domain near the N-terminus.
 - RING fingers: cysteine-rich sequences that facilitate ubiquitination

BRCA1

- BRCA1 activa los 3 check points del ciclo celular (G1/S, S y G2/M) a través de distintas fosforilaciones que le confieren funciones reguladoras distintas
- Interacciona con p53 facilitando la fosforilación de p53 por ATM en respuesta al daño

1) P53 -P → ↑ P21^{WAF1} → Arresto en G1/S

2) BRCA1 detiene la replicación del DNA (mecanismo desconocido)

3) Detiene la mitosis para permitir reparación del DNA regulando la actividad de ChK1 quinasa

"A Genetic Counselor's Nightmare"

- Many genes control breast cancer— it's not a Mendelian trait
- Not all mutations in BRCA1 cause cancer
- Not all individuals with a mutant BRCA1 allele develop cancer
 - BRCA1 is "incompletely penetrant"

PTEN Tumor suppressor gene

- Mapea en cromosoma 10q23
- Es fundamental para el desarrollo embrionario
- Está mutado y delecionado en múltiples cánceres (mama, pulmón) y desórdenes linfoproliferativos letales
- Presenta haploinsuficiencia (no todos los tumores pierden el alelo normal)
- Pérdida de PTEN en línea germinal genera síndromes hereditarios:

Hamartomas

Alta susceptibilidad al cáncer

Desórdenes neurológicos

- Biopsias de cánceres esporádicos tienen menor expresión de PTEN pero el segundo alelo no está mutado

PTEN Tumor suppressor gene

Función:

1- Fostasa de lípidos: defosforila PIP3 (3' fosfoinosítido-3,4,5 trifosfato)

PIP3 activa la vía PI3K \therefore proliferación celular- inhibición apoptosis

PTEN es un regulador negativo de vía PI3K

2- Actividad supresora tumoral nuclear directa sin actividad fosfatasa:

- regula inestabilidad genómica
- Progresión en el ciclo celular
- diferenciación celular
- expresión génica

PTEN Tumor suppressor gene

Ratones heterocigotas (penetrancia incompleta) ca. próstata intraepitelial e in situ

Ratones con fenotipo hypo/-, presentan reducción de proteína PTEN de 70-80% y forman tumores de próstata con 100% penetrancia, invasivos y metastásicos

Ratones hyper/+ , presentan reducción de proteína PTEN del 20% y forman tumores de mama, ganglios linfáticos, pulmón. No poseen mutación en el alelo wt.

Existe una exquisita sensibilidad tejido específica a la disminución de los niveles de PTEN con tejidos muy poco sensibles (próstata) y otros muy sensibles (mama y ganglio)

Haploinsuficiencia convierte a PTEN en TSG

(ca. mama, reducción del 35%, asociados a signature génica ; reducción del 15 % en sanas, predisposicion?)

PTEN Tumor suppressor gene

¿Qué factores pueden disminuir los niveles de PTEN?

- factores del ambiente (dieta, zinc, drogas)
- eventos genéticos (mutaciones, deleciones)
- Control transcripcional de PTEN por vía de MAPK y c-Jun (ras mutado resulta en reducción de PTEN)
- Control post-transcripcional (miRNA, pérdida de ceRNA (competitivo endógeno))
- Control post-traducciona (drogas, E3-ligasas, enzimas deubiquitinantes, DUB)

