Emergent Properties Common to both Stem Cells and Tumor Cells

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Properties of Stem Cells

Self-renewal

Multi-lineage potential

Response to injury

Cell fate decisions require epigenetic plasticity and exogenous signals

pluripotent stem cell

Condition:

A

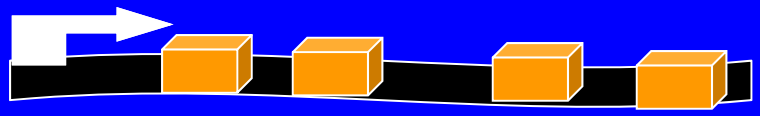
B

C

differentiated for liver

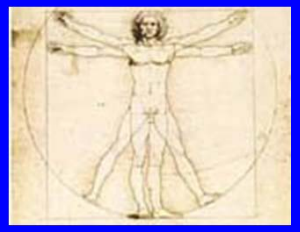
differentiated for heart

differentiated for eye



undifferentiated

Condition:



stem cell

A

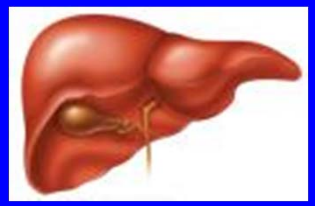
B

C

distinct epigenomes

Epigenetic plasticity is a distinguishing characteristic of stem cells

Condition:

