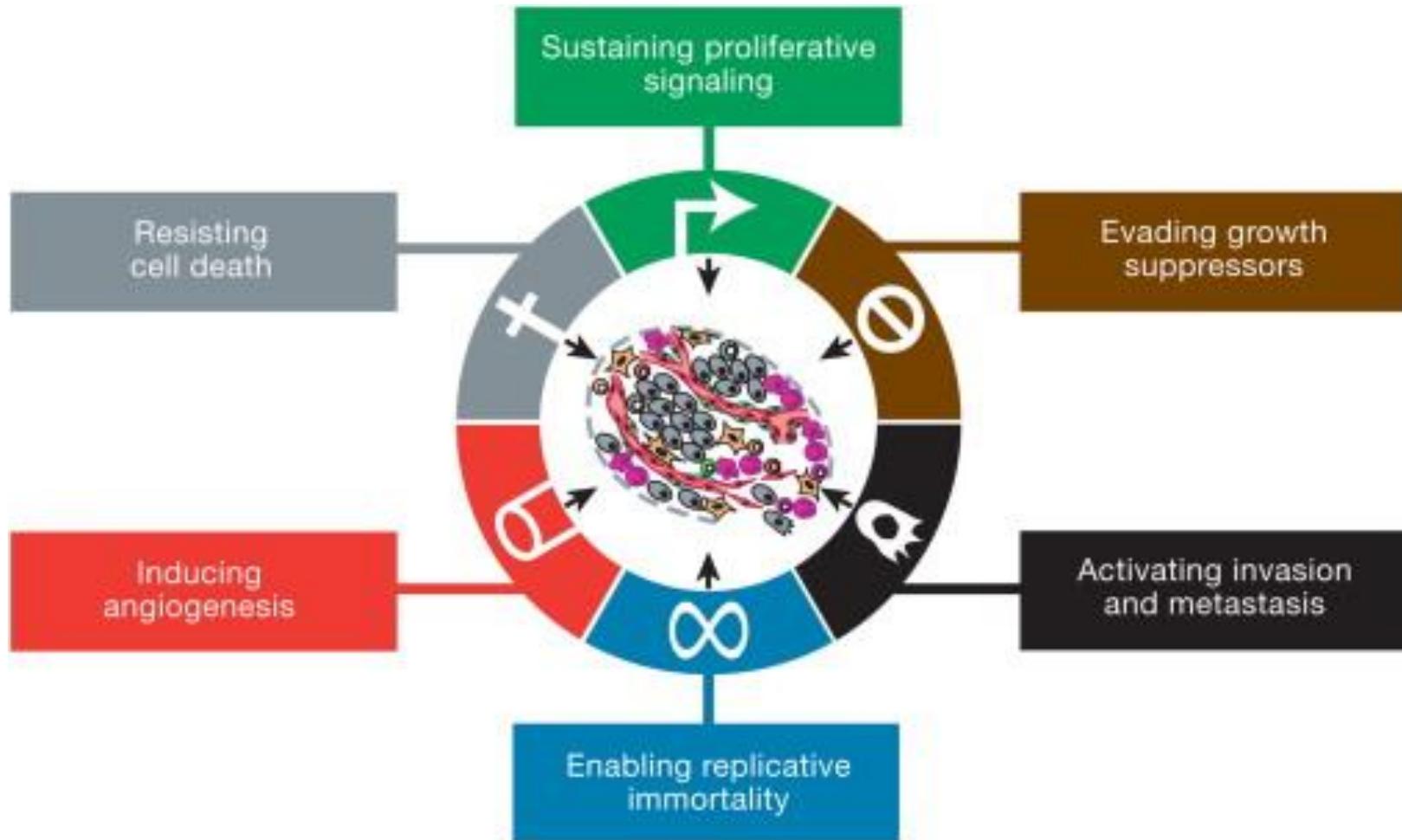


Cancer Biology and Clinical Response of Normal Tissues:

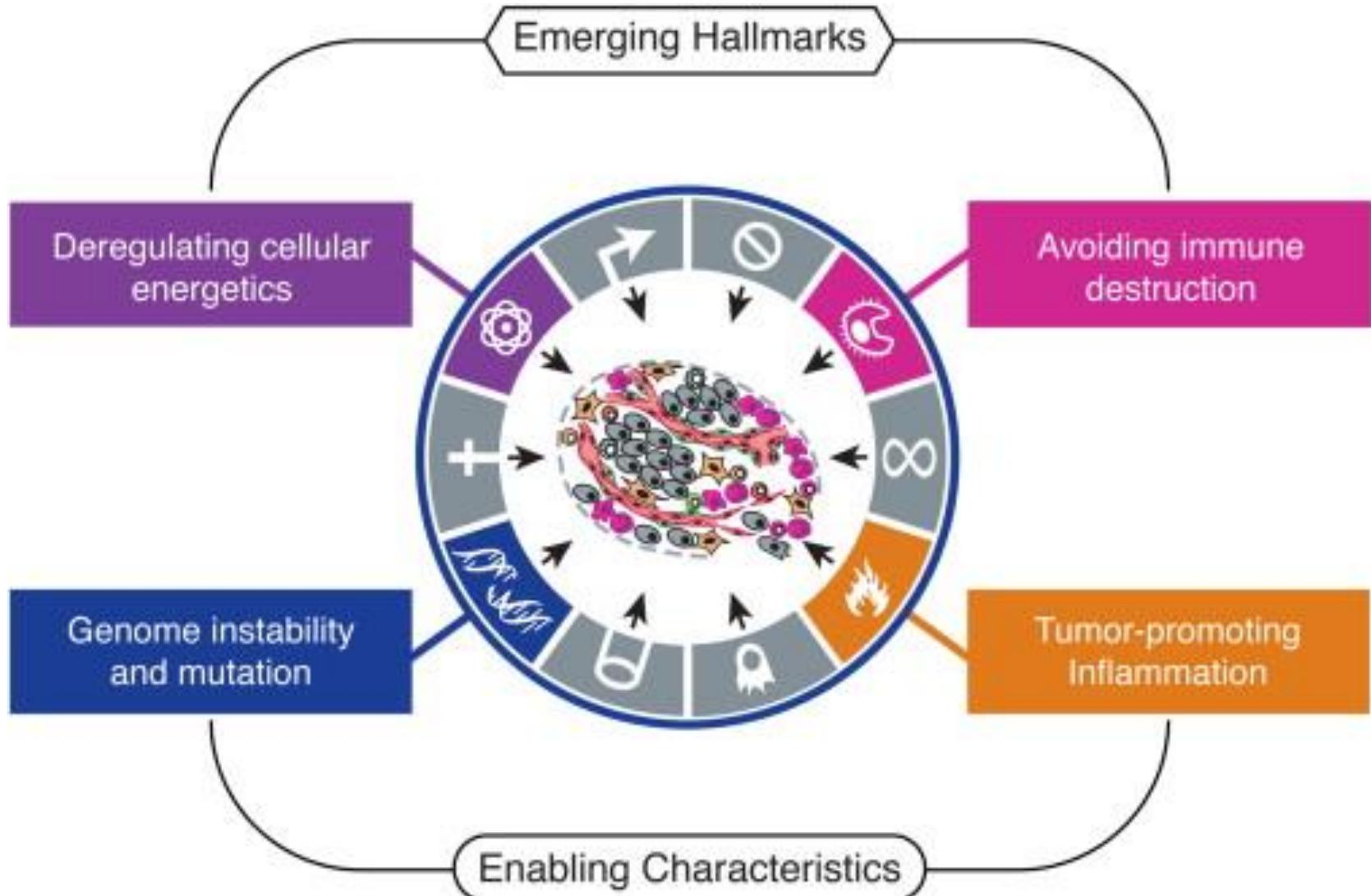
Simon N. Powell MD PhD

Memorial Sloan-Kettering Cancer Center
Radiation Oncology Department
& Molecular Biology Program

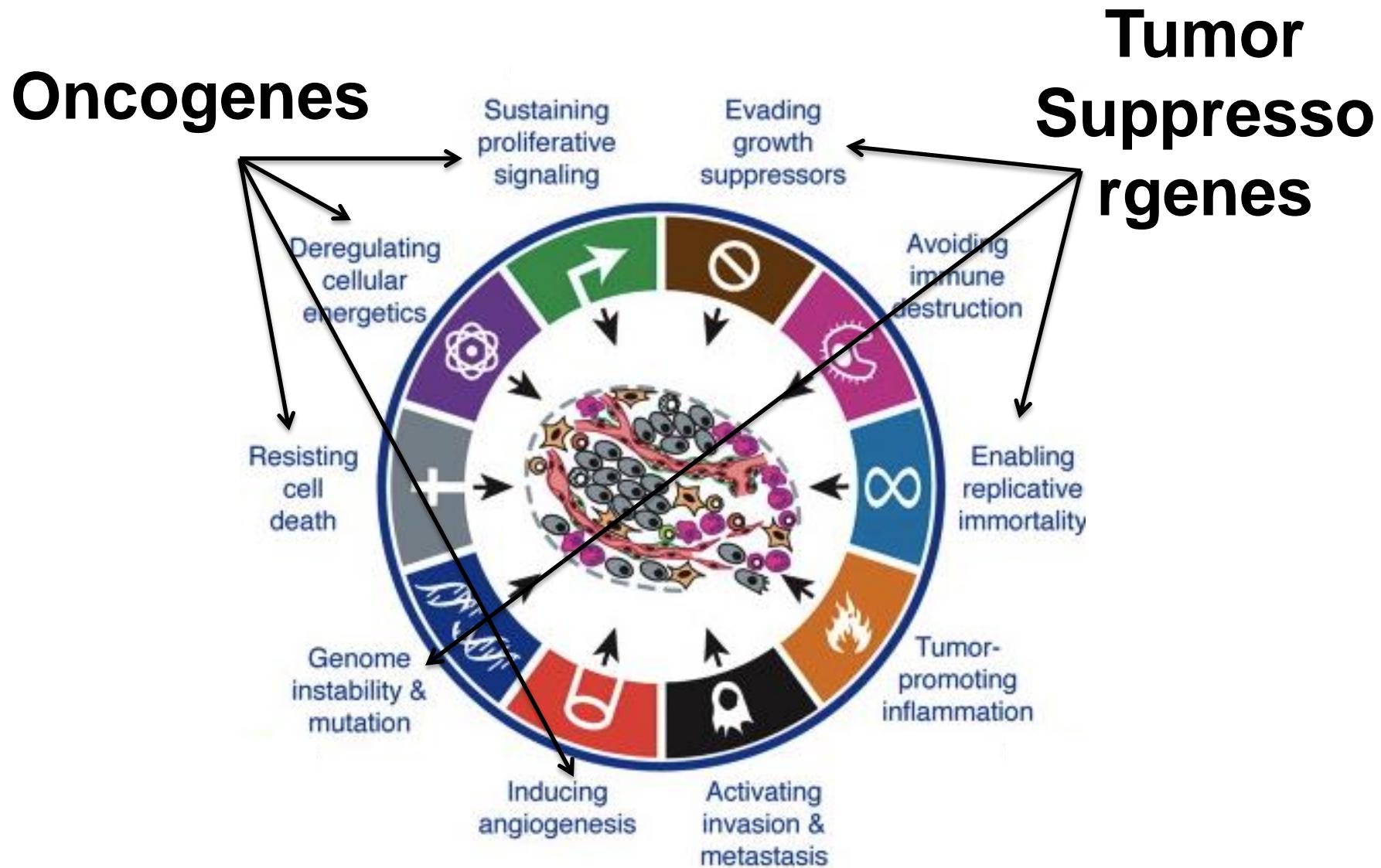
The Hallmarks of Cancer



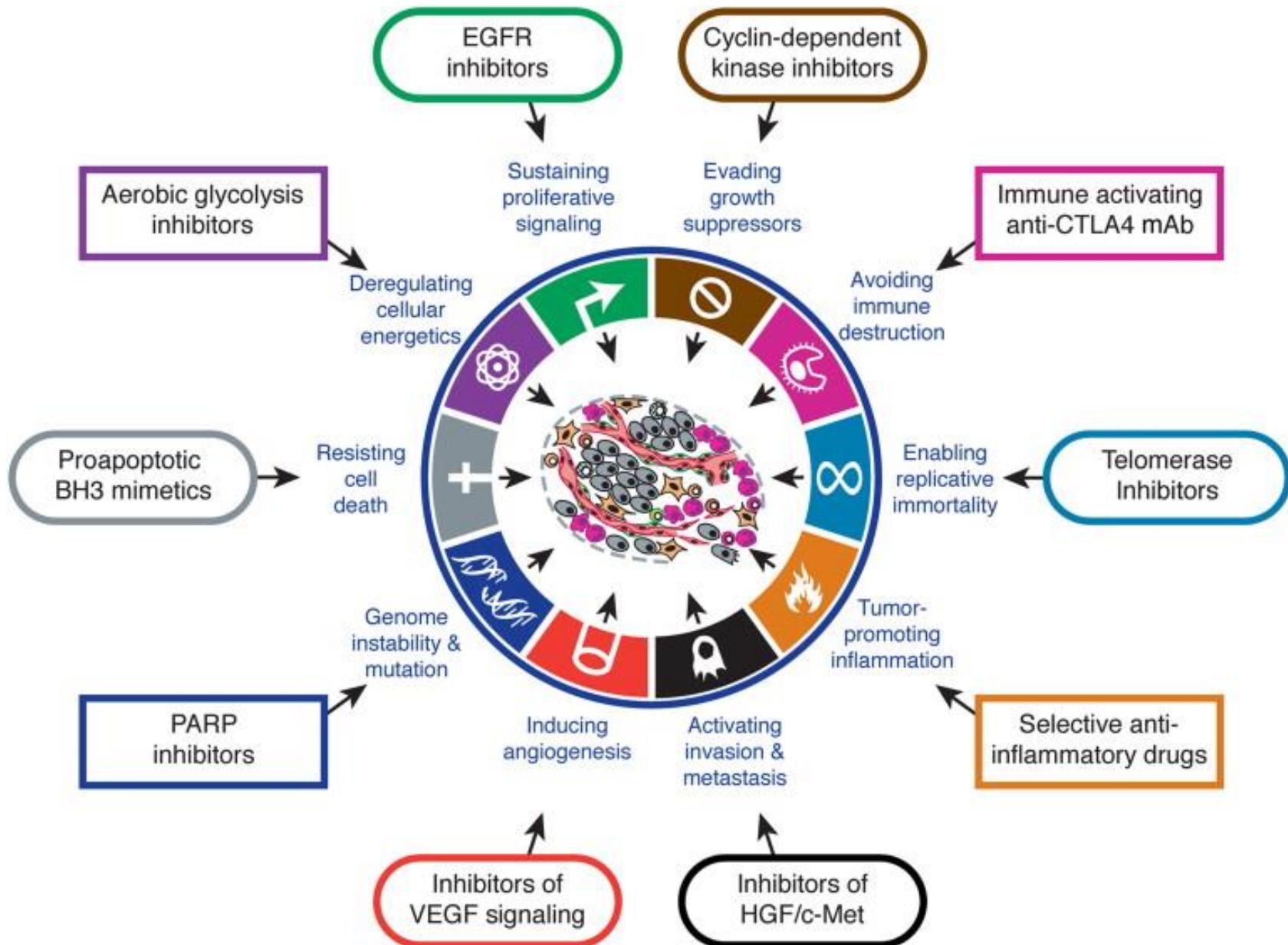
The Hallmarks of Cancer



The Hallmarks of Cancer



The Hallmarks of Cancer



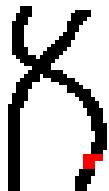
Functions of Oncogenes

1. Growth Factors (e.g. *Epithelial Growth Factor EGF; Platelet Derived Growth Factor PDGF*)
2. Growth Factor Receptors (*EGFR, PDGFR, IGFR*)
3. Signal transduction (*PI3-kinase, Ras, Raf, MEK*)
4. Transcription Factors (*myc, Jun, Fos, Elk-1*)

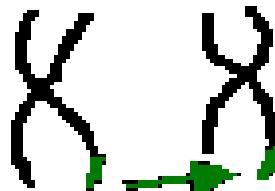
How does a Proto-oncogene become an Oncogene?

Proto-Oncogene → Oncogene

1. Mutation
2. Gene translocation
3. Amplification
4. Activation



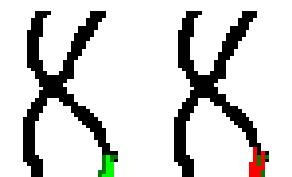
Point Mutation



Translocation



Amplification



Activation

Table 4.2 A list of point-mutated *ras* oncogenes carried by a variety of human tumor cells

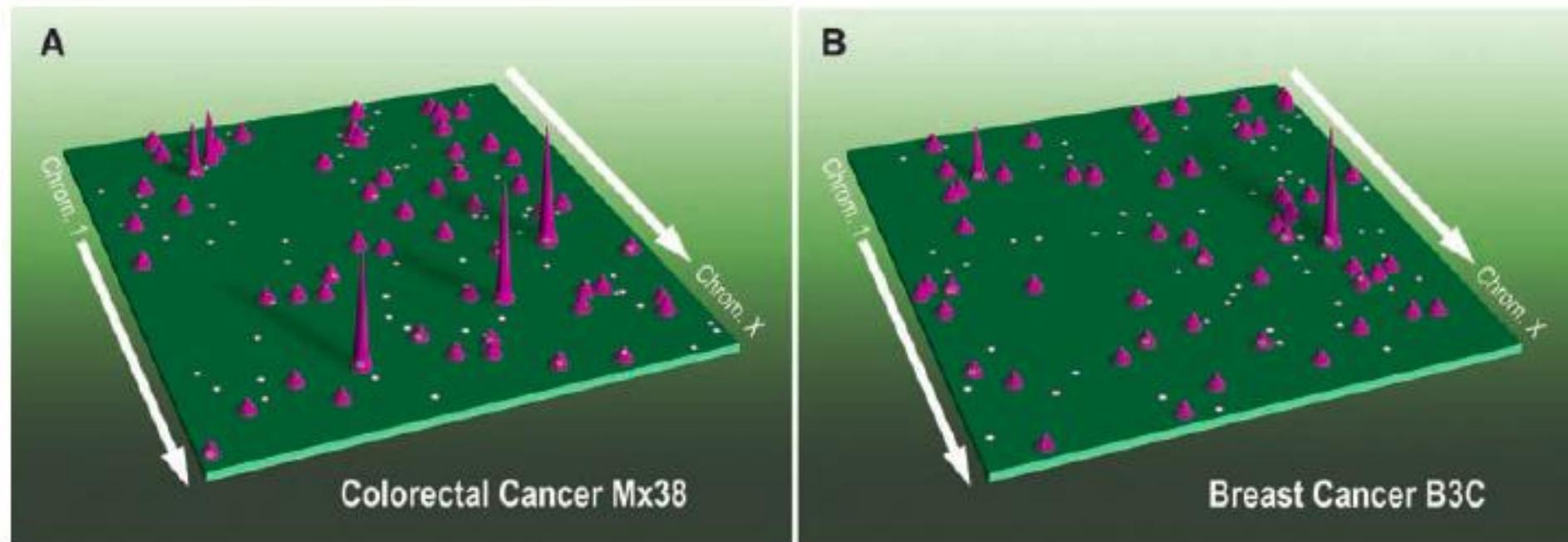
Tumor type	Proportion (%) of tumors carrying a point-mutated <i>ras</i> gene ^a
Pancreas	90 K
Thyroid (papillary)	60 (H, K, N)
Thyroid (follicular)	55 (H, K, N)
Colorectal	45 (K)
Seminoma	45 (K, N)
Myelodysplasia	40 (N, K)
Lung (non-small-cell)	35 (K)
Acute myelogenous leukemia	30 (N)
Liver	30 (N)
Melanoma	15 (K)
Bladder	10 (K)
Kidney	10 H

^aH, K, and N refer to the human *H-RAS*, *K-RAS*, and *N-RAS* genes, respectively.