PTEN

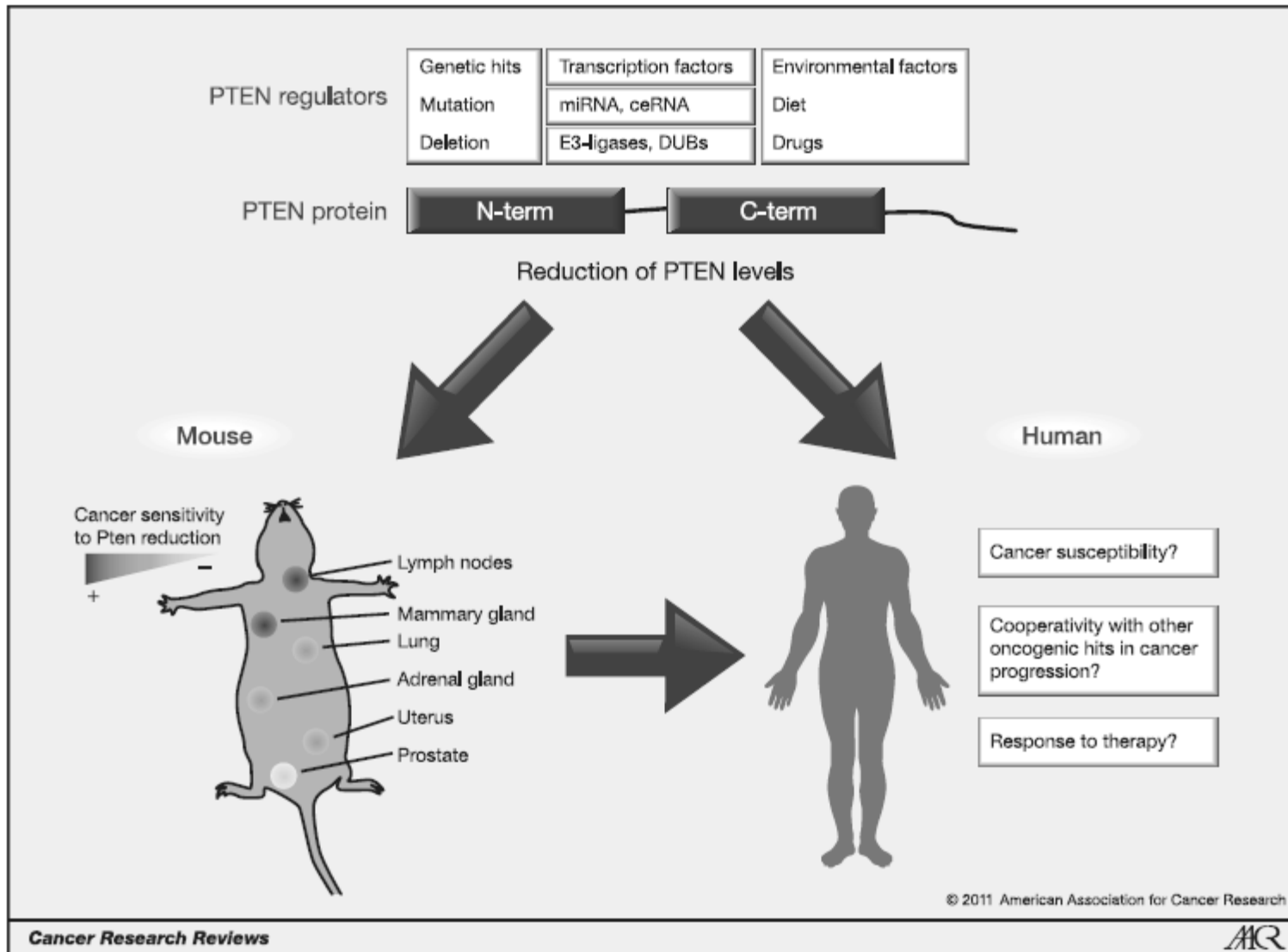


Figure 1. Impact of PTEN regulatory cues in tumorigenesis. Schematic of the tissue-specific cancer sensitivity to PTEN downregulation and the regulatory factors that could lead to the reduction of PTEN levels. PTEN expression is regulated at multiple levels, genomic (mutation and deletions), transcriptional (transcription factors), post-transcriptional (miRNAs, ceRNAs, such as the PTEN pseudogene), and post-translational [drugs, E3-ligases, deubiquitinating enzymes (DUB)]. In turn, the reduction of PTEN levels has a gradual and tissue-specific impact on cancer initiation in the mouse, with the mammary tissue being among the most sensitive tissues to PTEN level reduction. These data suggest that, in humans, subtle reduction of PTEN levels may lead to cancer susceptibility, cooperate with other oncogenic events in cancer progression, or affect the response to therapy.

Control epigenético de la supresión tumoral

-Modificaciones epigenéticas son cambios heredables en la expresión génica que ocurren sin alterar la secuencia de DNA

(ej: metilación del DNA y modificaciones en las histonas)

- Metilación del DNA : 1- preserva la integridad del DNA
2-silenciamiento de genes a nivel transcripcional

- cpG son targets de metilación (CpG islands en promotores, DNA repetitivo, regiones pericentroméricas, y non-CpG islands)