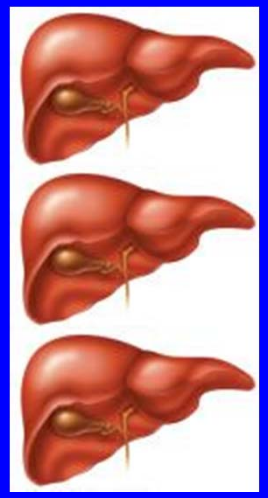


differentiated cell

A

B

C

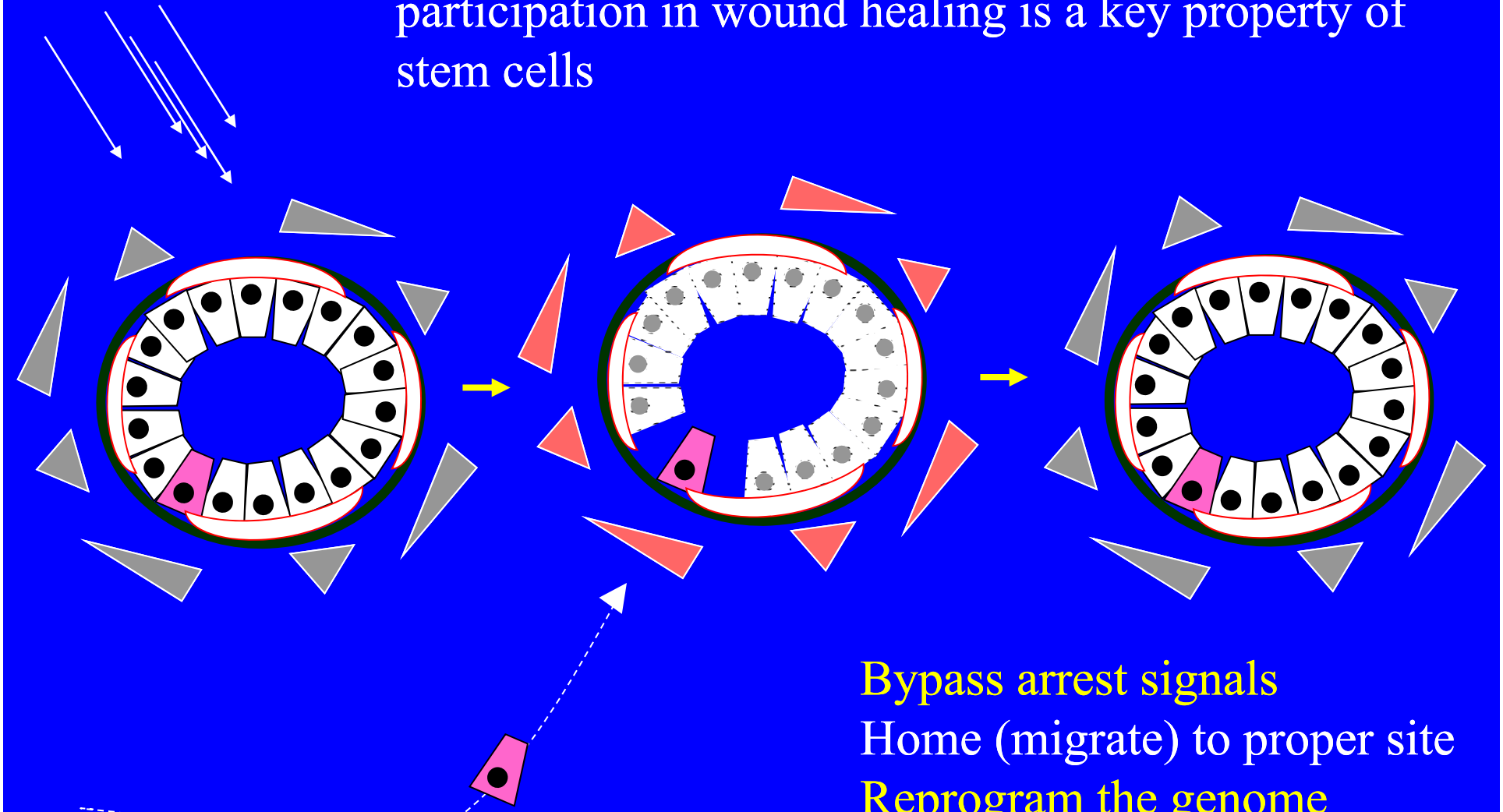


identical epigenomes

Acute Wounding → Healing

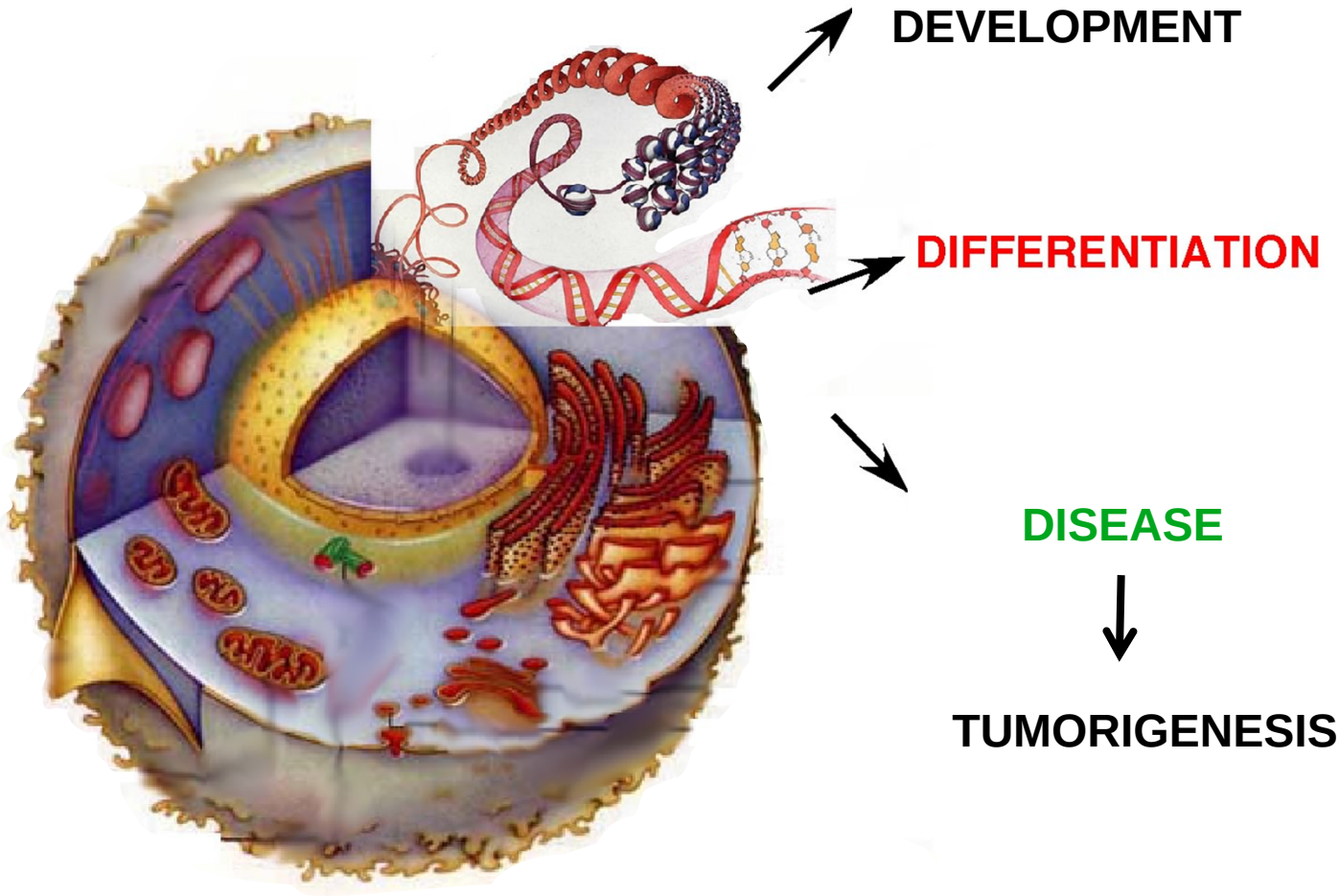
damage

In addition to Self-renewal and Multi-lineage potential, participation in wound healing is a key property of stem cells