Adapted from J. Downward, *Nat. Rev. Cancer* 3:11–22, 2003.

Modern Human Genomics

Number of mutations in cancers? 80!

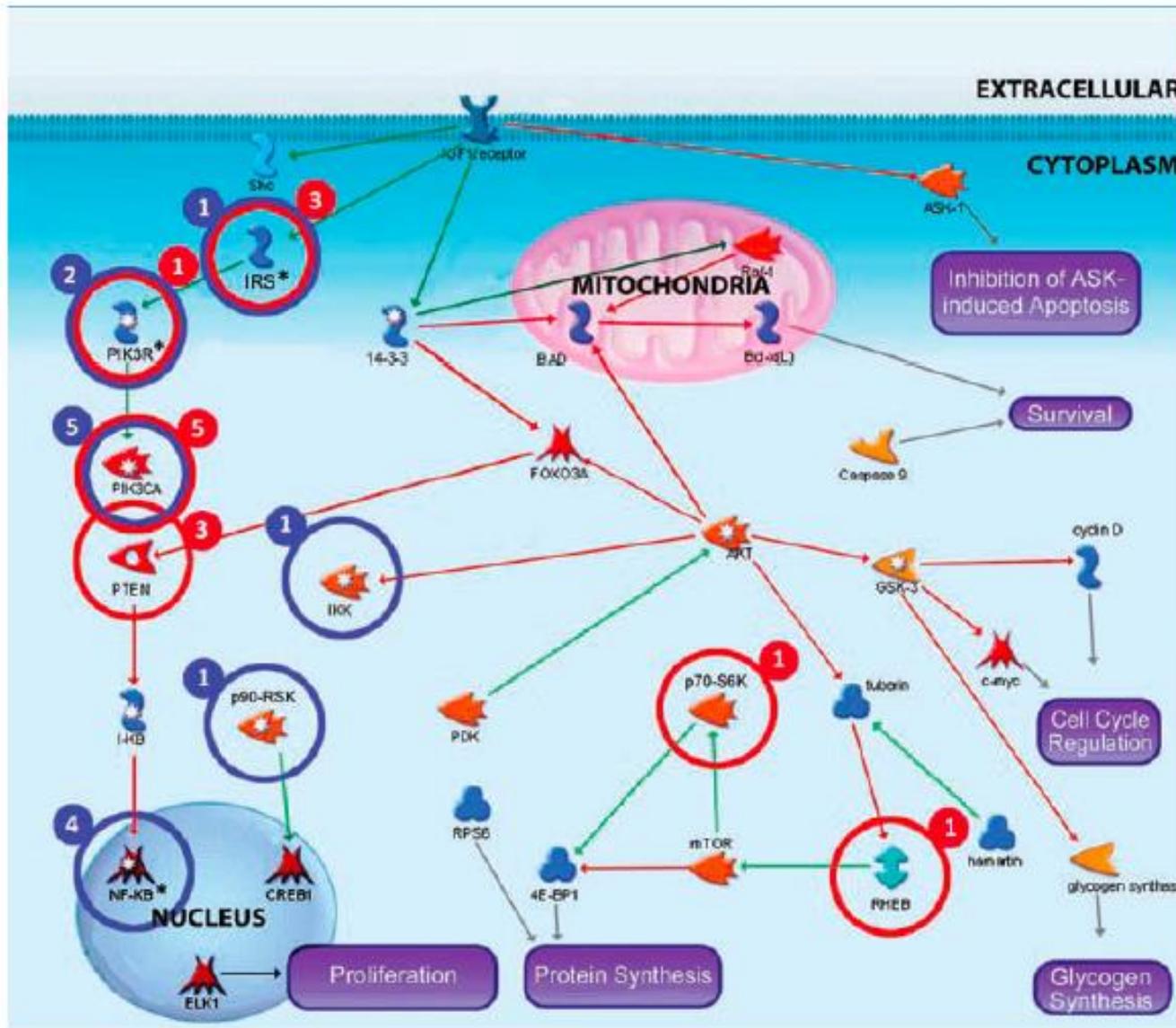


The Genomic Landscapes of Human Breast and Colorectal Cancers

Laura D. Wood,^{1*} D. Williams Parsons,^{1*} Siân Jones,^{1*} Jimmy Lin,^{1*} Tobias Sjöblom,^{1†} Rebecca J. Leary,¹ Dong Shen,¹ Simina M. Boca,^{1,2} Thomas Barber,^{1,‡} Janine Ptak,¹ Natalie Silliman,¹ Steve Szabo,¹ Zoltan Dezso,³ Vadim Ustyanksky,³ Tatiana Nikolskaya,^{3,4} Yuri Nikolsky,³ Rachel Karchin,⁵ Paul A. Wilson,⁵ Joshua S. Kaminker,⁶ Zemin Zhang,⁶ Randal Croshaw,⁷ Joseph Willis,⁸ Dawn Dawson,⁸ Michail Shipitsin,⁹ James K. V. Willson,¹⁰ Saraswati Sukumar,¹¹ Kornelia Polyak,⁹ Ben Ho Park,¹¹ Charit L. Pethiyagoda,¹² P. V. Krishna Pant,¹² Dennis G. Ballinger,¹² Andrew B. Sparks,¹² § James Hartigan,¹³ Douglas R. Smith,¹³ Erick Suh,¹³ Nickolas Papadopoulos,¹ Phillip Buckhaults,⁷ Sanford D. Markowitz,¹⁴ Giovanni Parmigiani,¹ || Kenneth W. Kinzler,¹ || Victor E. Velculescu,¹ || Bert Vogelstein¹ ||

Modern Human Genomics

Number of altered pathways in cancers? 8?



Translocation and Chromosome Fusion in the activation of endogenous cellular oncogenes

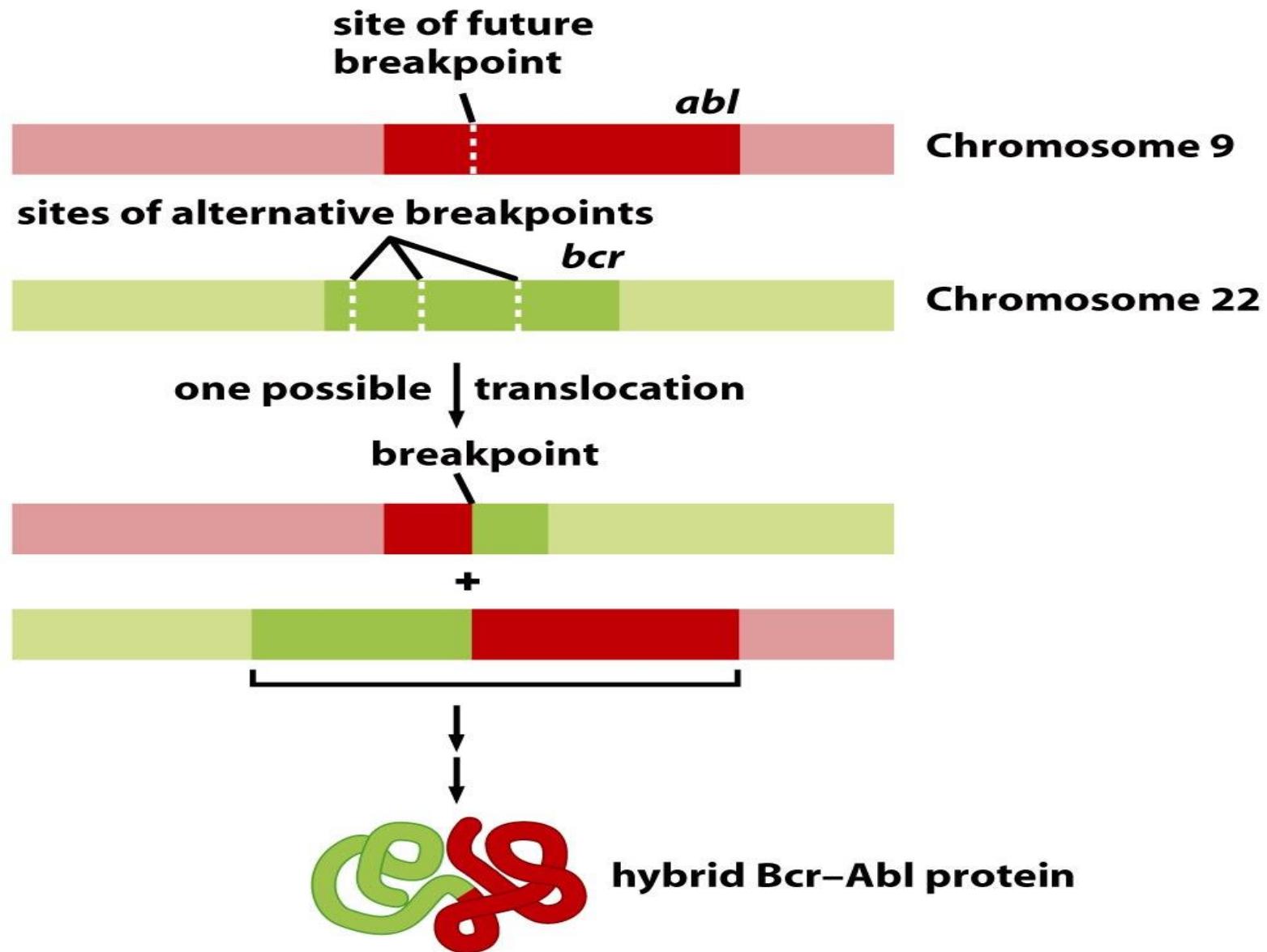
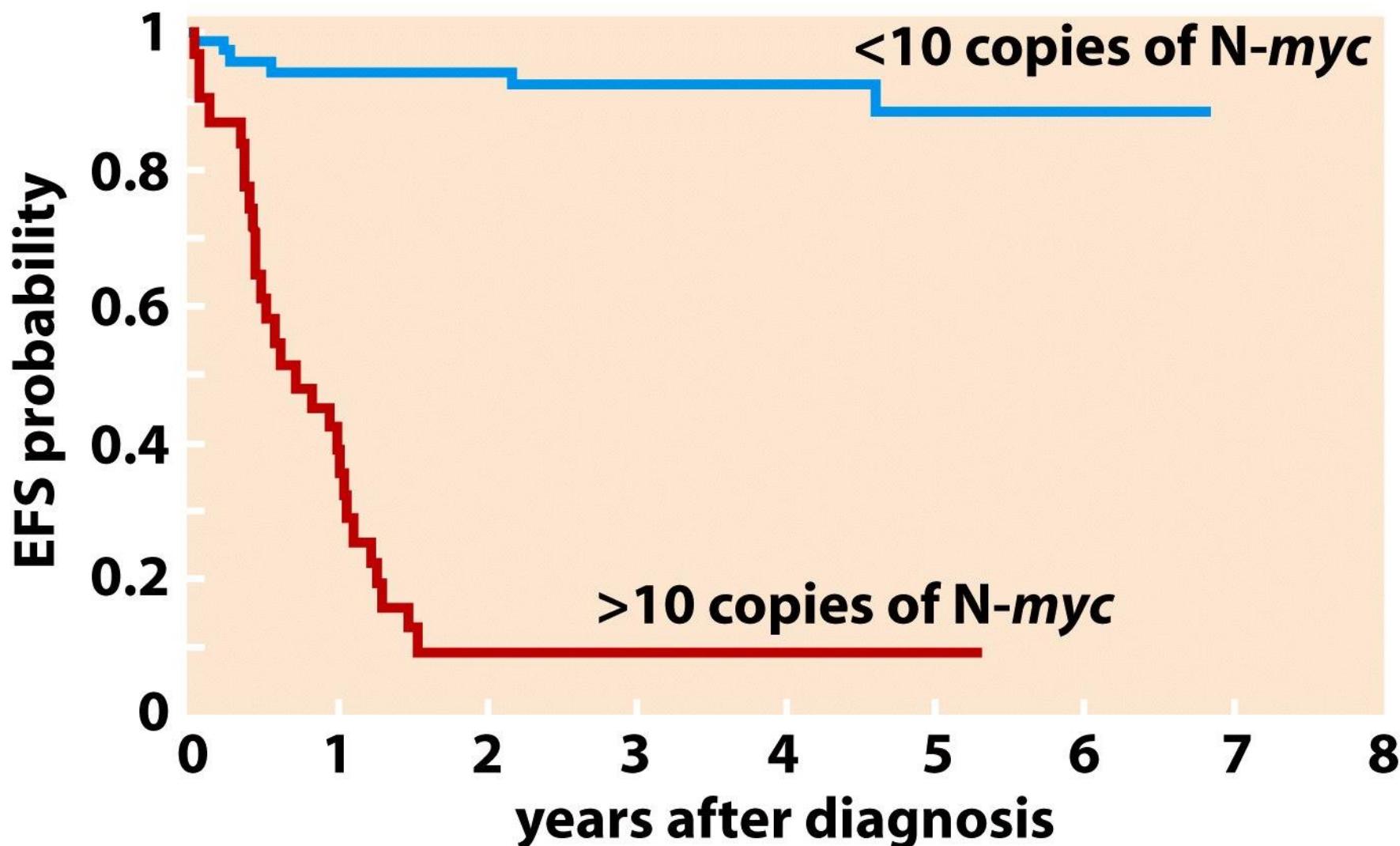


Table 4.4 Translocations in human tumors that deregulate proto-oncogene expression and thereby create oncogenes

Oncogene	Neoplasm
<i>myc</i>	Burkitt's lymphoma; other B- and T-cell malignancies
<i>bcl-2</i>	follicular B-cell lymphomas
<i>bcl-3</i>	chronic B-cell lymphomas
<i>bcl-6</i>	diffuse B-cell lymphomas
<i>hox1</i>	acute T-cell leukemia
<i>lyl</i>	acute T-cell leukemia
<i>rhom-1</i>	acute T-cell leukemia
<i>rhom-2</i>	acute T-cell leukemia
<i>tal-1</i>	acute T-cell leukemia
<i>tal-2</i>	acute T-cell leukemia
<i>tan-1</i>	acute T-cell leukemia

Adapted from G.M. Cooper, Oncogenes, 2nd ed. Boston and London: Jones and Bartlett, 1995.

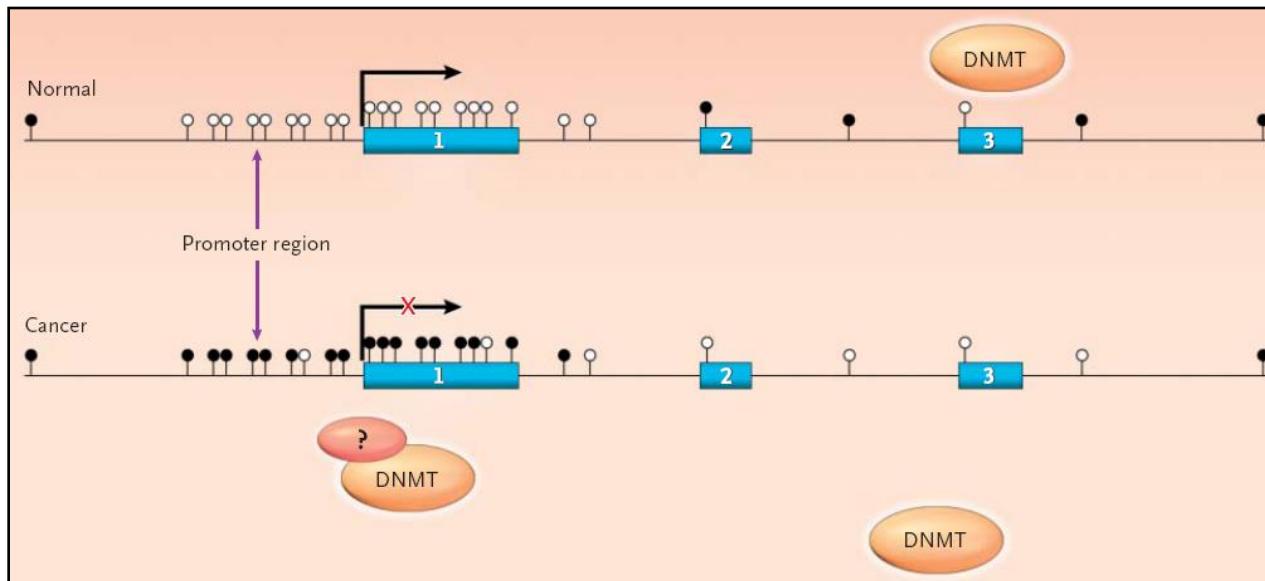
Amplification of Endogenous Oncogene in Human Cancer: N-Myc in Human Neuroblastoma.