-Metilación en promotores y cuerpo de los genes tiene función regulatoria

- Metilación fisiológica para imprinting de genes e inactivación de X en hembras

Control epigenético de la supresión tumoral

- Maquinaria metilante fisiológica está regulada por el ciclo celular para mantener la metilación y preservar los patrones durante la replicación
- En tumores todo lo contrario: existe deregulación de la maquinaria demetilante y una gradual de-metilación del genoma así como la adquisición de hipermetilación aberrante en CpG islands (puede reprimir TSG).
- Hipometilación puede activar oncogenes
- Hipoacetilación de histonas también resultar en silenciamiento transcripcional de TSG ; también fosforilación, sumoilación, ubiquitinación, biotinilación, etc)
- Hipermetilación de promotores interfiere con la unión de factores de transcripción o alterando la cromatina, existiendo cooperatividad con la marcas de histonas acetiladas
- Se pueden usar como target, cio agentes de-metilantes o inhibidores de la HDAC para re-expresar los genes.
(ej: 5-azadC, tricostatina)

TABLE 2

Partial Listing of Proven and Putative TSGs Exhibiting Epigenetic Modifications in Different Tumor Types

Gene	Pathway	Cancer type(s)	Initial report(s)
p15 ^{INK4}	Cell cycle	Hematologic, hepatic, lung, colorectal	210
p16 ^{INK4}	Cell cycle	Hematologic, lung, gliomas, head and neck, squamous cell	211, 212
p14 ^{ARF}	Cell cycle	Breast, lung, gastrointestinal, hepatocellular, salivary gland	97
RB	Multifunctional, cell cycle	Retinoblastoma, lung, bladder, pancreatic, pituitary adenoma, hepatic, glioblastoma	213
MLH1	DNA repair	Colorectal, endometrial, gastric, squamous cell	214–216
MGMT	DNA repair	Colorectal, medulloblastoma, melanoma, testicular, esophageal	217, 218
BRCA1	DNA repair	Breast, ovarian, cervical	219
DAPK	Apoptosis	Cervical, lung, hematologic, glioma, laryngeal, esophageal, mesothelioma	220, 221
TMS1	Apoptosis	Breast, neuroblastoma, cervical, prostate	222
CDH1	Cell adhesion	Breast	223
GSTP1	Carcinogen detoxification	Prostate, breast, renal	223
SERPINB5 (maspin)	Cell adhesion, invasion, metastasis	Breast, thyroid, prostate, melanoma	224
MDG1	Cell growth and proliferation	Breast, gastric	225
RIZ1	Transcriptional regulation	Gall bladder, gastric, thyroid	226
VHL	Transcriptional regulation	Esophageal, renal, oral	227
DIRAS3	Growth suppression	Ovarian, breast, thyroid	113
SFRP1	Cell adhesion, migration	Colorectal, breast	228
APC	Nuclear export	Colorectal, endometrial, breast, lung, stomach, melanoma	229
FHIT	Proliferation, survival	Esophageal; hematologic, lung	230
HIC1	Transcriptional regulation	Glioblastoma, hematologic, cervical	231–233
RASSF1A	DNA repair, cell cycle	Gastric, lung, breast, kidney, medulloblastoma, esophageal	234
RARB2	Signal transduction	Esophageal, breast	235
GADD45G	DNA damage, apoptosis	Lymphoma, nasopharyngeal, esophageal, cervical, lung	236
14-3-3 σ	DNA damage	Breast, gastric, liver, lung skin, ovarian, neuroblastoma	237
CST6	Metastasis suppressor	Breast, glioma	238–240
BEX1	Unknown	Glioma	120
BEX2	Putative transcriptional regulator	Glioma	120
CEBPA	Transcription factor	Endometrial, lung, hepatoma	241
p27 ^{KIP1}	Cell cycle	Melanoma	242
p21 ^{WAF1/CIP1}	Cell cycle	Prostate	243
RUND3	Transcription factor, apoptosis	Yolk sac, colon, nasopharyngeal, lung	244

Bibliografía sugerida:

- 1- Epigenetic control of tumor suppression. *Critical Reviews in Eukaryotic Gene Expression* 17(4): 295-316 (2007).
- 2- PTEN level in tumor suppression: How much is too little? *Cancer Research* 71(3): 629-33, 2011
- 3- p53: The attractive tumor suppressor in the cancer research field. *Journal of Biomedicine and Biotechnology* volume 2011, article ID 603925, 13 pages (2010)
Doi: 10.1155/2011/ 603925
- 4- The p53 tumor suppressor : A master regulator of diverse cellular processes and therapeutic target in cancer. *BBRC* 396: 85-89, 2010.
- 5- The retinoblastoma tumor suppressor gene, the exception that proves the rule. *Oncogene* 25(38): 5233-4243, 2006.
- 6- The tumor suppressor genetics. *Carcinogenesis* 26: 2031-2045, 2005.