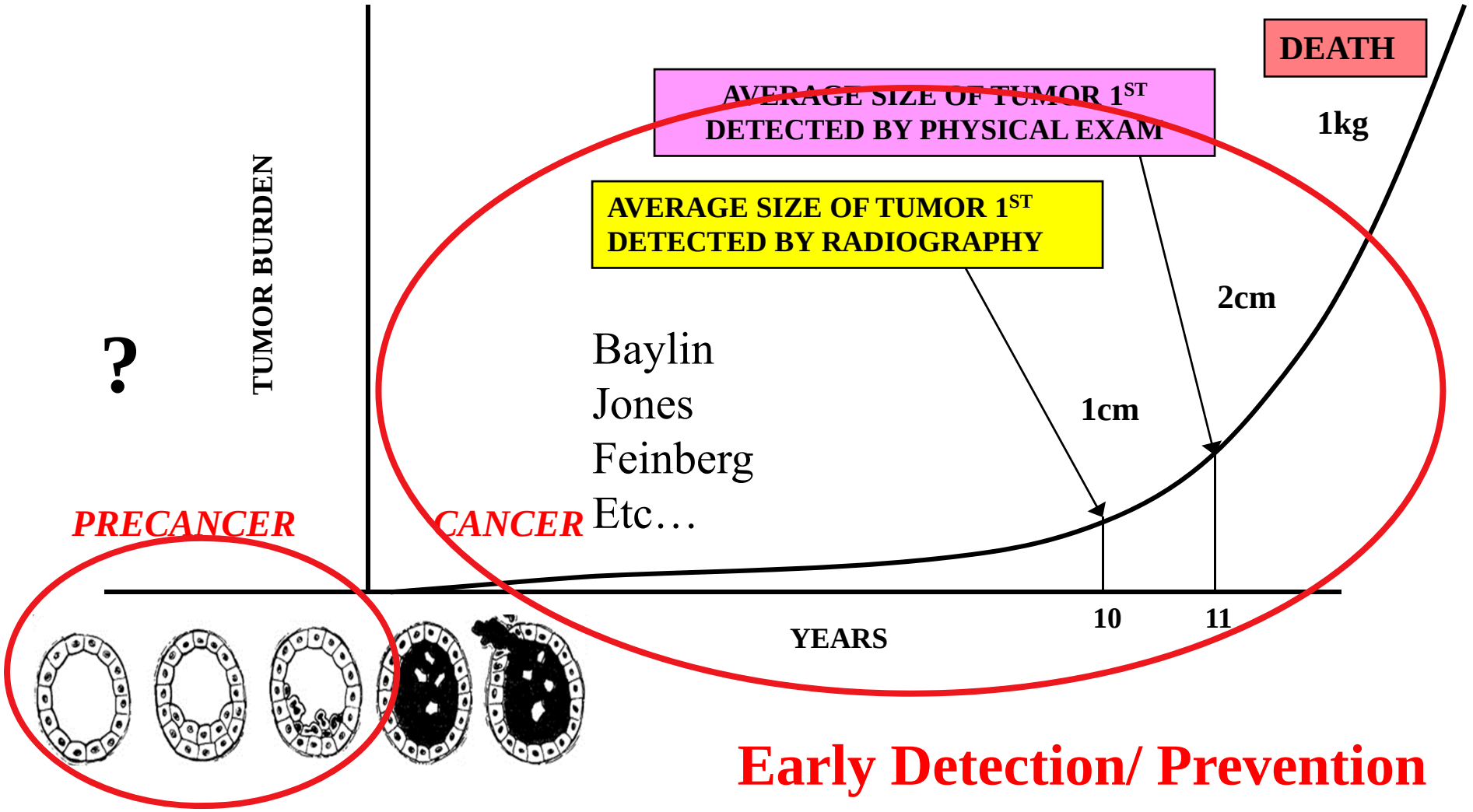
Bypass arrest signals
Home (migrate) to proper site
Reprogram the genome
Complete differentiation

The active acquisition of epigenetic changes is a poorly understood but important process in development, differentiation, and disease.



Both stem cells and cancer cells exhibit epigenetic plasticity - the ability to reprogram the genome in a heritable fashion.