Activation of Oncogenes by Chromatin Modification

Methylome changes in neoplasia:

- Global hypo-methylation
- Site-specific hyper-methylation

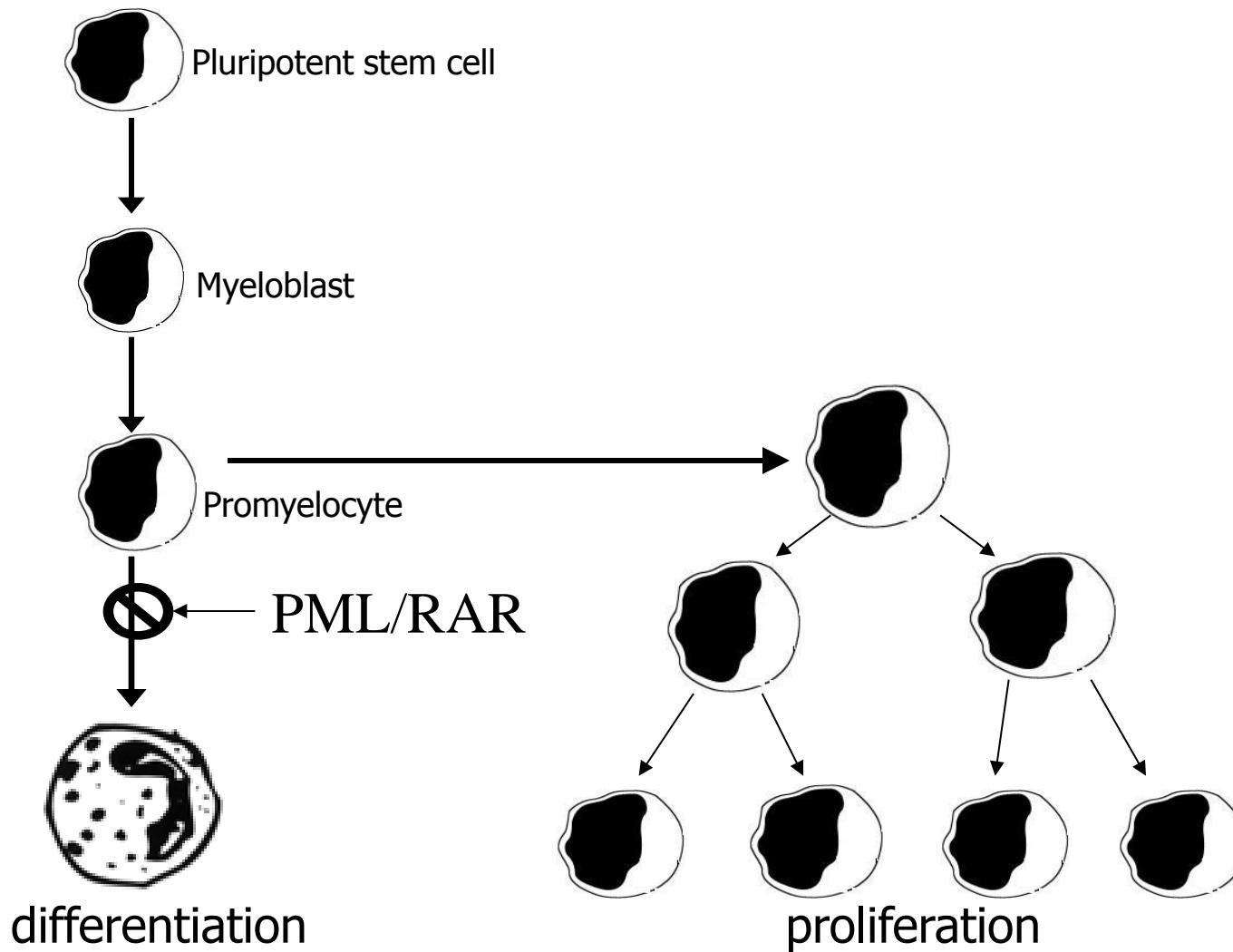


Oncogenes

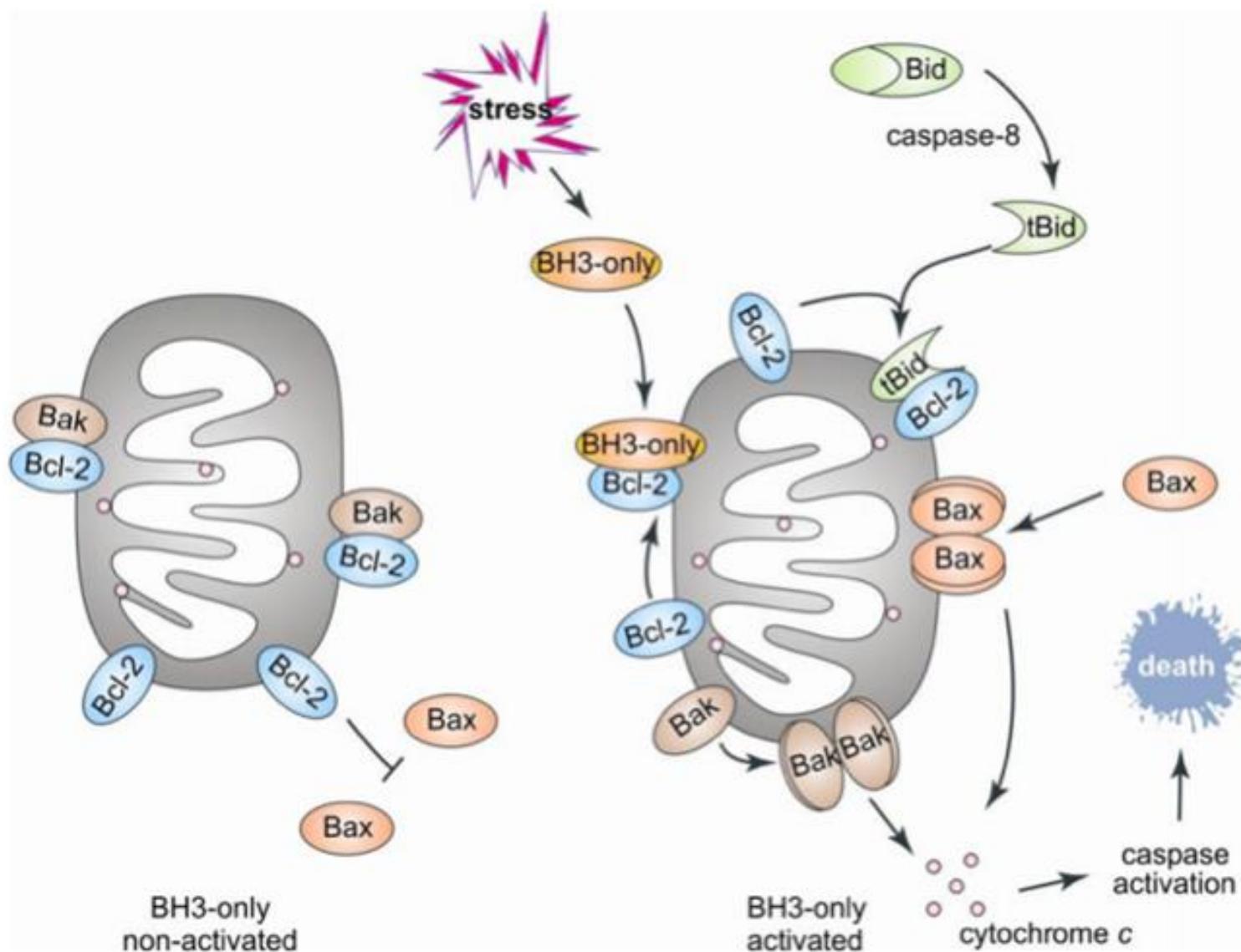
Cause cancer by affecting:

1. Cell Proliferation: (*Ras, Raf, EGF*)
2. Cell differentiation (*PML/RAR* that inhibits the differentiation of promyelocyte to granulocyte which will maintain the cell in its active proliferate state)
3. Cell Survival (*PI-3/AKT*) which will activate *BCL-2* inhibit apoptosis & maintain cell survival.

PML/RAR Action

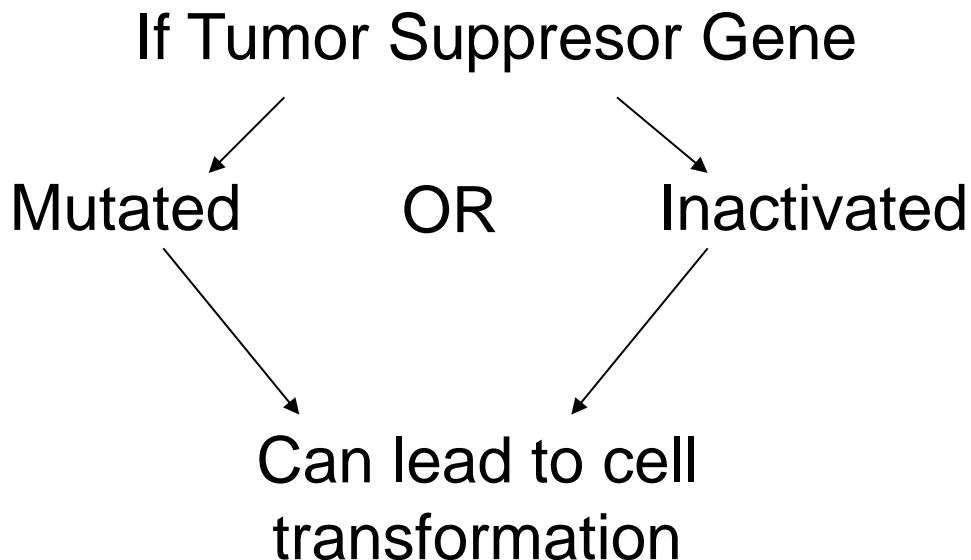


Bcl-2 Action



Tumor Suppressor Genes

Tumor Suppressor genes: are genes that act to inhibit cell proliferation and tumor development.



Tumor Suppressor Genes

- Mutation of tumor suppressor genes

Knudsen Two-hit Model:

- Rb gene and retinoblastoma
- The development of retinoblastoma can be either:
 - Hereditary: a defective copy of Rb gene is inherited from the affected parents.
 - Nonhereditary: in which 2 normal Rb genes are inherited and both develop mutation during life.
- Retinoblastoma is developed if 2 somatic mutations inactivate both copies of Rb in the same cell.

germline

constitutional

tumor

first
mutation



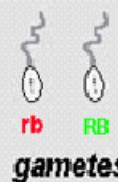
second
mutation



$rb \times RB$

$RB\ rb$

$rb\ rb$



Hereditary
Mutation

Non-
hereditary
Mutation

germline

constitutional

tumor

first & second
mutation



$RB \times RB$

$RB\ RB$

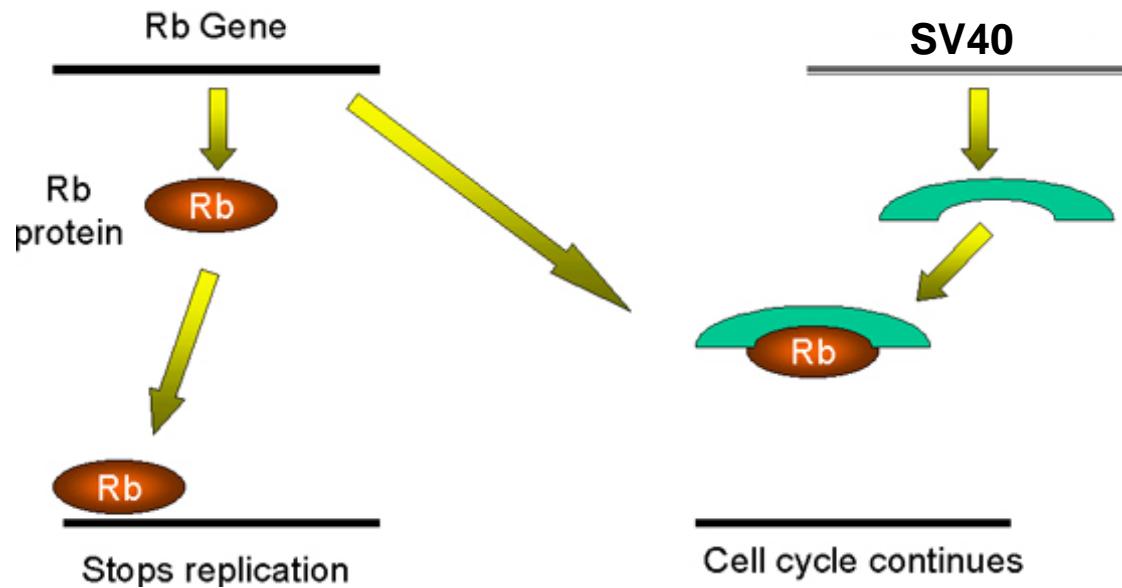


$rb\ rb$



Tumor Suppressor Genes

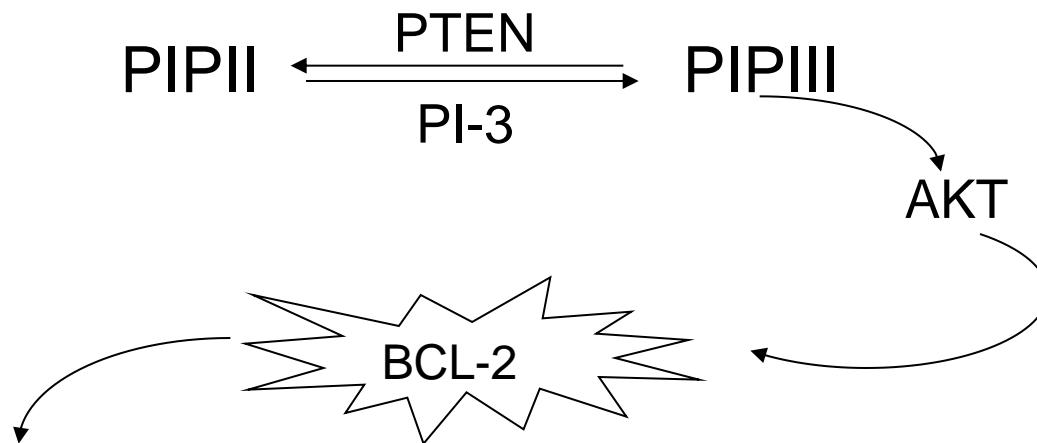
- Inactivation of Tumour suppressor gene
- If the Rb gene interacts with DNA tumour virus (SV40) it will induce cell transformation.



Tumor Suppressor Genes

can antagonize the action of oncogene

PTEN which converts PIP_{III} to PIP_{II} because PIP_{III} will activate PI-3/AKT that activates BCL-2 that will inhibit apoptosis and induce cell transformation



Inhibit apoptosis & induce cell
transformation

Function of Tumor Suppressor Genes

Transcription factors

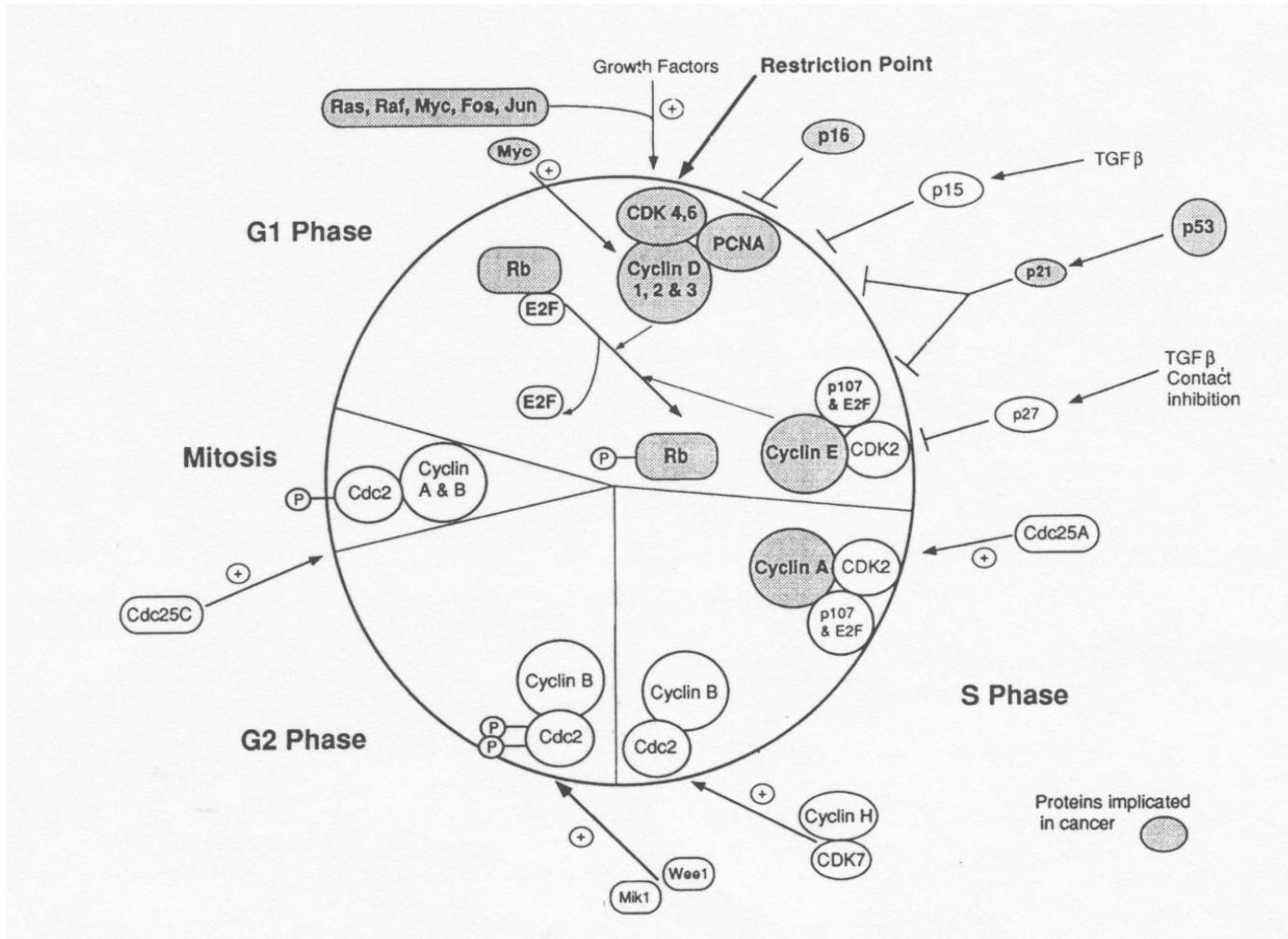
- Repressor transcription factors: example; WT1 is a repressor that appears to suppress transcription factor (Insulin-like Growth Factor, IGF) which will contribute in the development of tumour.
- Activator transcription factors: example; SMAD family that are activated by TGF- β , leading to inhibition of cell proliferation.