Epigenetic Changes are Frequent in Late Stage Cancer



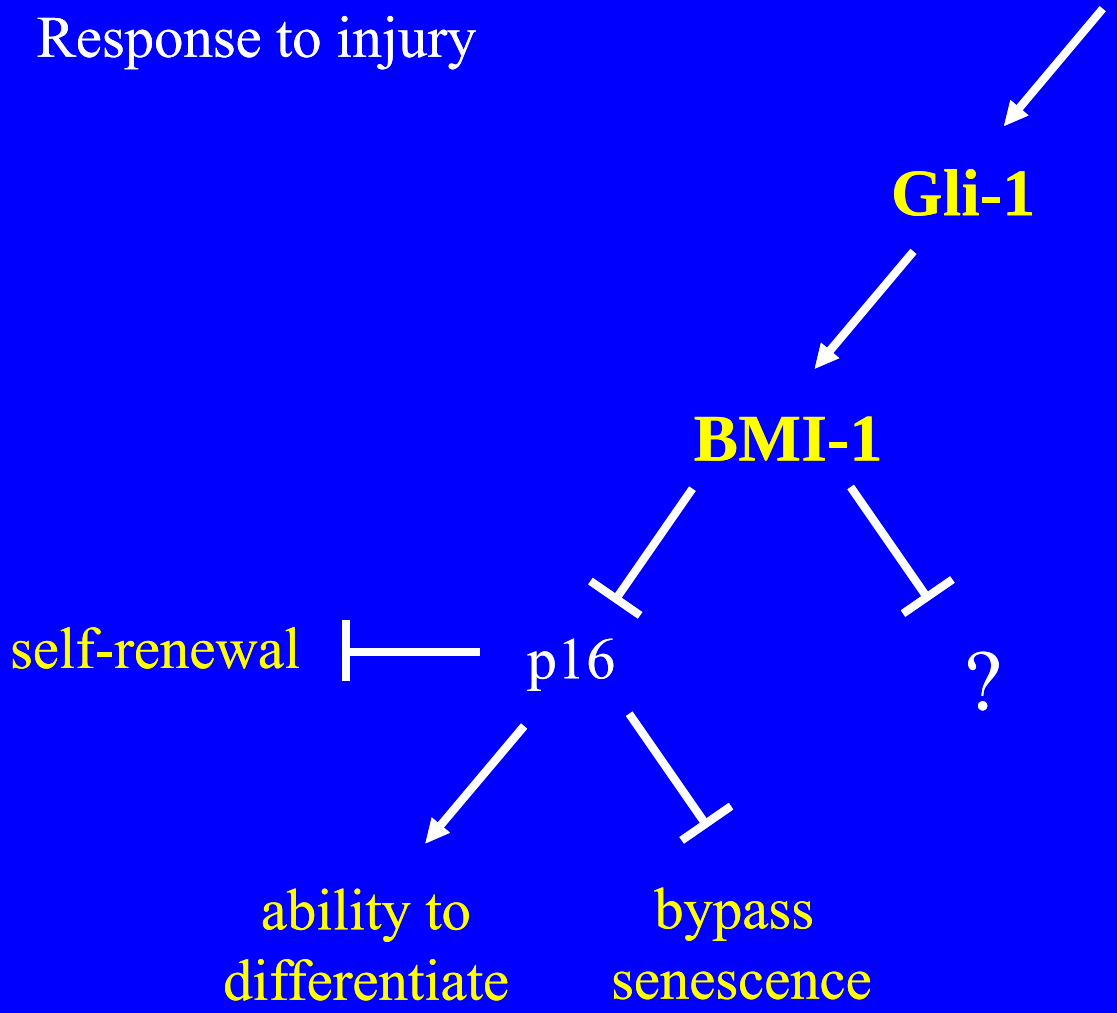
Early Detection/ Prevention

Properties of Stem Cells

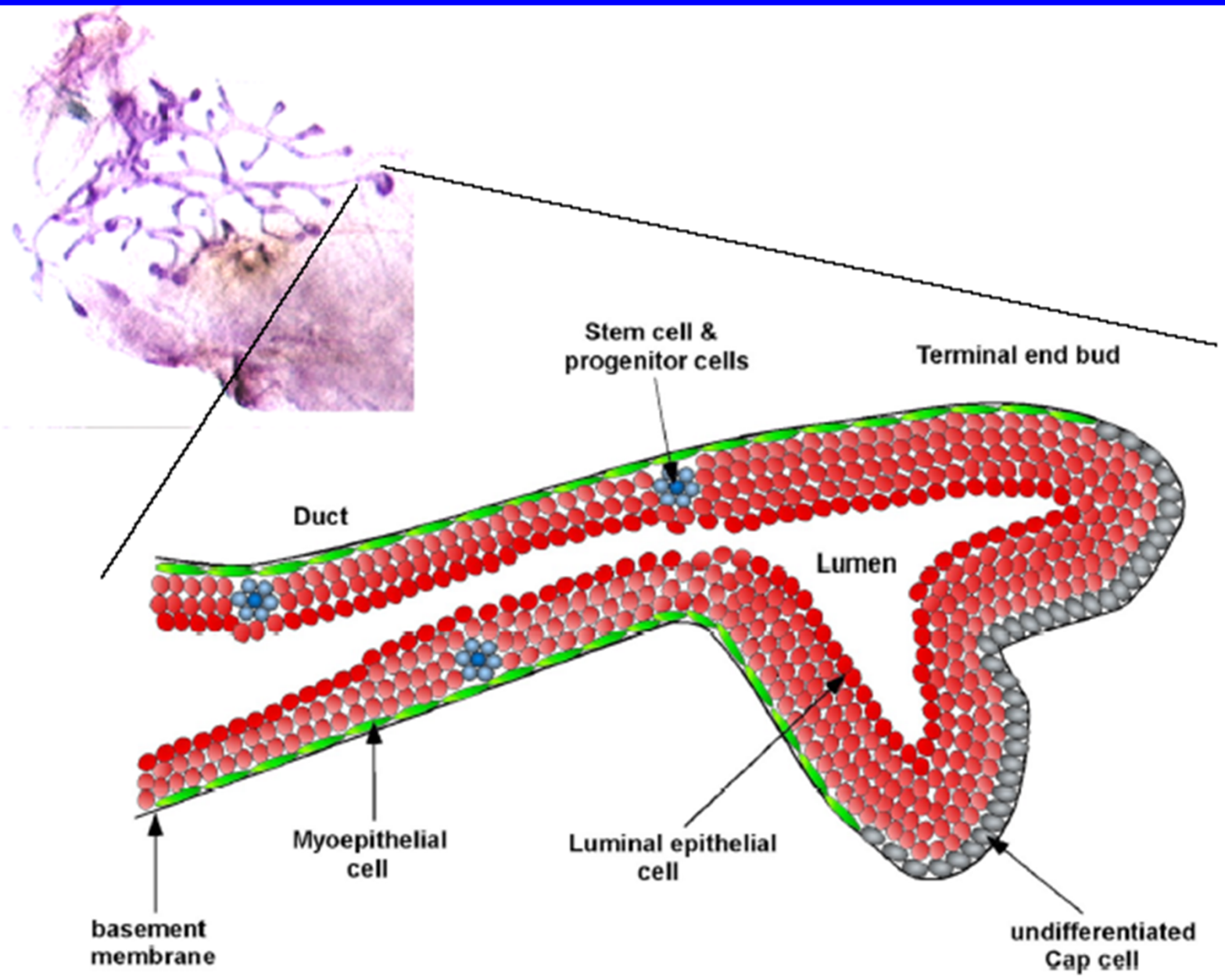
Self-renewal
Multi-lineage potential
Response to injury

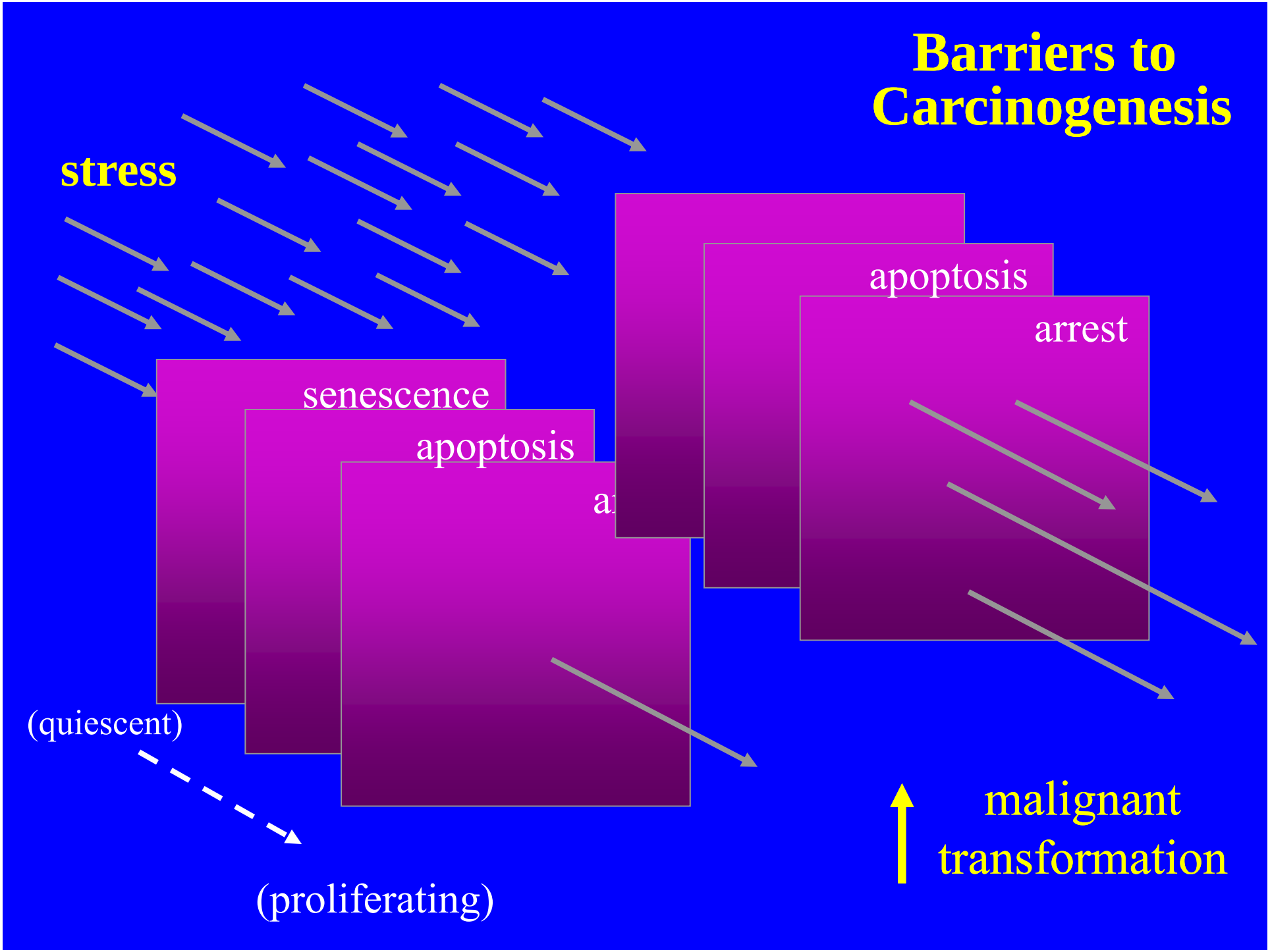
Bmi-1 is required for maintenance of adult self-renewinghaematopoietic stem cells
In-kyung Park, et al. Nat 423, 302-305, 2003

Bmi-1 promotes neural stem cell self-renewal and neural development…
by repressing the p16Ink4a and p19Arf senescence pathways
Molofsky AV, et al.
Genes Dev. 19,1432-7 , 2005