Function of Tumour Suppressor Genes

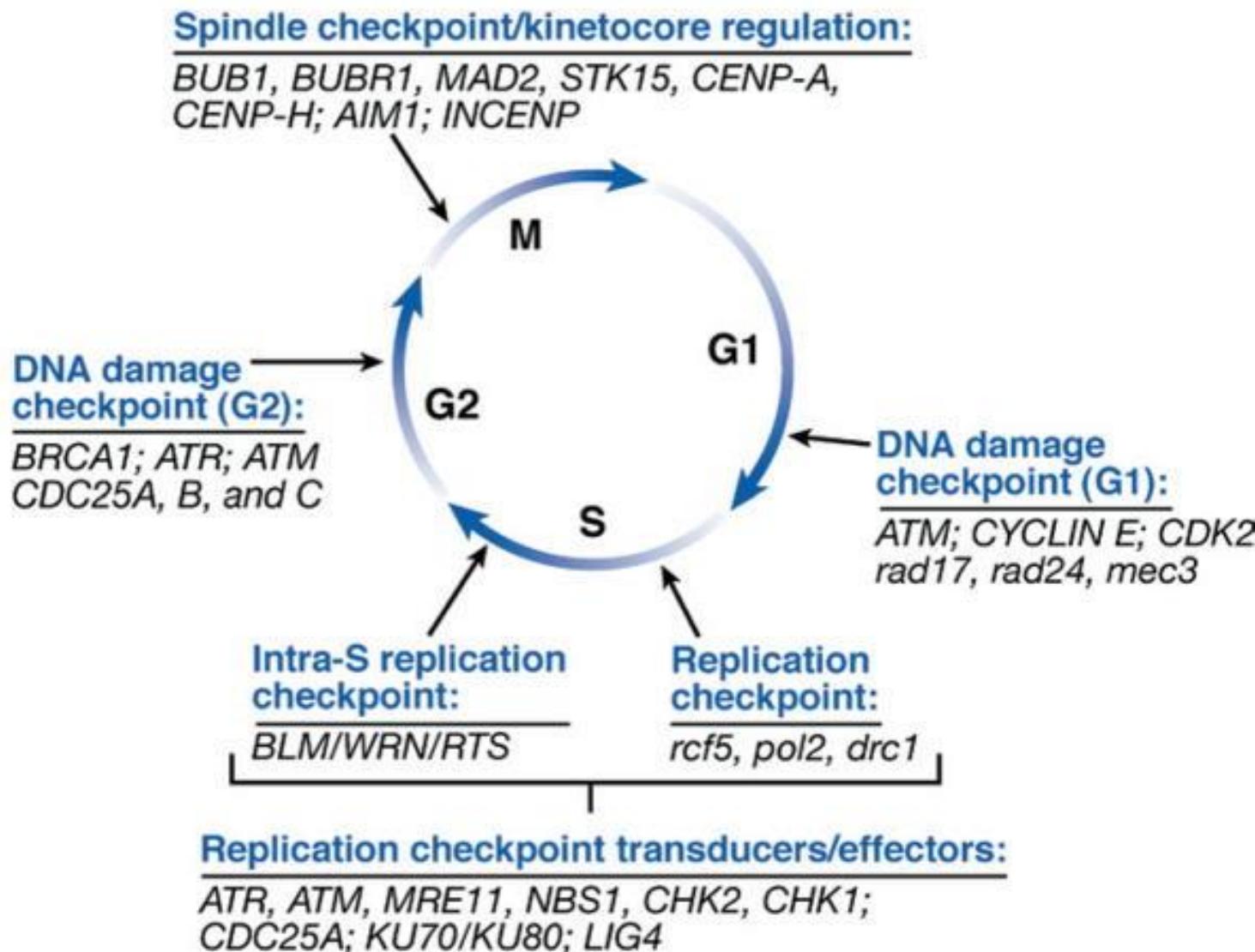
Regulate cell cycle:

- *Rb gene*: that inhibits the cell cycle in the G1 phase decrease cell proliferation.
- *INK-4 gene*: that produces P16 —→ that inhibits cdk4/cyclin D action (to phosphorylate Rb gene to inactivate its action)
- *P53*: that produces P21 that has the same action of P16 in inhibiting the action of cdk4/cyclin D

Cell Cycle Regulators and Cancer



Cell Cycle Regulators and Cancer



Function of Tumor Suppressor Genes

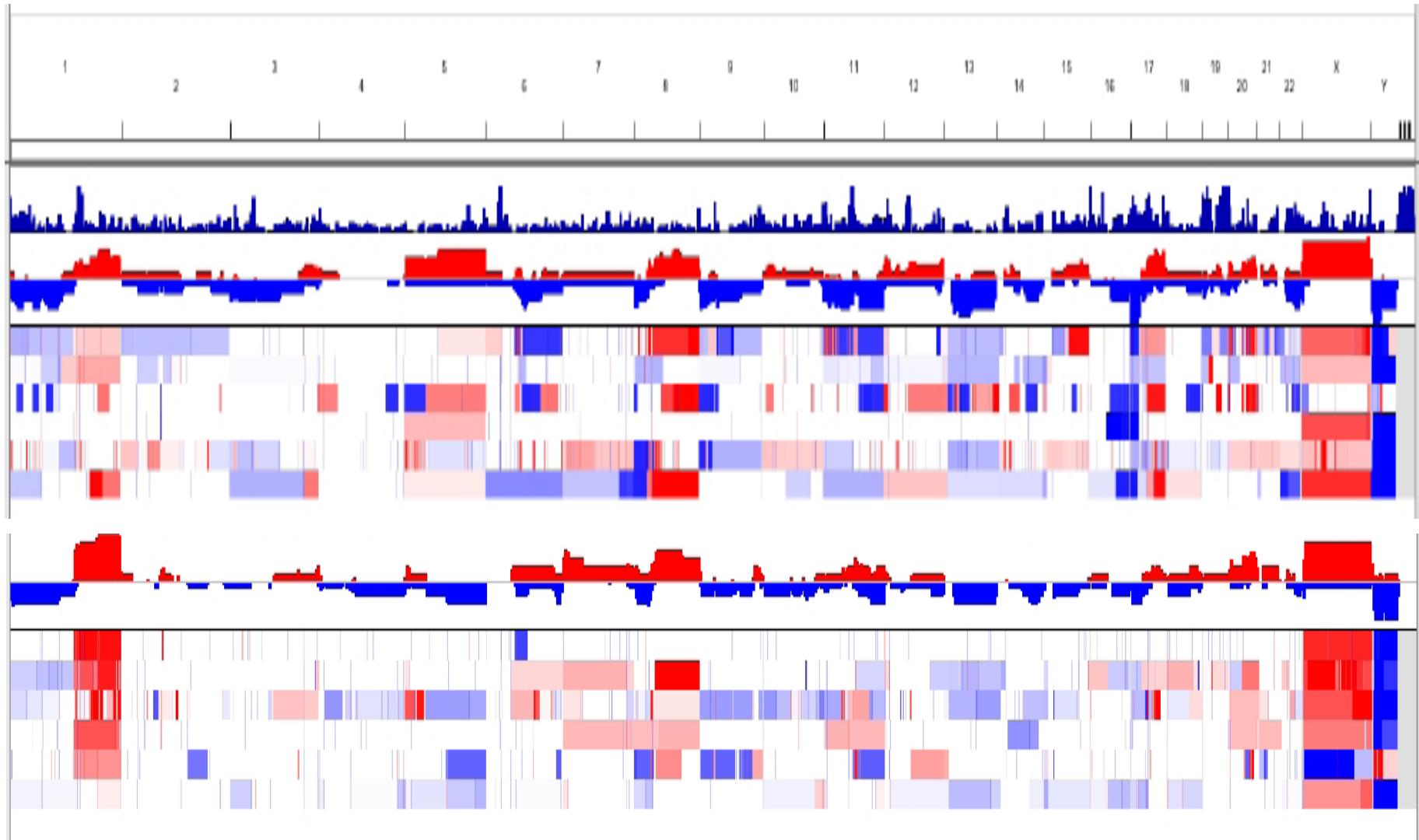
Induce apoptosis:

- An increase in *P53* will:
- increase Bax
- form holes in the mitochondria
- release cytochrome c
- activate apoptosis

Chromosomal Deletions Associated with Specific Neoplasms

1p	Melanoma, Neuroblastoma, Thyroid Cx, Ductal Breast Cx Multiple Endocrine Neoplasia
1q	Breast Carcinoma
3p	Small-cell Lung Cx, Renal Cell Cx, Cervical Cx
5q	Familial Polyposis Coli, Colorectal Cx
11q	Wilm's Kidney Tumor, Breast Cx, Rhabdomyosarcoma, Bladder Cx
13q	Retinoblastoma, Osteogenic Sarcoma Small-cell Ling Cx, Ductal Breast Cx
17p	Small-cell Lung Cx, Colorectal Cx, Breast Cx, Osteosarcoma
17q	Neurofibroma
18q	Colorectal Cx
22	Meningioma, Acoustic neuroma, Pheochromocytoma

Array-CGH of tumors

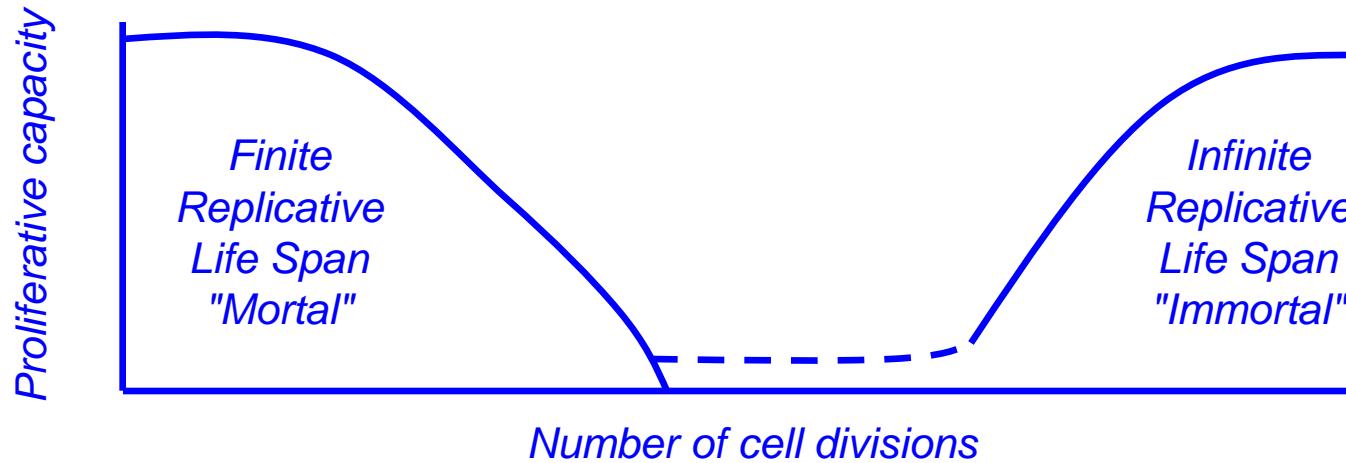


Cellular Senescence

Response of normal cells to potentially cancer-causing events

The senescence response is an anti-cancer mechanisms that prevents the proliferation (growth) of damaged or dysfunctional cells (potential cancer cells)

The Hayflick Limit



EXCEPTIONS
Germ line
Early embryonic cells (stem cells)
Many tumor cells

What happens when cells exhaust their replicative life span?

What happens when cells exhaust their replicative life span?



REPLICATIVE SENESCENCE

- *Irreversible arrest of cell proliferation
(universal)*
- *Resistance to apoptosis
(certain cell types)*
- *Altered function
(universal but cell type specific)*

Cellular Senescence

Cell proliferation (replicative senescence)
= *TELOMERE SHORTENING*

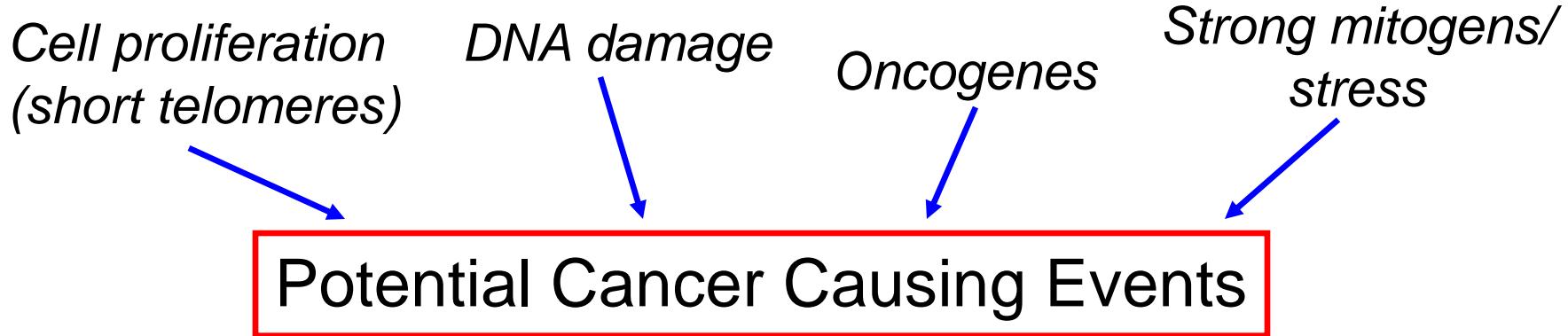
DNA damage

Oncogene expression

Super-mitogenic/stress signals

What do inducers of the senescent phenotype have in common?

Inducers of cellular senescence



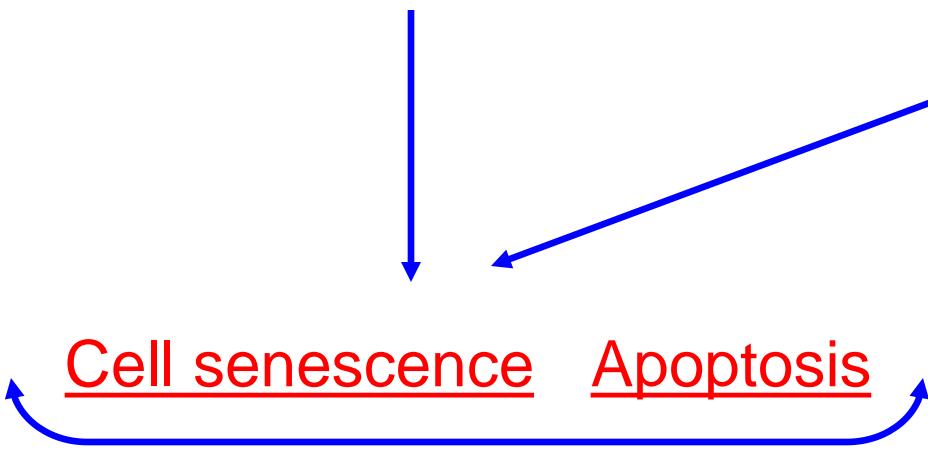
Normal cells
('mortal')

'Immortal' cells
(precancerous)

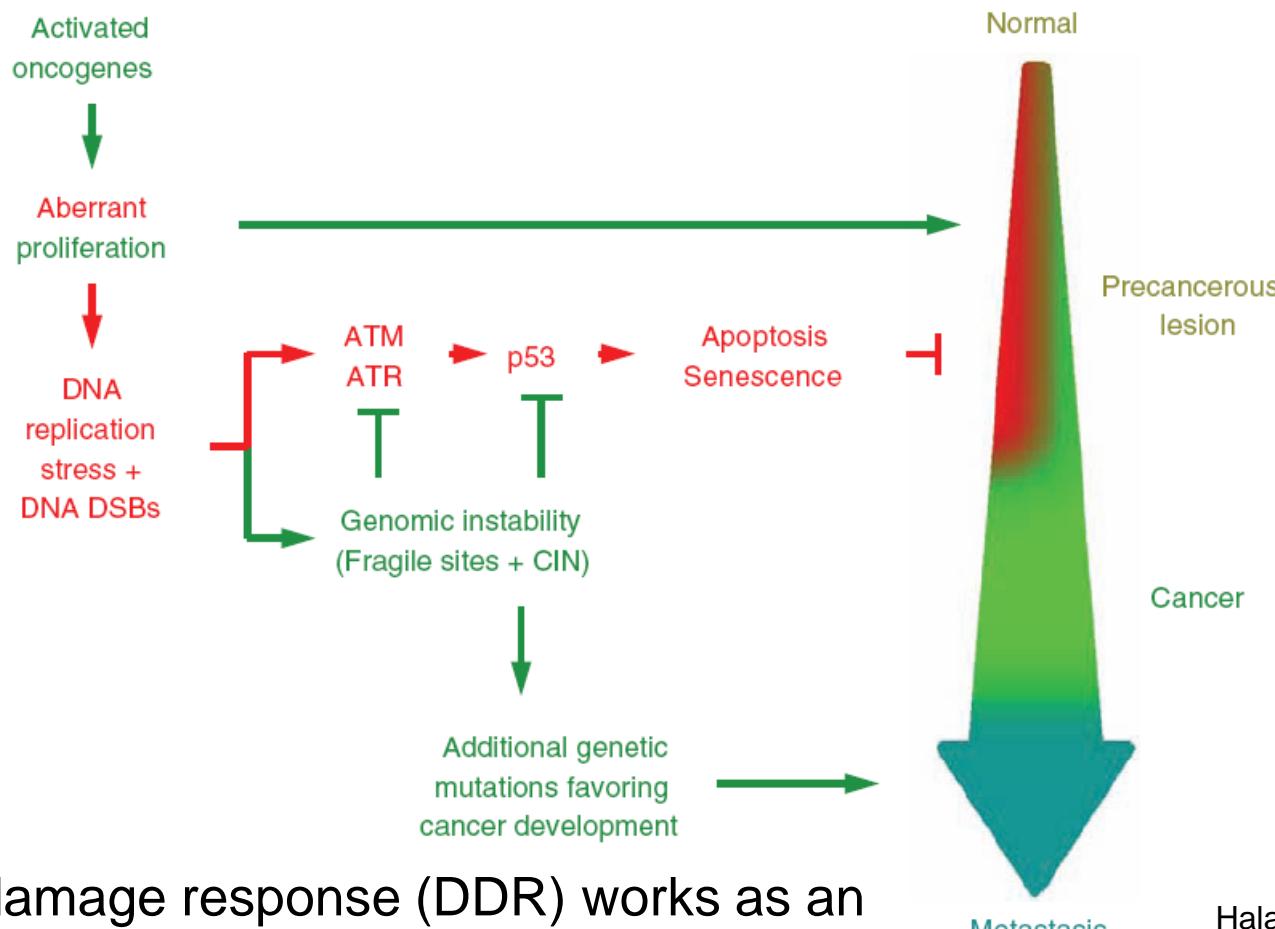
Cell senescence Apoptosis

Transformation

Tumor suppressor mechanisms



DNA Damage Response = Anticancer Barrier



The DNA damage response (DDR) works as an oncogene-inducible biological barrier against progression of cancer beyond its early stages. This model may explain the high frequency of DDR defects in cancer.