Cellular Composition of Mammary Gland

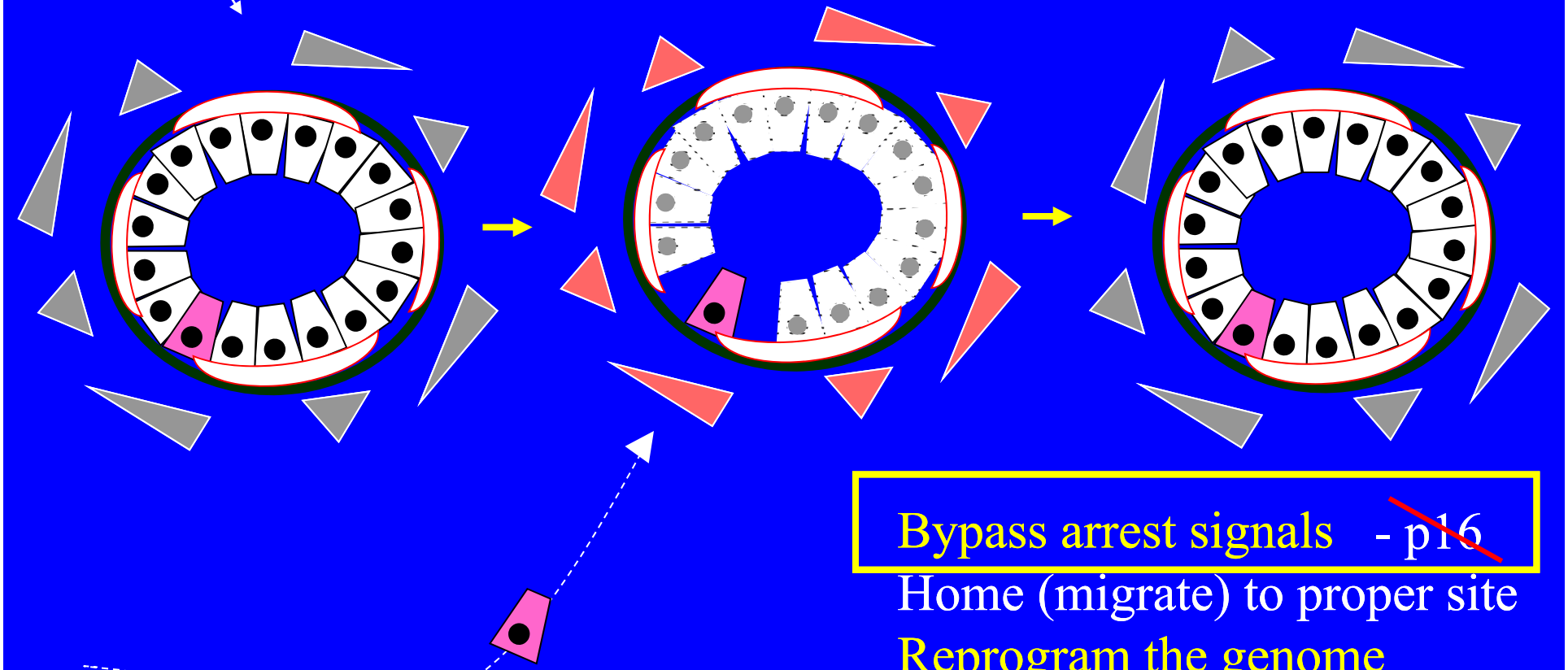



Barriers to Carcinogenesis
stress
senescence
apoptosis
apoptosis
arrest
(quiescent)
(proliferating)
malignant transformation

Acute Wounding → Healing

damage

In addition to Self-renewal and Multi-lineage potential, participation in wound healing is a key property of stem cells



Bypass arrest signals ~~- p16~~

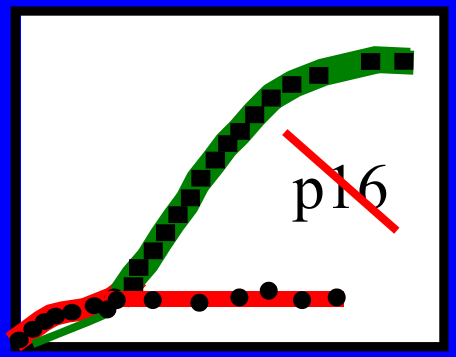
Home (migrate) to proper site

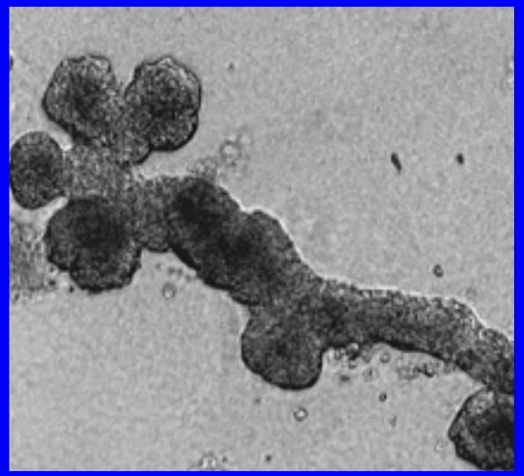
Reprogram the genome

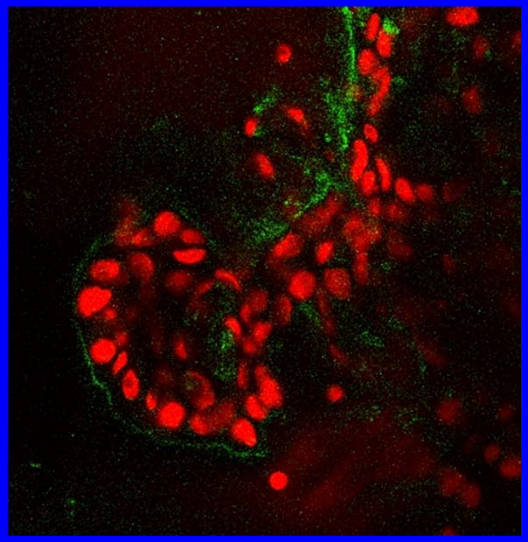
Complete differentiation

Epithelial cells that can bypass stress signals are found in disease free women and can be propagated in culture

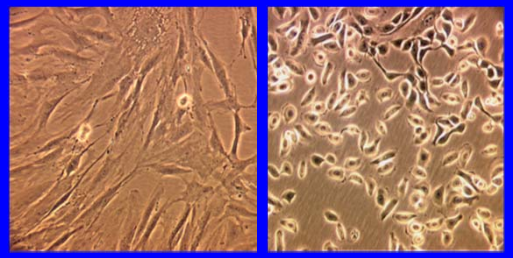
These cells provide an excellent opportunity to study precursors to cancer (and stem cells)



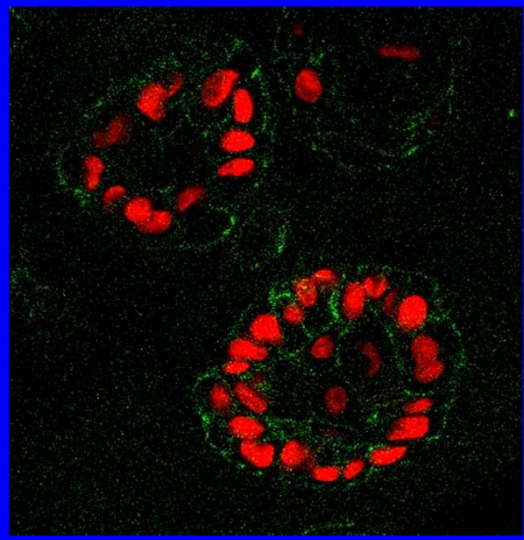




In vivo

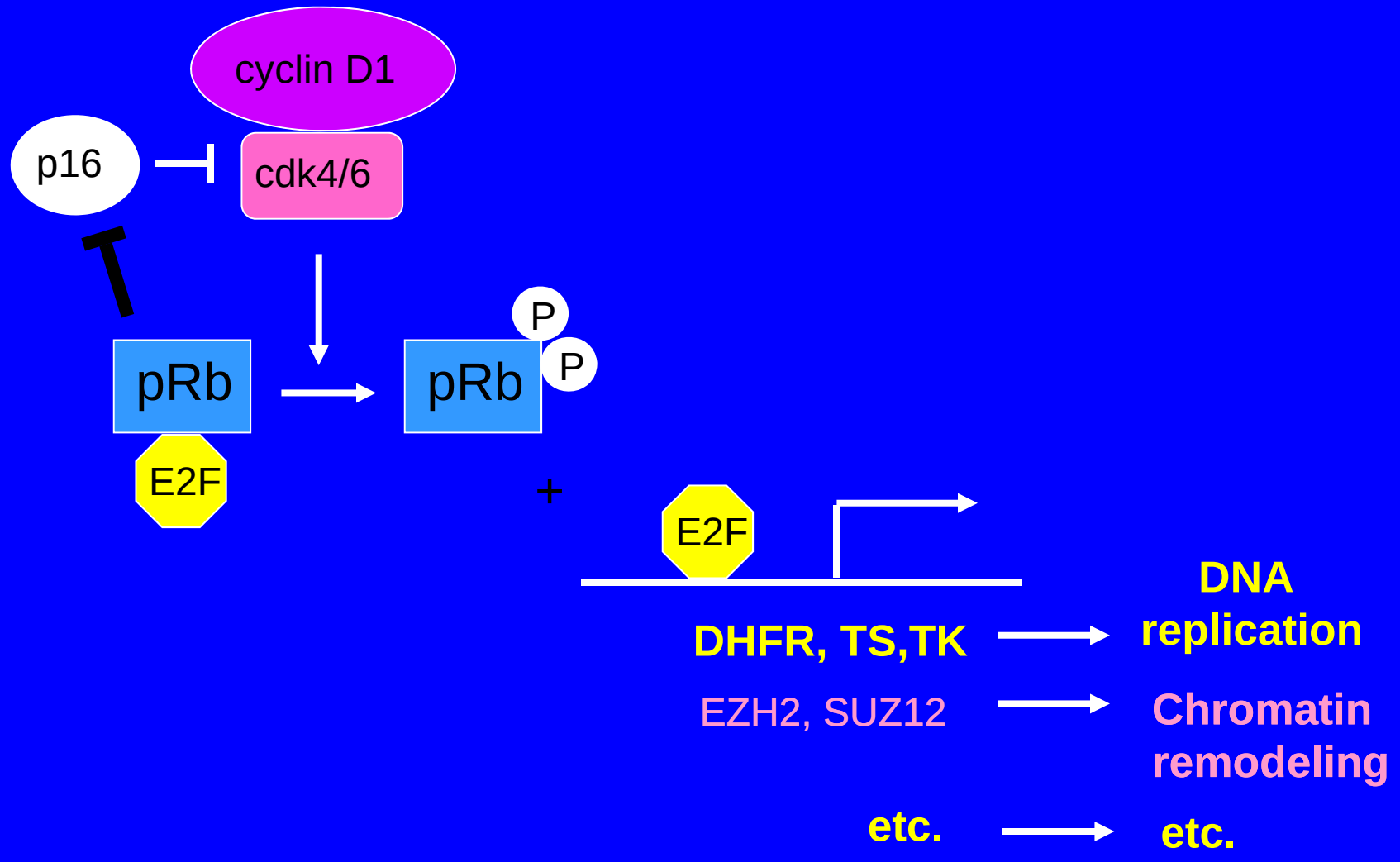


Fibroblasts **Epithelial cells**



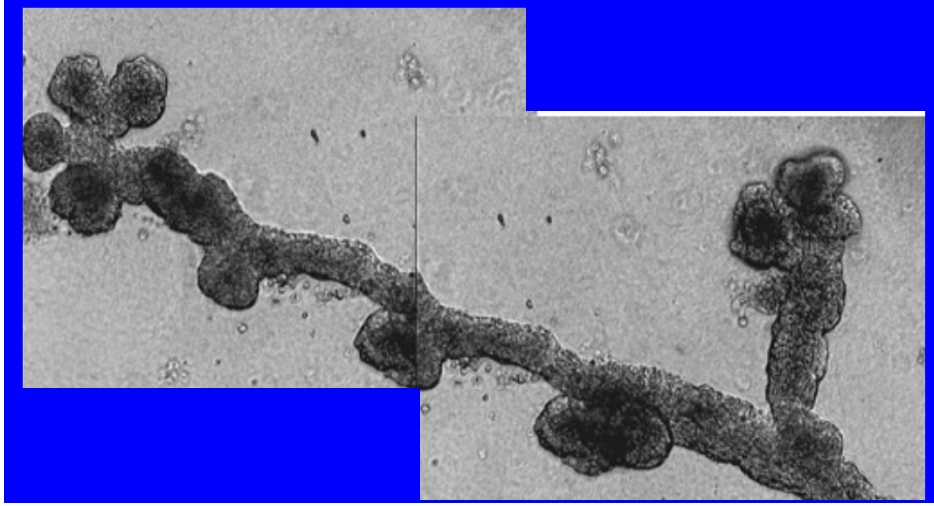
In vitro

p16 Contributes to Cell Cycle Regulation and Negatively Regulates E2F Target Gene Expression