Halazonetis et al. 2008,
Science 319, 1352

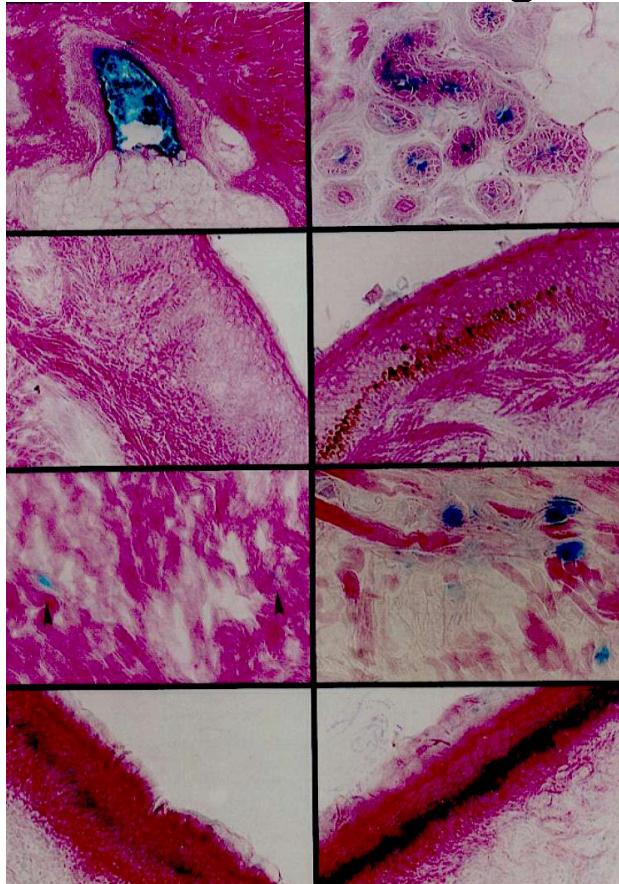
Cellular Senescence

A crucial tumor suppressor mechanism

- Induced by potentially oncogenic events
- Most tumor cells are replicatively immortal
- Many oncogenic mutations allow cells to bypass the senescence response
- Senescence is controlled by the two most important tumor suppressor genes -- **p53** and **pRB**
- Mice with cells that do not senesce die young of cancer

Cellular senescence: Markers in culture and in vivo

*Human skin,
stained for SA-Bgal*



p16 expression

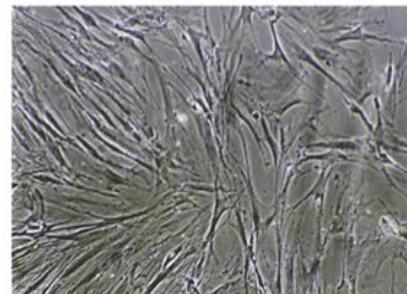
Heterochromatic foci

Telomeric-DNA damage foci

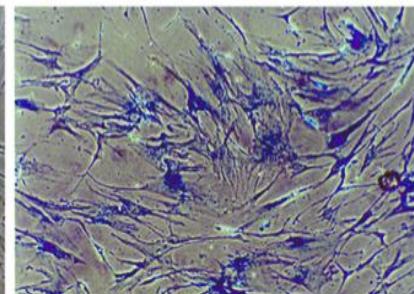
DNA damage foci

Human Fibroblasts, Stained for SA-Bgal

Presenescent

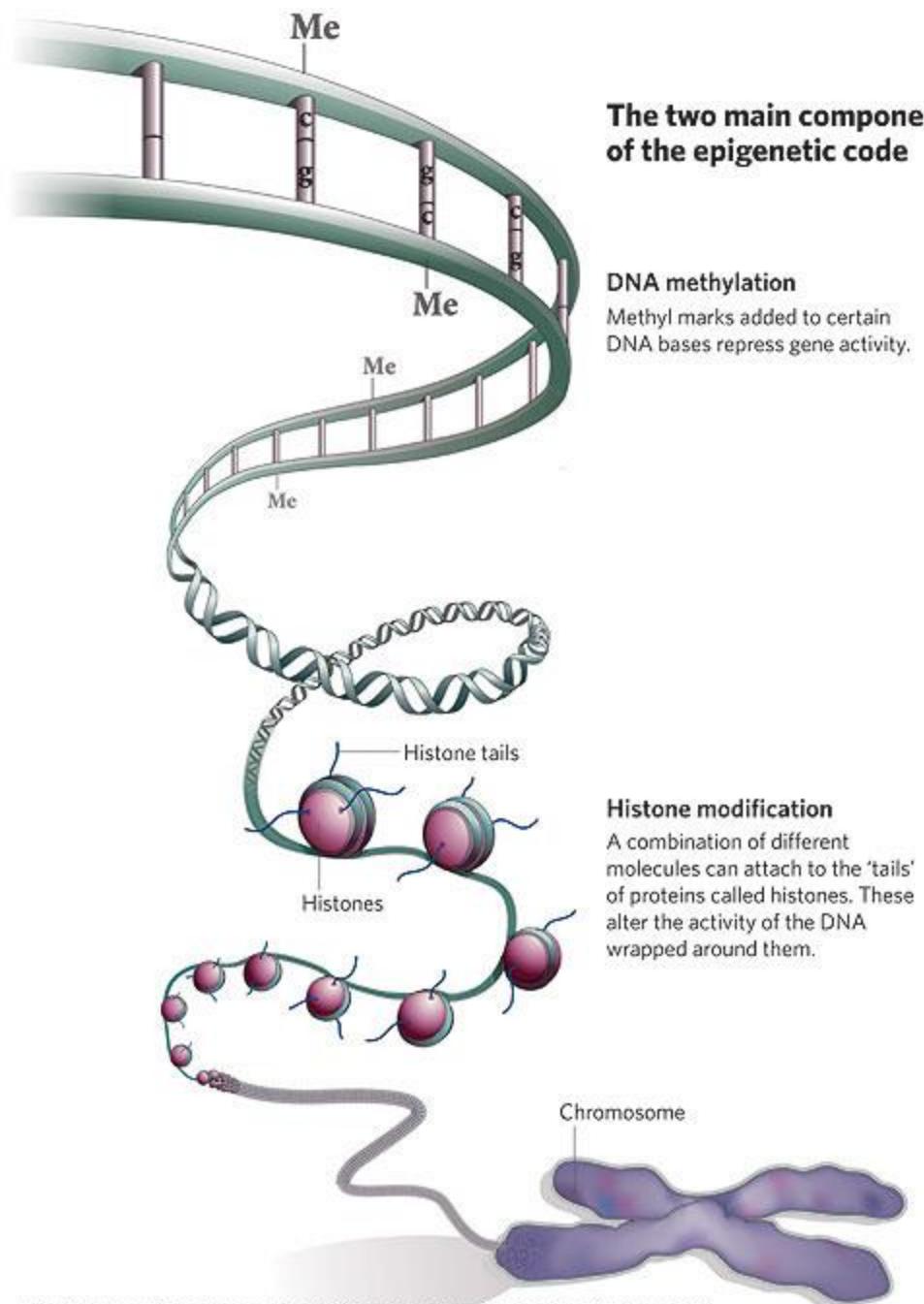


Senescent



Epigenetics

- Epigenetic alterations are heritable changes in the structure and function of the genome that occur without changes in DNA sequence
- In mammalian cells, epigenetic marks consist primarily of DNA methylation, post-translational histone modifications
- Also includes miRNAs and long, noncoding RNAs (we will not cover these today)
- Play key roles in both normal development and differentiation as well as in tumorigenesis



The two main components of the epigenetic code

DNA methylation

Methyl marks added to certain DNA bases repress gene activity.

Histone modification

A combination of different molecules can attach to the 'tails' of proteins called histones. These alter the activity of the DNA wrapped around them.

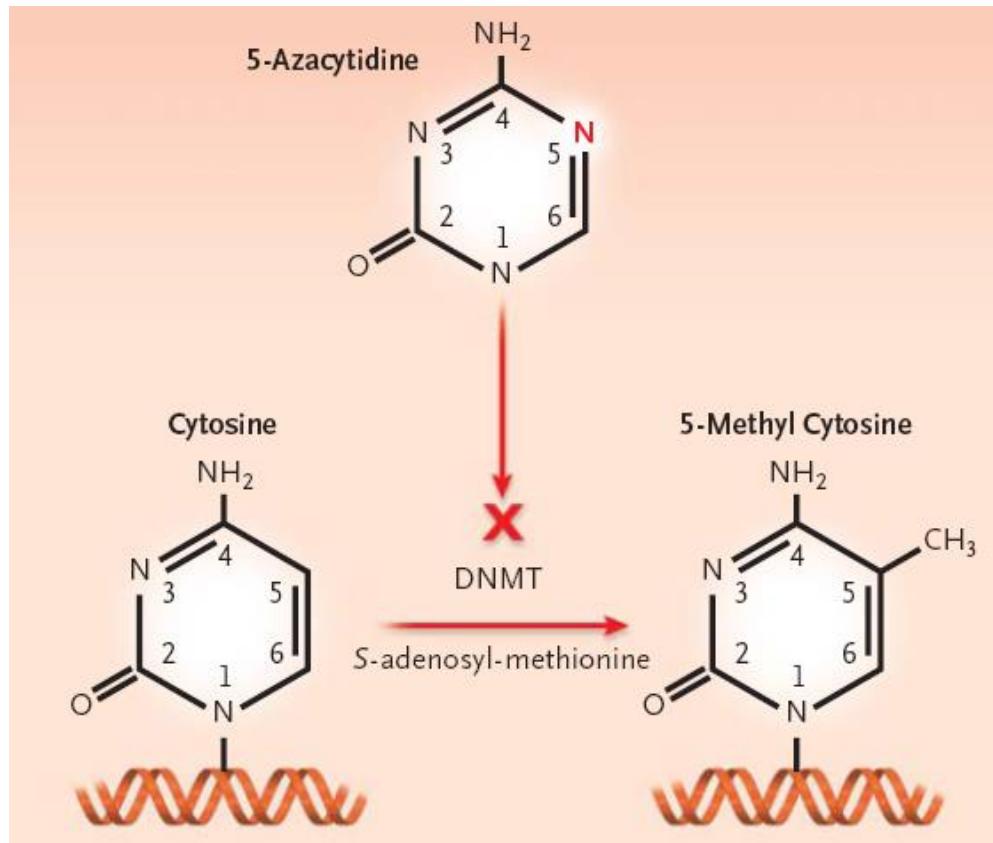
Fundamental Role of Epigenetics

- Widespread changes in DNA methylation and histone code act to properly activate and repress appropriate transcriptional programs during development
- Global changes also affect genome stability and division of euchromatin and heterochromatin

DNA methylation

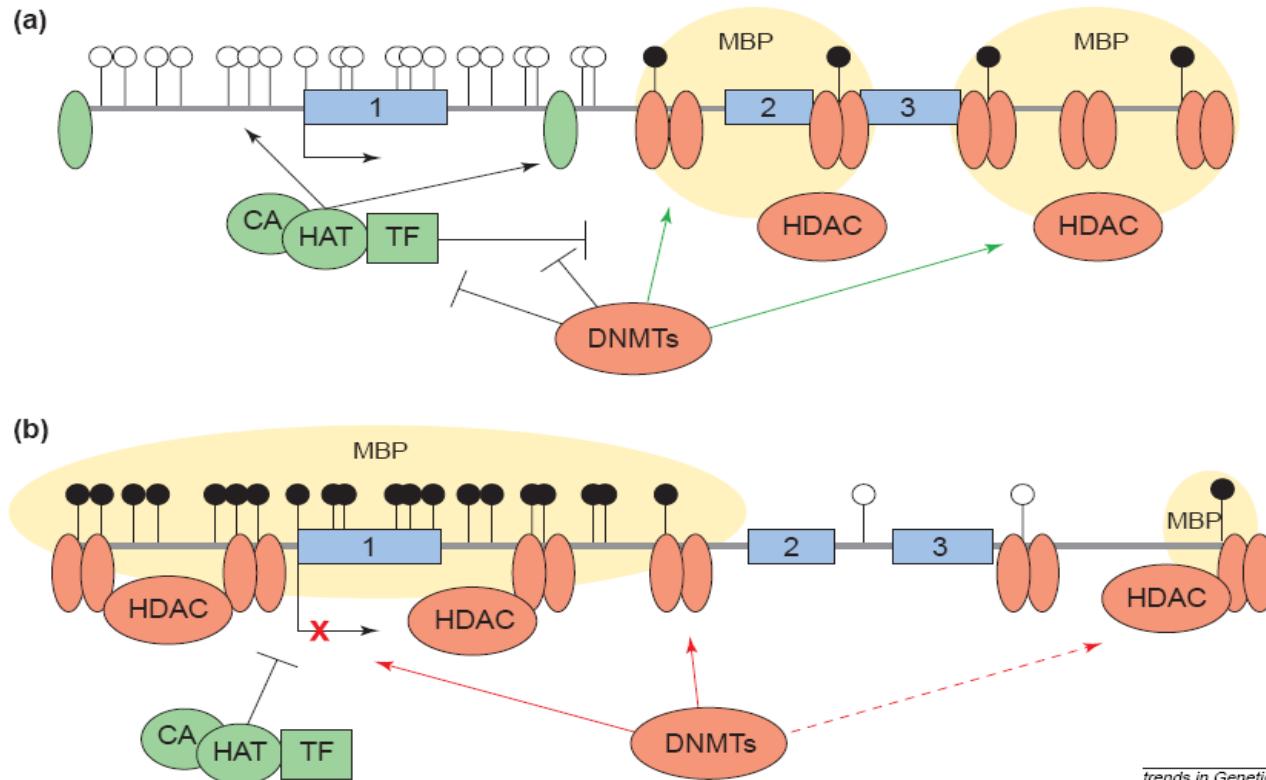
- methylation takes place on the 5' position of cytosine bases that are part of CpG dinucleotides
- CpG dinucleotides have been progressively depleted from the eukaryotic genome over the course of evolution
- The remaining CpG dinucleotides in the mammalian genome are often methylated. m5C constitutes approximately 1-2% of total DNA bases and thus affects about 70% of all CpG dinucleotides in the genome
- This methylation is thought to play an important role in the coordination of chromosomal structure and integrity, facilitating the functional segregation of active and silent chromatin.

DNA methylation

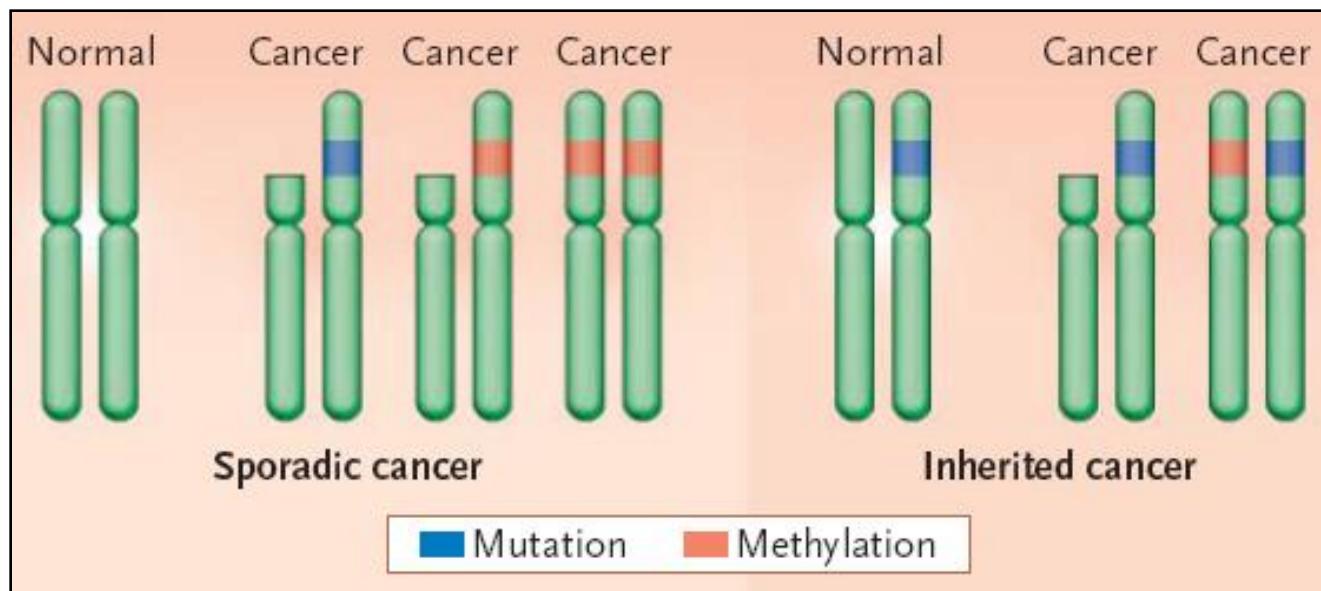


DNA methylation

- Profound rearrangement of genome-wide methylation in cancer



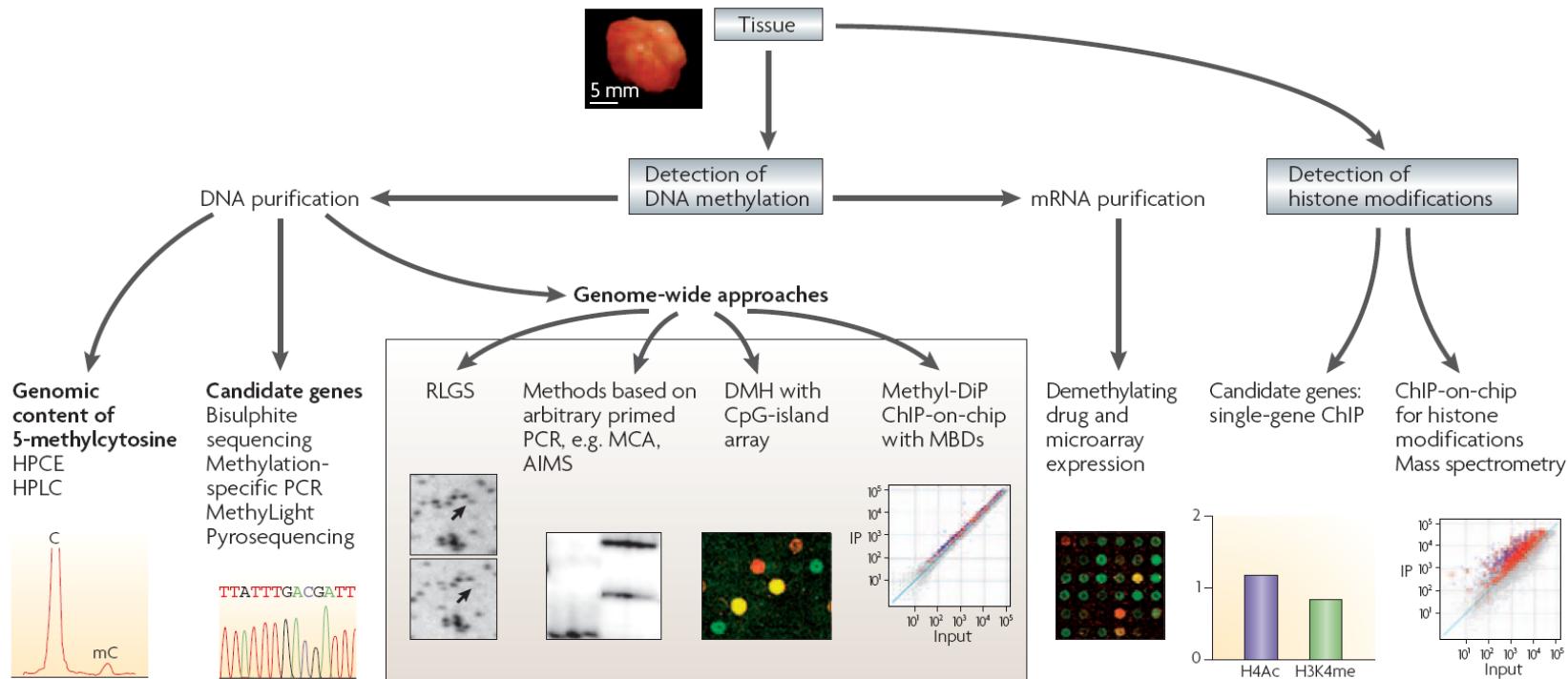
Epimutations help drive oncogenesis



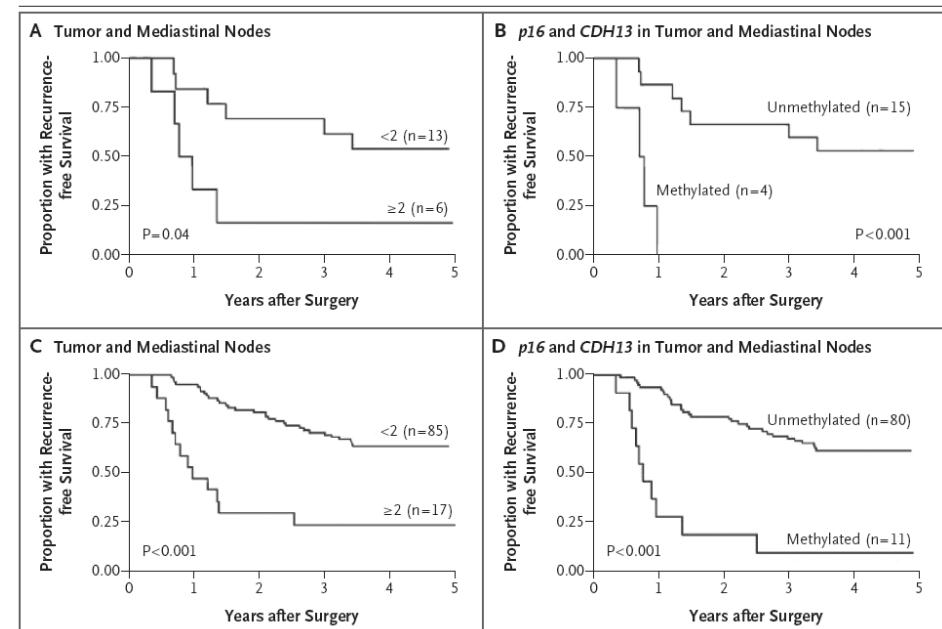
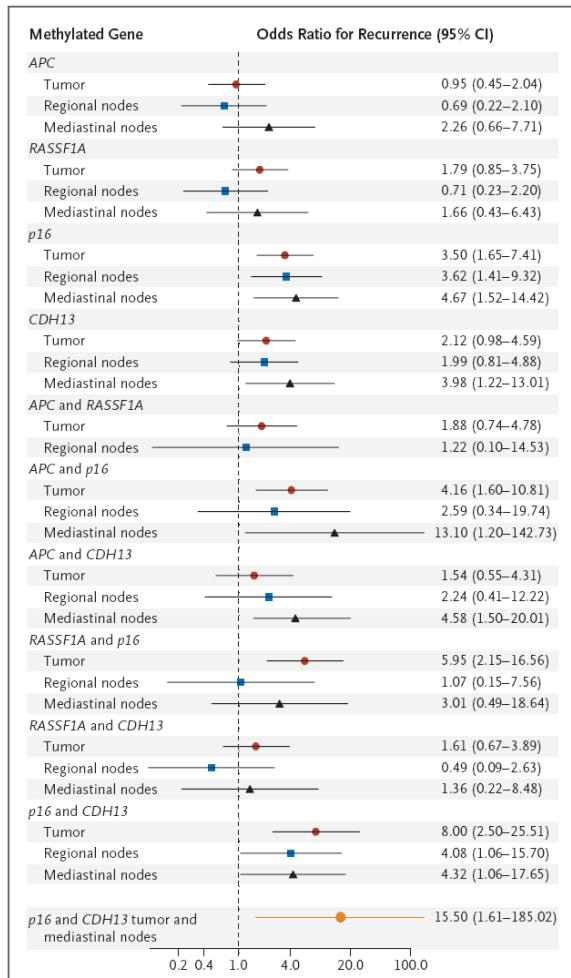
Hypermethylated genes

Gene	Function	Location	Tumour type	Consequences
<i>MLH1</i>	DNA mismatch repair	3p21.3	Colon, endometrium, stomach	Frameshift mutations
<i>BRCA1</i>	DNA repair, transcription	17q21	Breast, ovary	Double-strand breaks?
<i>p16^{INK4a}</i>	Cyclin-dependent kinase inhibitor	9p21	Multiple types	Entrance in cell cycle
<i>p14^{ARF}</i>	MDM2 inhibitor	9p21	Colon, stomach, kidney	Degradation of p53
<i>p15^{INK4b}</i>	Cyclin-dependent kinase inhibitor	9p21	Leukaemia	Entrance into cell cycle
<i>MGMT</i>	DNA repair of 06-alkyl-guanine	10q26	Multiple types	Mutations, chemosensitivity
<i>GSTP1</i>	Conjugation to glutathione	11q13	Prostate, breast, kidney	Adduct accumulation?
<i>p73</i>	p53 homologue	1p36	Lymphoma	Unknown
<i>LKB1/STK11</i>	Serine-threonine kinase	19p13.3	Colon, breast, lung	Unknown
<i>ER</i>	Oestrogen receptor	6q25.1	Breast	Hormone insensitivity
<i>PR</i>	Progesterone receptor	11q22	Breast	Hormone insensitivity
<i>AR</i>	Androgen receptor	Xq11	Prostate	Hormone insensitivity
<i>PRLR</i>	Prolactin receptor	5p13-p12	Breast	Hormone insensitivity
<i>TSHR</i>	Thyroid-stimulating hormone receptor	14q31	Thyroid	Hormone insensitivity
<i>RARβ2</i>	Retinoic acid receptor- β 2	3p24	Colon, lung, head and neck	Vitamin insensitivity?
<i>CRBP1</i>	Retinol-binding protein	3q21-q22	Colon, stomach, lymphoma	Vitamin insensitivity?
<i>RASSF1A</i>	Ras effector homologue	3p21.3	Multiple types	Unknown
<i>NORE1A</i>	Ras effector homologue	1q32	Lung	Unknown
<i>VHL</i>	Ubiquitin ligase component	3p25	Kidney, haemangioblastoma	Loss of hypoxic response?
<i>Rb</i>	Cell-cycle inhibitor	13q14	Retinoblastoma	Entrance into cell cycle
<i>THBS1</i>	Thrombospondin-1, Anti-angiogenic	15q15	Glioma	Neovascularization
<i>CDH1</i>	E cadherin, cell adhesion	16q22.1	Breast, stomach, Leukaemia	Dissemination
<i>CDH13</i>	H cadherin, cell adhesion	16q24	Breast, lung	Dissemination?
<i>FAT</i>	Cadherin, tumour suppressor	4q34-35	Colon	Dissemination?
<i>HIC1</i>	Transcription factor	17p13.3	Multiple types	Unknown
<i>APC</i>	Inhibitor of β -catenin	5q21	Aerodigestive tract	Activation β -catenin route
<i>SFRP1</i>	Secreted frizzled-related protein 1	8p12-p11	Colon	Activation Wnt signalling
<i>DKK1</i>	Extracellular Wnt inhibitor	10q11.2	Colon	Activation Wnt signalling

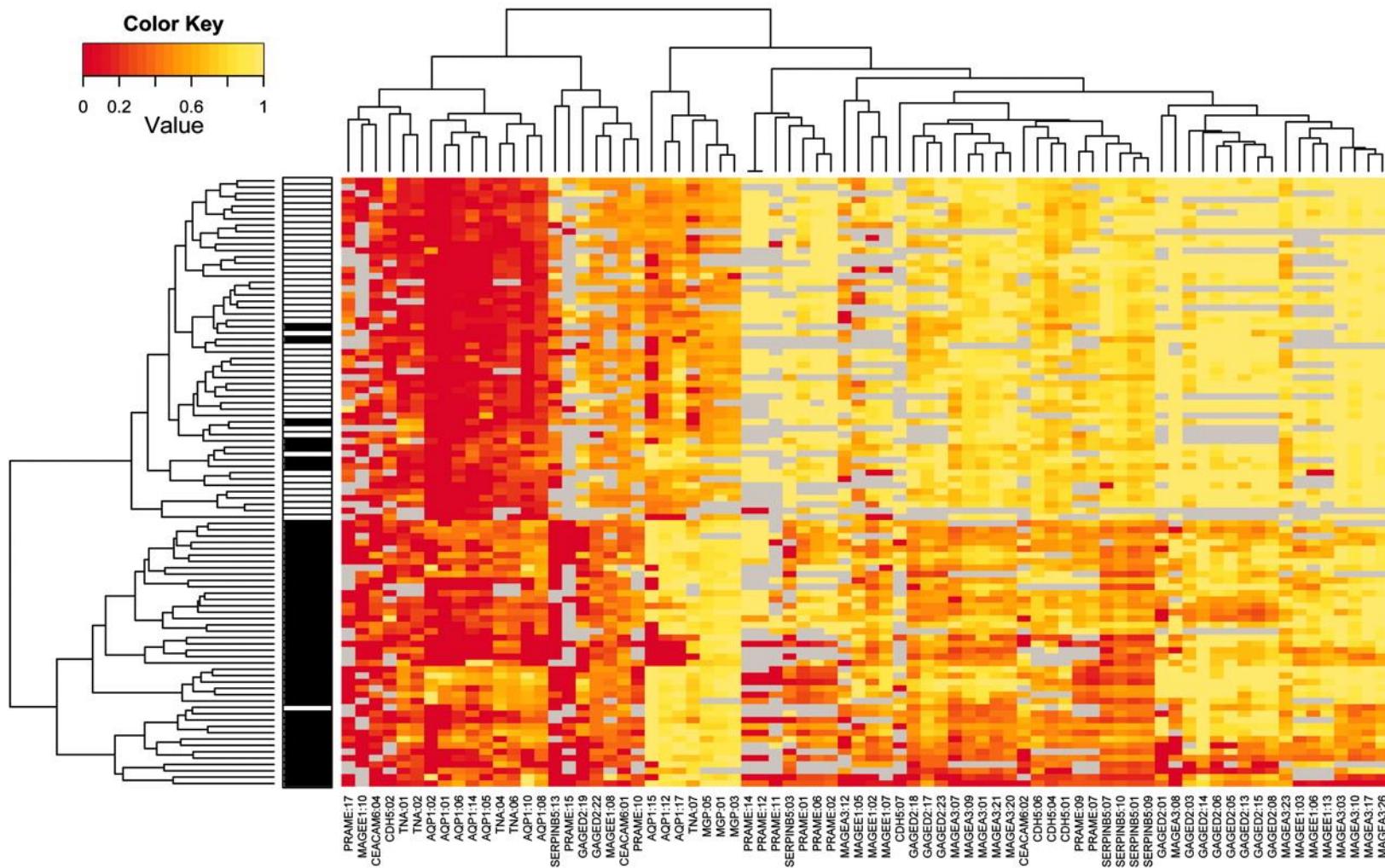
Techniques for studying the epigenetics of cancer



Methylation and lung cancer prognosis



A two-way hierarchical cluster analysis of the relative methylation of 68 CpG units (columns) measured on 96 tissue samples from 48 lung cancer cases (rows).



Ehrich M et al. PNAS 2005;102:15785-15790

MGMT methylation predicts for response to treatment in GBM patients

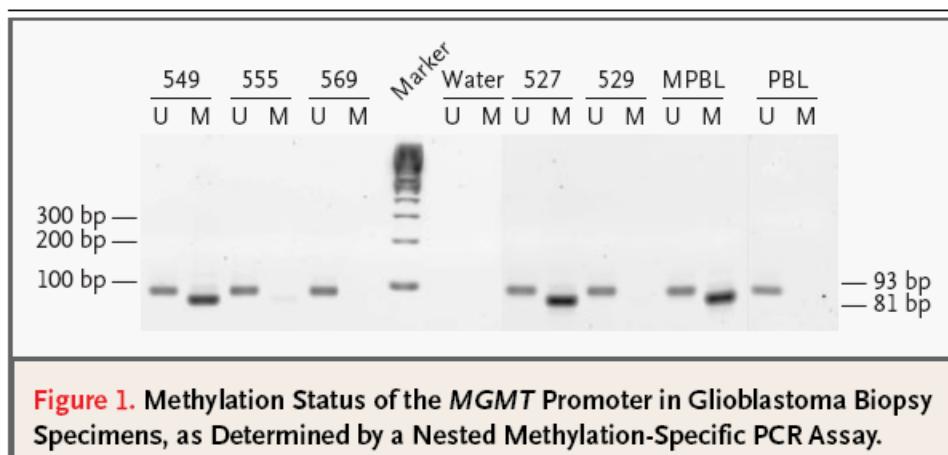
The NEW ENGLAND JOURNAL of MEDICINE

ORIGINAL ARTICLE

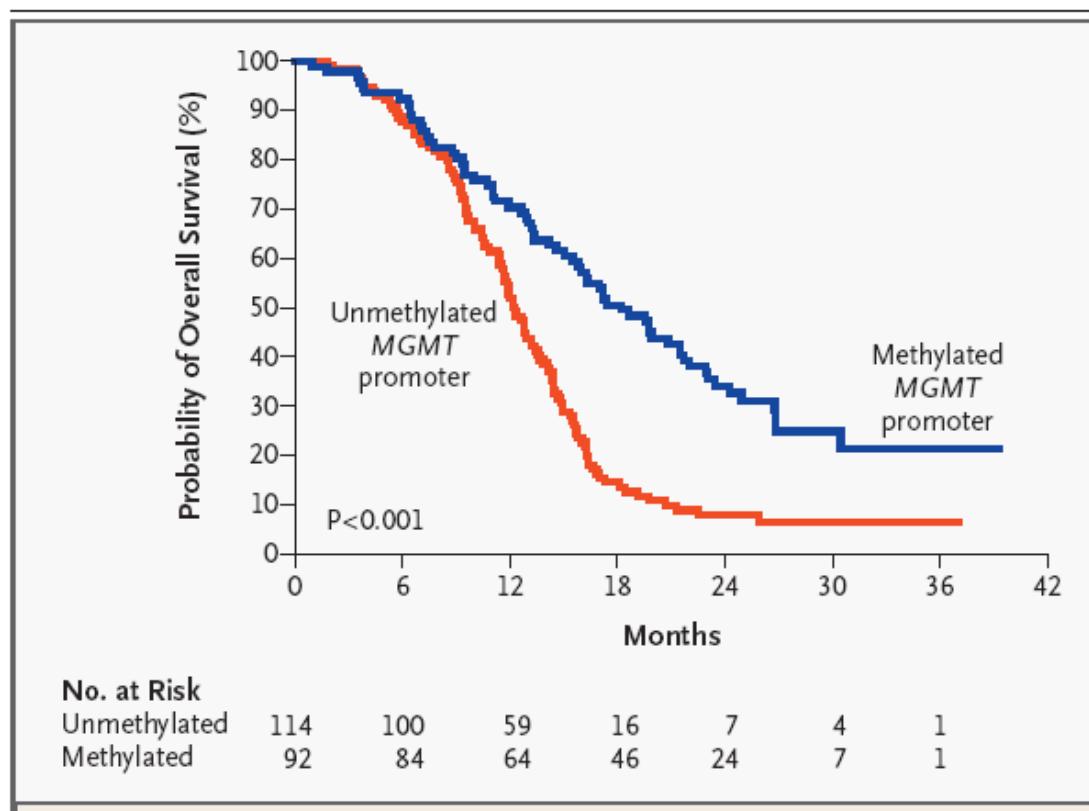
MGMT Gene Silencing and Benefit from Temozolomide in Glioblastoma

Monika E. Hegi, Ph.D., Annie-Claire Diserens, M.Sc., Thierry Gorlia, M.Sc.,
Marie-France Hamou, Nicolas de Tribolet, M.D., Michael Weller, M.D.,
Johan M. Kros, M.D., Johannes A. Hainfellner, M.D., Warren Mason, M.D.,
Luigi Mariani, M.D., Jacqueline E.C. Bromberg, M.D., Peter Hau, M.D.,
René O. Mirimanoff, M.D., J. Gregory Cairncross, M.D., Robert C. Janzer, M.D.,
and Roger Stupp, M.D.

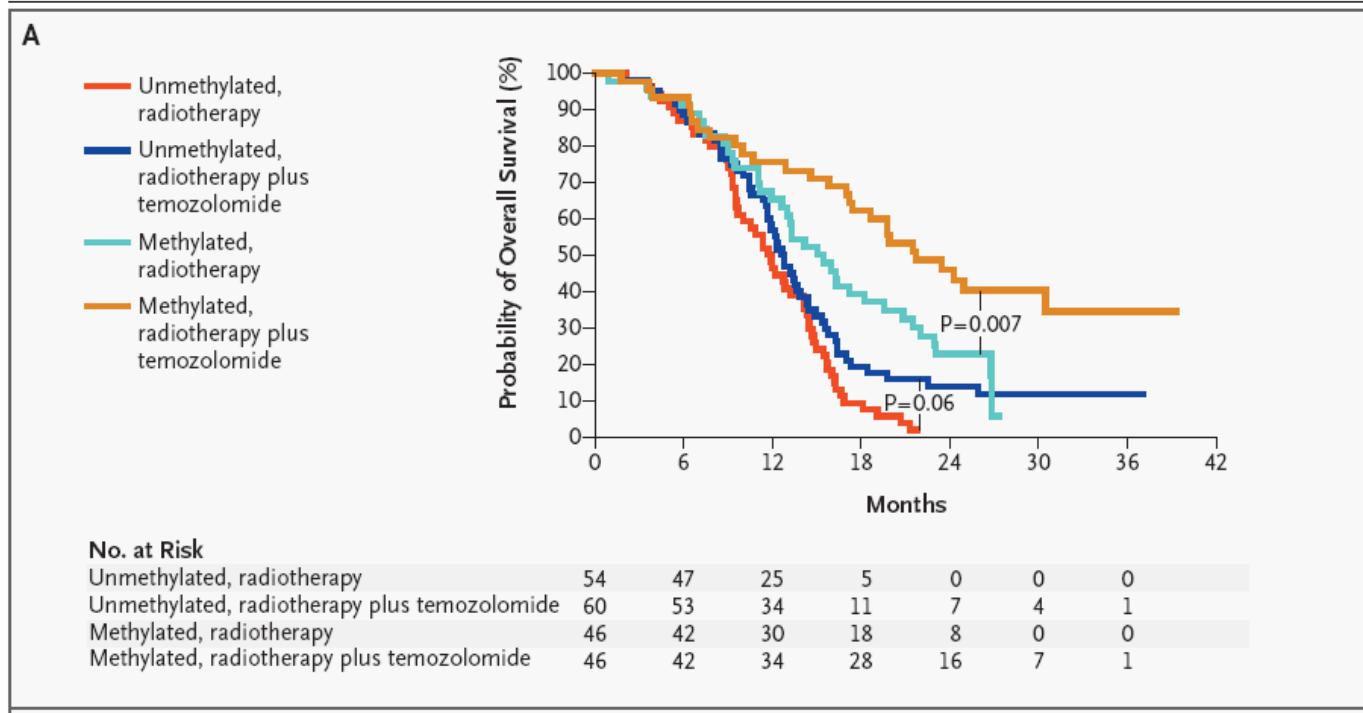
O6-methylguanine DNA methyltransferase
-DNA repair enzyme
-Reverses alkylator damage to DNA



Overall survival by MGMT promoter methylation status

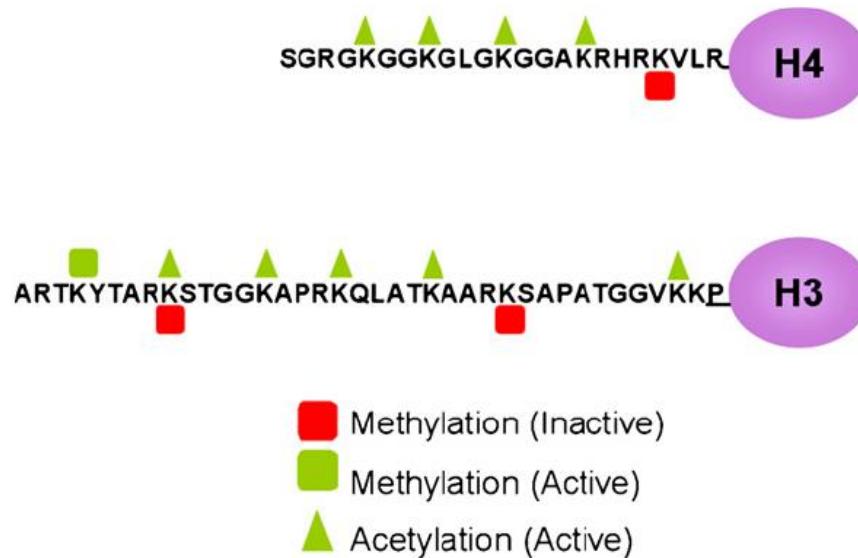


Overall survival by MGMT methylation status and treatment arms

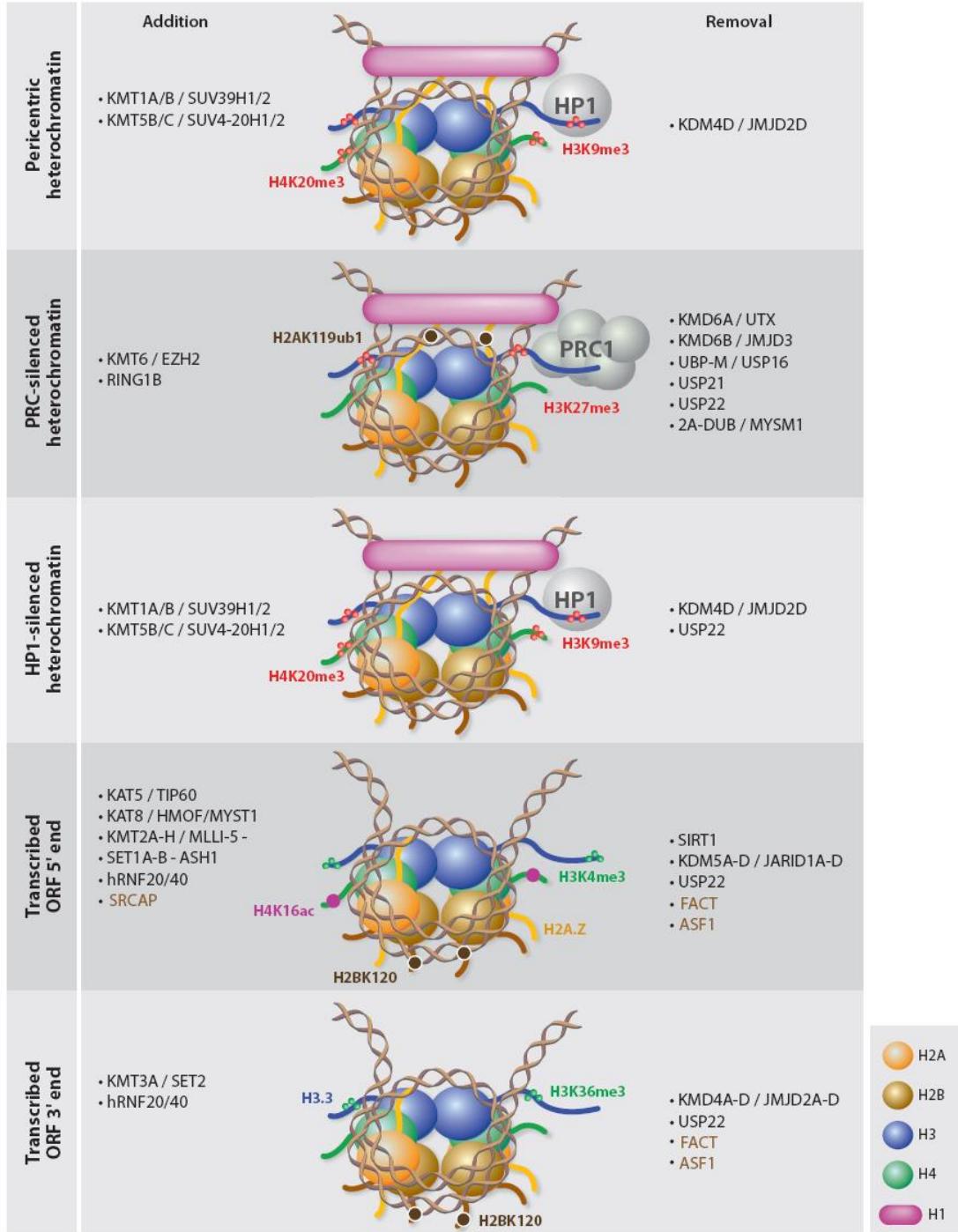


Post-translational histone modifications (histone code)

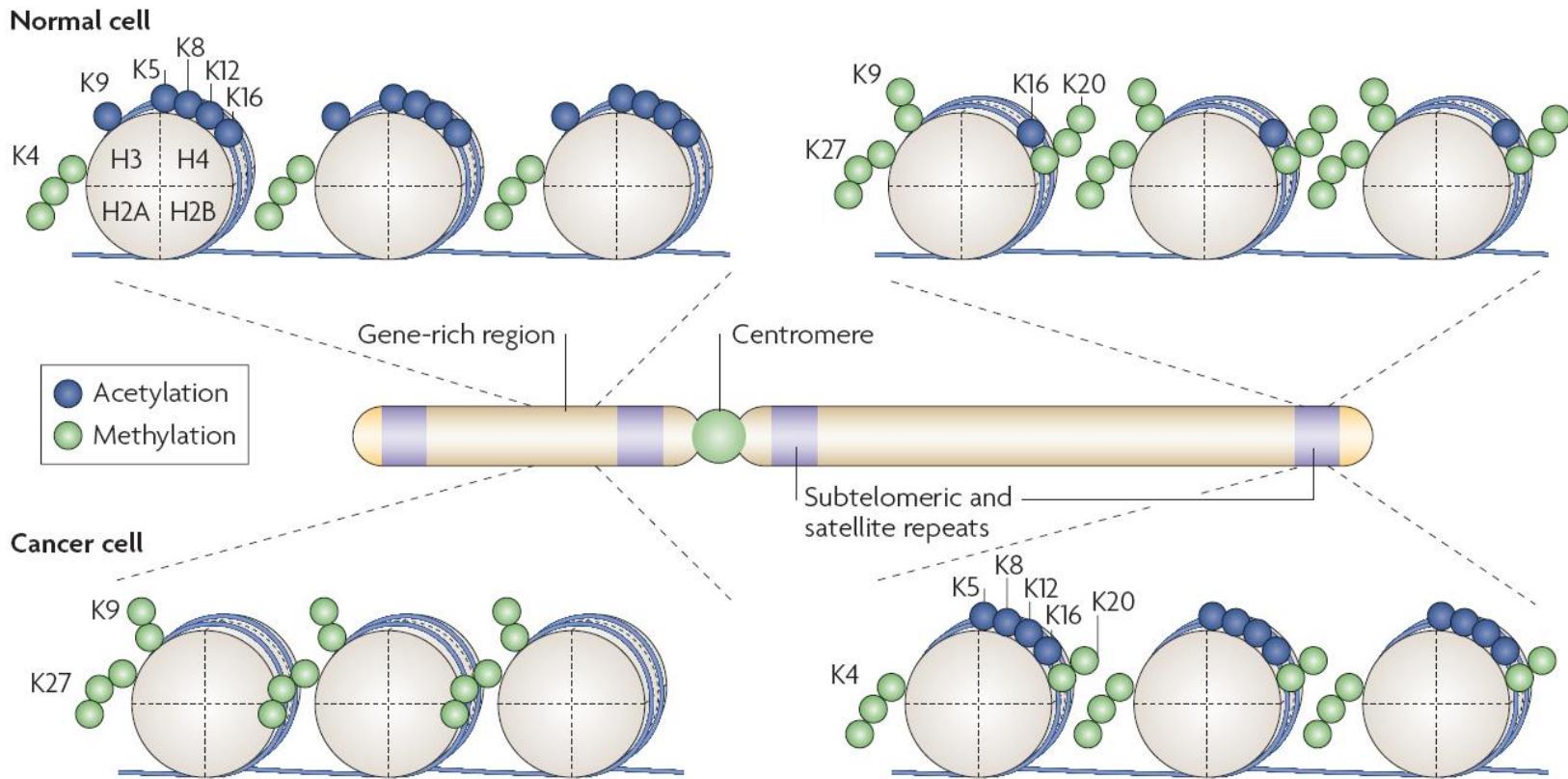
- Modulators of transcription and histone function
- Regulators of chromatin state and compaction



Histone modifications



Histone code alterations in cancer

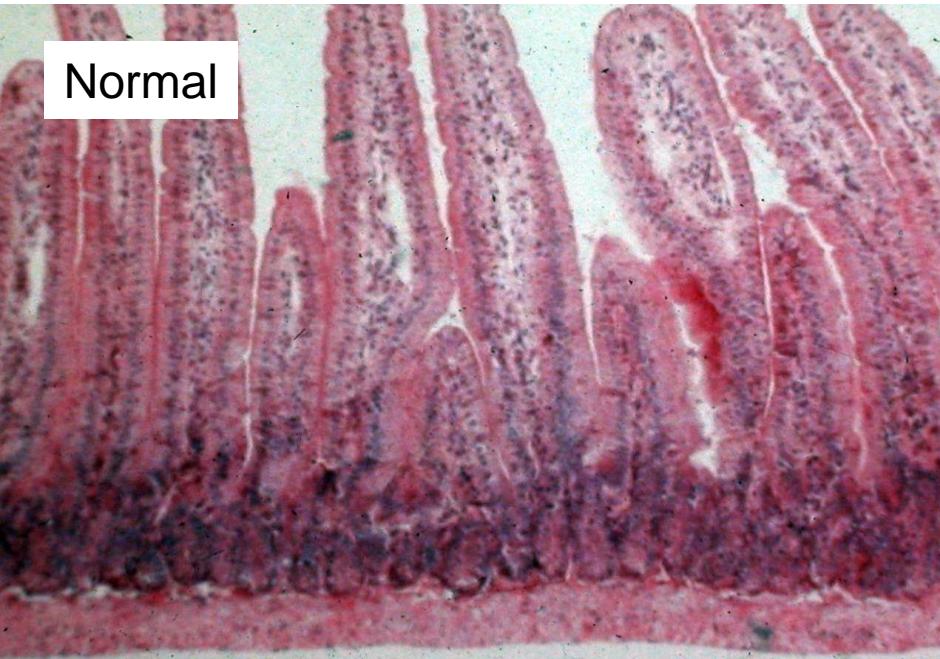


Factors that Influence Tissue Tolerance

- Intrinsic cellular radiosensitivity and repair
- Regeneration potential
- Structural organization of tissues
- Cytokines and Growth Factors

Murine Small Intestine

Normal



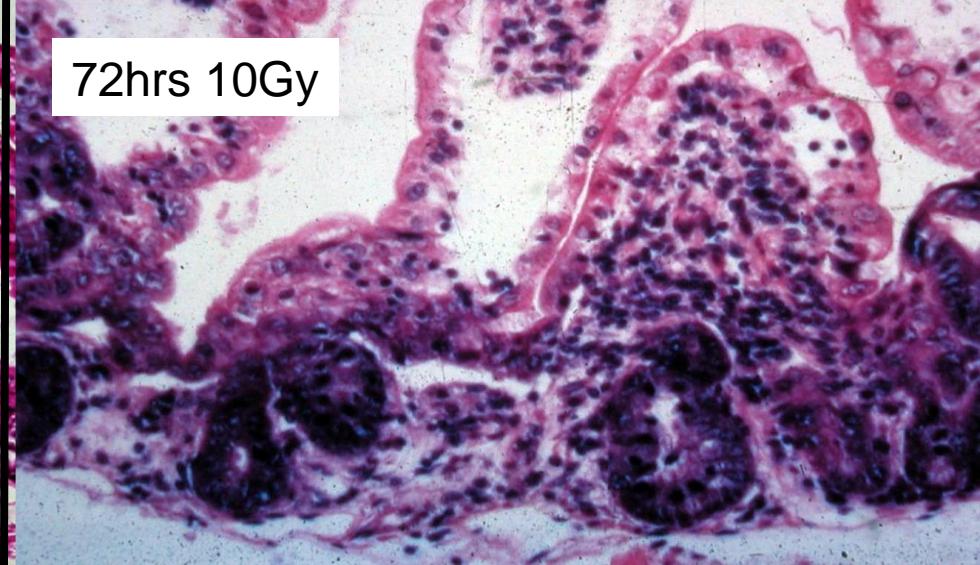
24hrs 10Gy



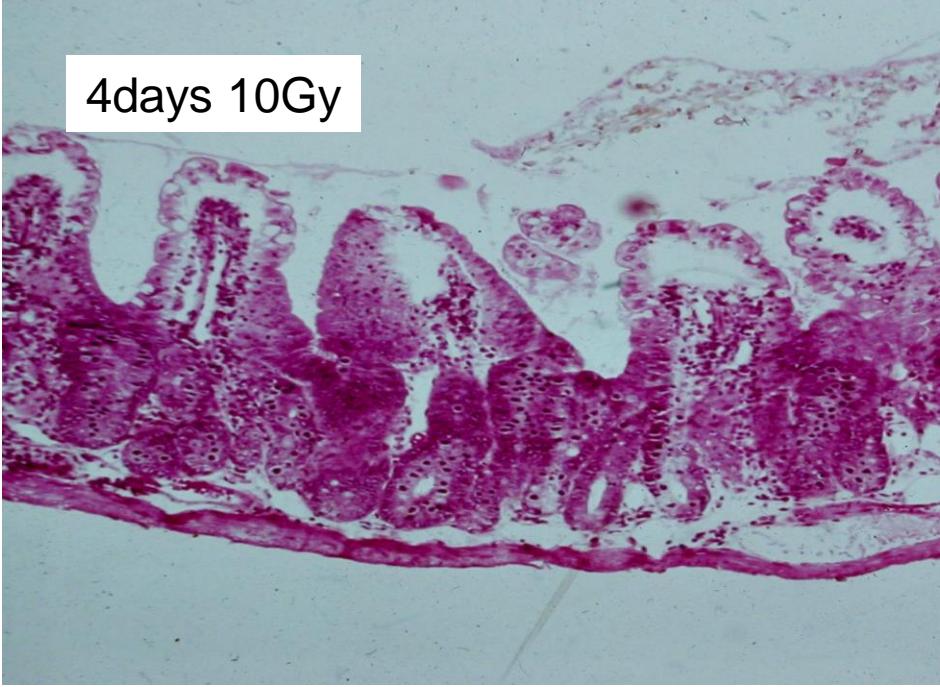
48hrs 10Gy



72hrs 10Gy



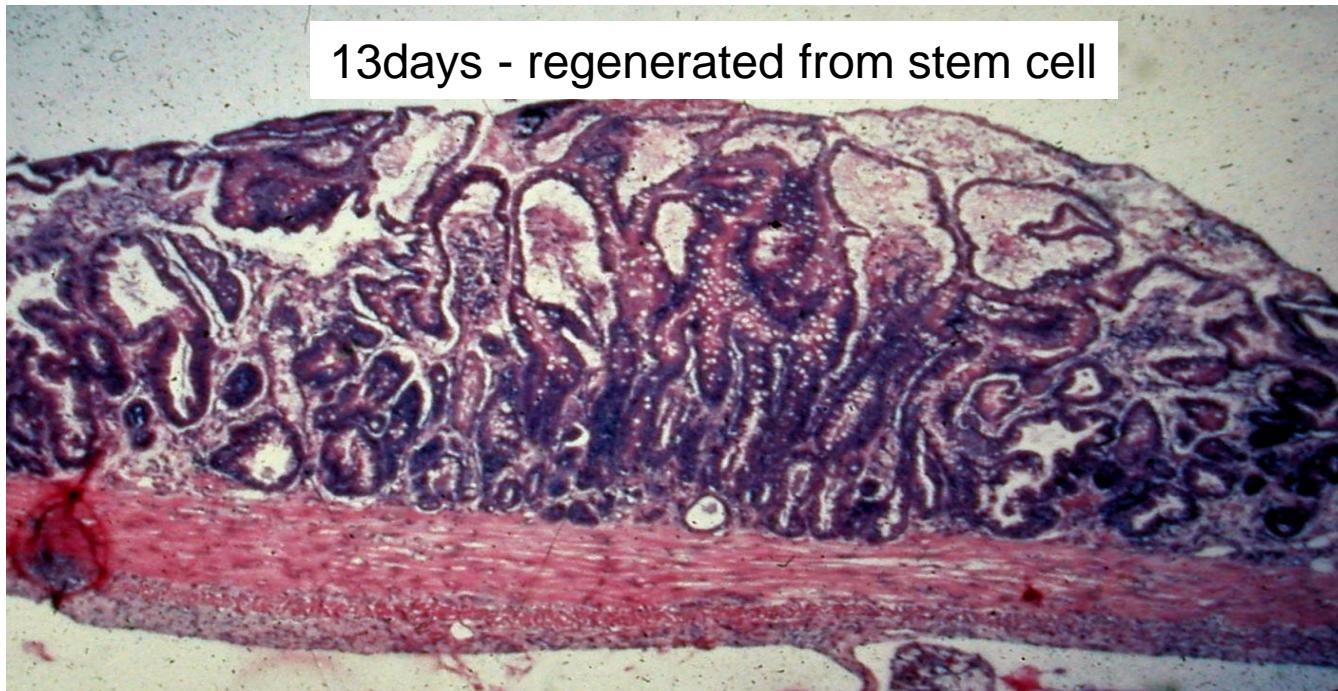
4days 10Gy



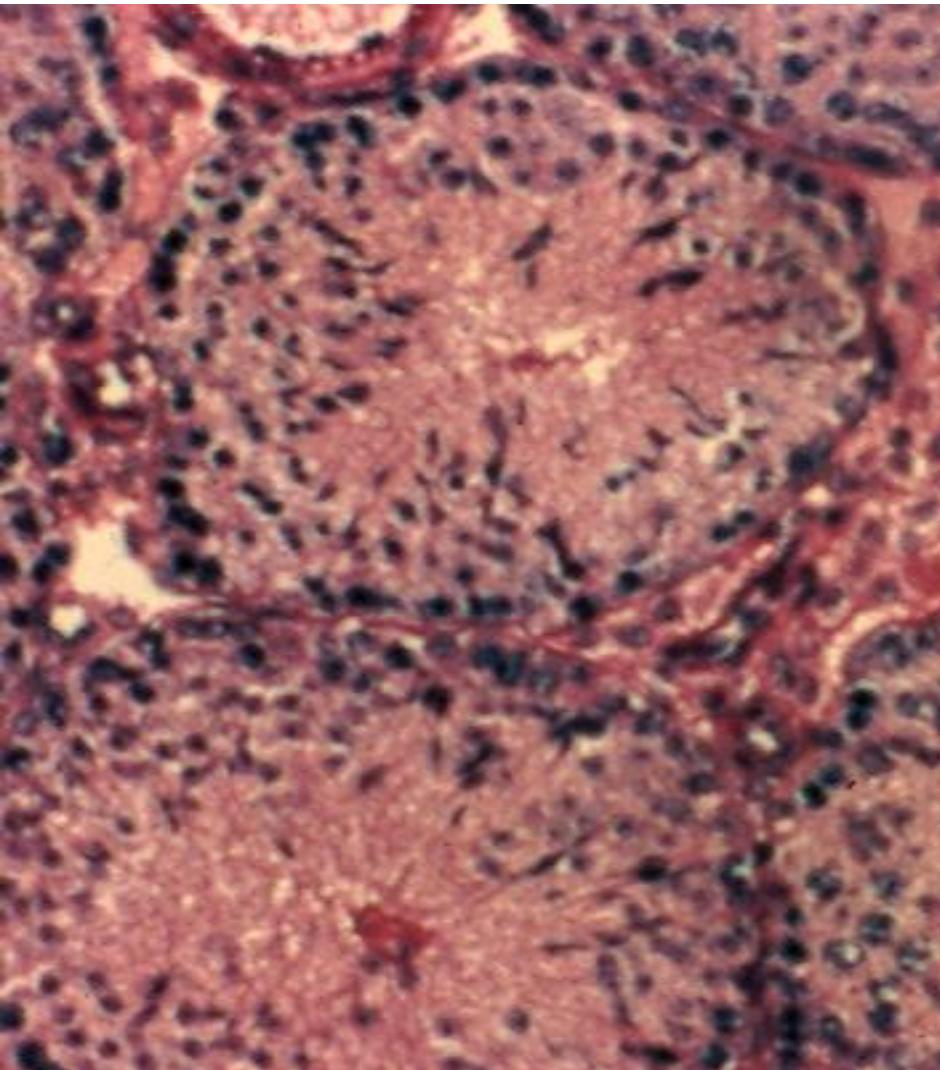
5days 10Gy



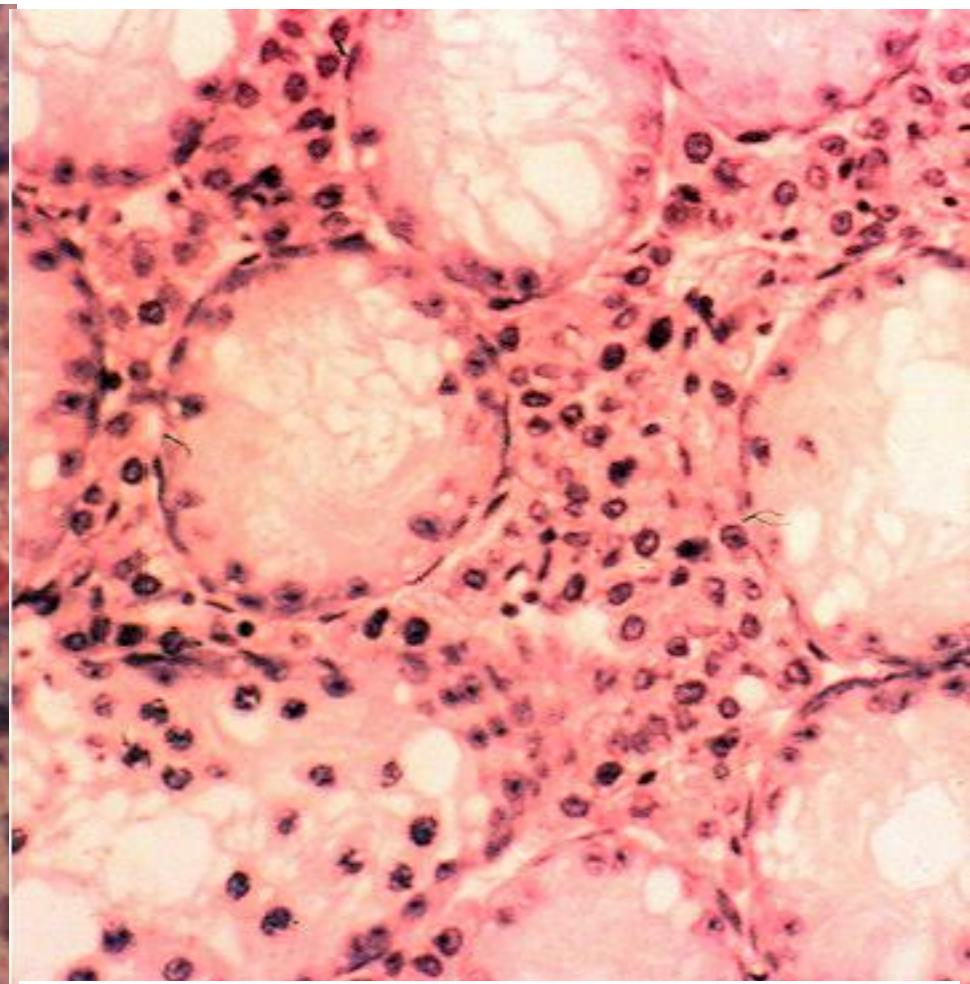
13days - regenerated from stem cell



Murine Testis



Testicular tubules containing spermatagonia



5 weeks after XRT - sertoli cells and surviving spermatogonia showing no repopulation

➤ Gut

- **Latency** = 14 days - time taken for epithelial cells to move up the villus and be shed into the lumen.
- **Tolerance** is about 50 Gy in 2 Gy fx for the small intestine and higher for the large intestine.
- **Regeneration** occurs with a lag time of <24hrs

➤ Testis

- **Latency** = 60 days - time for 1 spermatogenic stem cell to give 1000 sperm. This is why sperm counts remain normal for weeks after irradiation and then fall precipitously.
- **Tolerance** is about 4 - 8 Gy in 2 Gy fractions can cause permanent sterility. 0.1 - 0.15 Gy can cause temporary sterility.
- **Regeneration:** Unlike the gut, where recovery can be complete, sperm counts may not recover for years - there is little regeneration.

Clinical Relevance

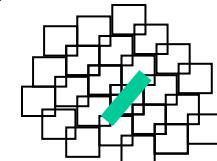
- Mucositis appears 2 to 3 weeks after the start of a standard RT course.
- Regeneration begins at about 10 to 12 days, before evident mucositis. This can increase the tolerance of the mucosa by about 1Gy/day, which is equivalent to clonogenic cell number doubling every 2 days.

FSUs and Volume Effects

➤ FSUs arranged in parallel e.g. lung, liver, kidney

In tissues that are intrinsically radiosensitive and rely on a functional reserve:

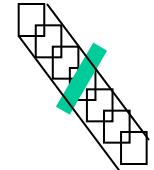
- a low dose to a large volume can be hazardous
- But a high dose to small volume may be innocuous
 - This is because function is determined by the amount of tissue that is not irradiated i.e. the “reserve function” determines the “volume” effect, which will be large.



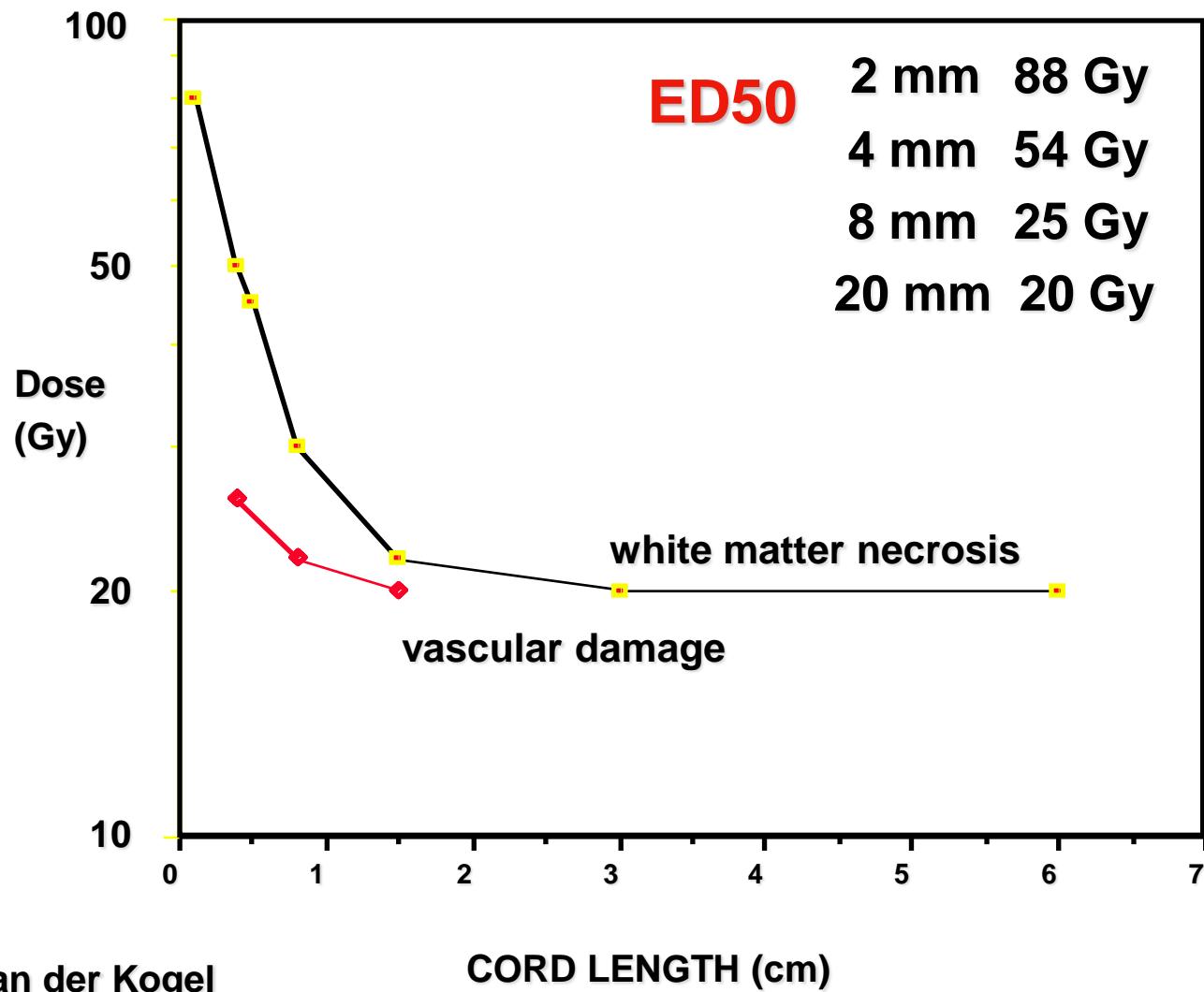
➤ FSUs arranged in series eg. spinal cord, nerves

In tissues that are intrinsically radioresistant, and rely on cell migration:

- A low dose to a large volume may be innocuous
- But a high dose to a small volume may be hazardous
 - i.e. a strong volume effect over a short distance

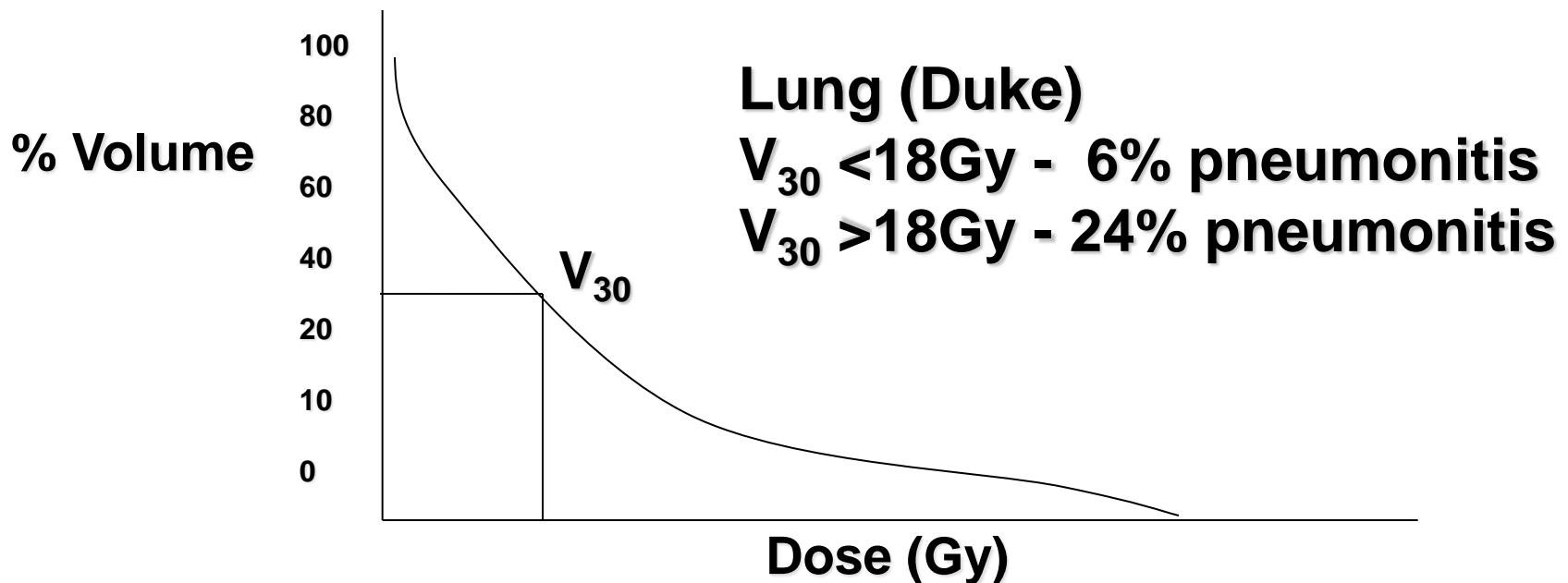


Rat Spinal Cord Volume Effect



Dose Volume Histograms

- The dose of radiation delivered can be distributed in a 3-D volume with smaller subunits each receiving different doses of radiation.
- May be a useful guide in comparing treatment plans to predict development of normal tissue complications, but there are potential pitfalls



DVH Analyses

- May hide “hot spots” resulting from dose heterogeneity
- Ignores structural heterogeneity within tissues
- Varies with endpoint
- The absolute volume is important rather than the area e.g. bladder surface
- The effects of changes in DVH will vary with the tissue, depending on FSU structure (series/parallel), physiological reserve, etc.

Cytokines and Growth Factors

- Inflammatory Cytokines
 - Tumor Necrosis Factor (TNF- α), Interleukin-1 (IL-1)
- Angiogenesis
 - Vascular Endothelial Growth Factor (VEGF), Basic Fibroblast Growth Factor (bFGF), TNF- α
- Immune Cytokines
 - IL-2 and IL-4
- Fibrotic Cytokines
 - Transforming Growth Factor beta (TGF- β), bFGF, IL-6, CTGF
- Growth Factors
 - Colony Stimulating Factors - G-CSF, GM-CSF, IL-3, EPO, SCF
 - Epidermal Growth Factor (EGF), TGF- α , bFGF

Cytokines and Growth Factors

- Cytokines are expressed in tissues within hours of irradiation
- Over subsequent weeks and months there is a “cyclical cascade” of cytokines released that is aimed at tissue regeneration.
- Complications such as fibrosis arise when the regenerative process is unsuccessful.

Late Effects Involve Wound Healing

- Wound healing involves cytokines and growth factors that mediate cellular responses including tissue **regeneration**
- They may also contribute to the **pathogenesis of complications** and may be responsible for some side effects of irradiation, such as
 - Fatigue
 - Edema
 - Erythema
 - Somnolence
 - Nausea

Late Effects Involve Fibrosis

- Fibrosis is a common complication of radiation exposure that is a response to cell loss.
- TGF- β and its target genes are involved in promoting tissue fibrosis.
- TGF- β induces fibroblast differentiation, senescence, and collagen production.
- Patients with persistent high serum levels of TGF- β , before and during a course of therapy for lung cancer have a higher risk of developing pneumonitis.

Conclusions

- The time to a normal tissue complication depends on the tissue turnover time.
- The time to and extent of regeneration in normal tissues also determines how much dose can be tolerated
- Late effects are complex. The balance of cytokines and growth factors determine both regeneration and normal tissue complications
- Tissue tolerance depends not only on intrinsic radiosensitivity of clonogens, but also on organization into FSUs, and the number of clonogens per FSU
- Volume effects are complex, but a major volume effect may be due to differences in FSU organization
- Biological readout of normal tissue effects are needed beyond TGF- β
- Radiogenomics may help to predict tissue complications in the future