This small distinctive subpopulation of cells exhibits several pre-malignant properties

	By pass senescence	Genomic instability	Apoptotic resistant	Invasive	Etc.
Normal cell	-	-	-	-	-
Precancerous	+	+	+	-	-
Cancer cell	+	+	+	+	+



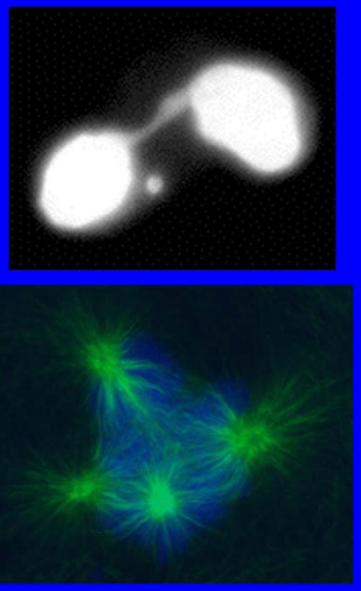
Silencing of p16 confers several properties:

(1) Silencing of p16 provides cells with pre-malignant properties: allows the bypass of senescence

confers genetic plasticity

activates and represses genes important for pre-malignant and stem/progenitor cell properties

confers epigenetic plasticity

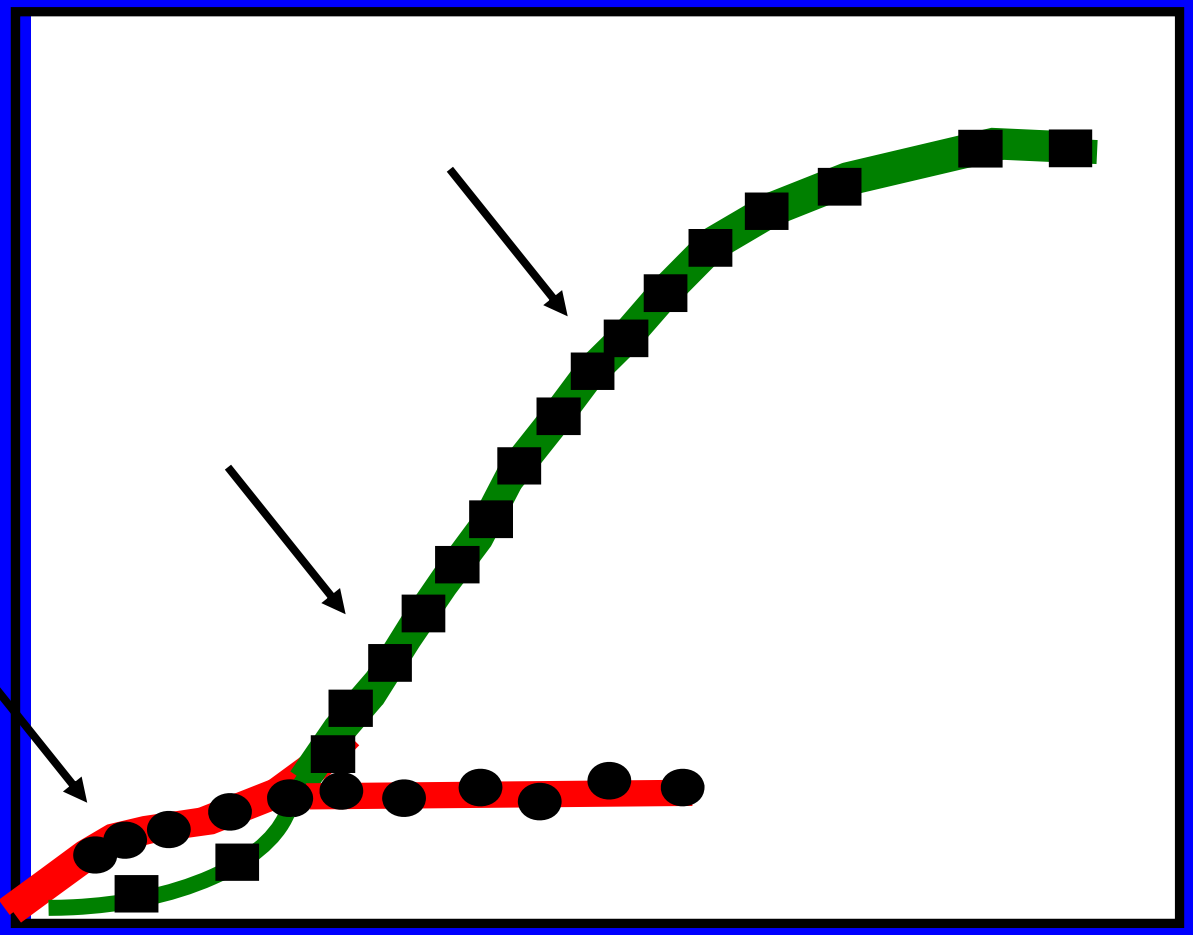


Romanov et al., 2001
Holst et al., 2003
Crawford et al., 2004
Gauthier et al., 2005
McDermott et al., 2006
Reynolds et al., 2006
Dumont et al., 2009

(2) Cells with these properties exist in healthy, disease-free individuals-
probably serve as precursors to cancer,
provide prognostic information.

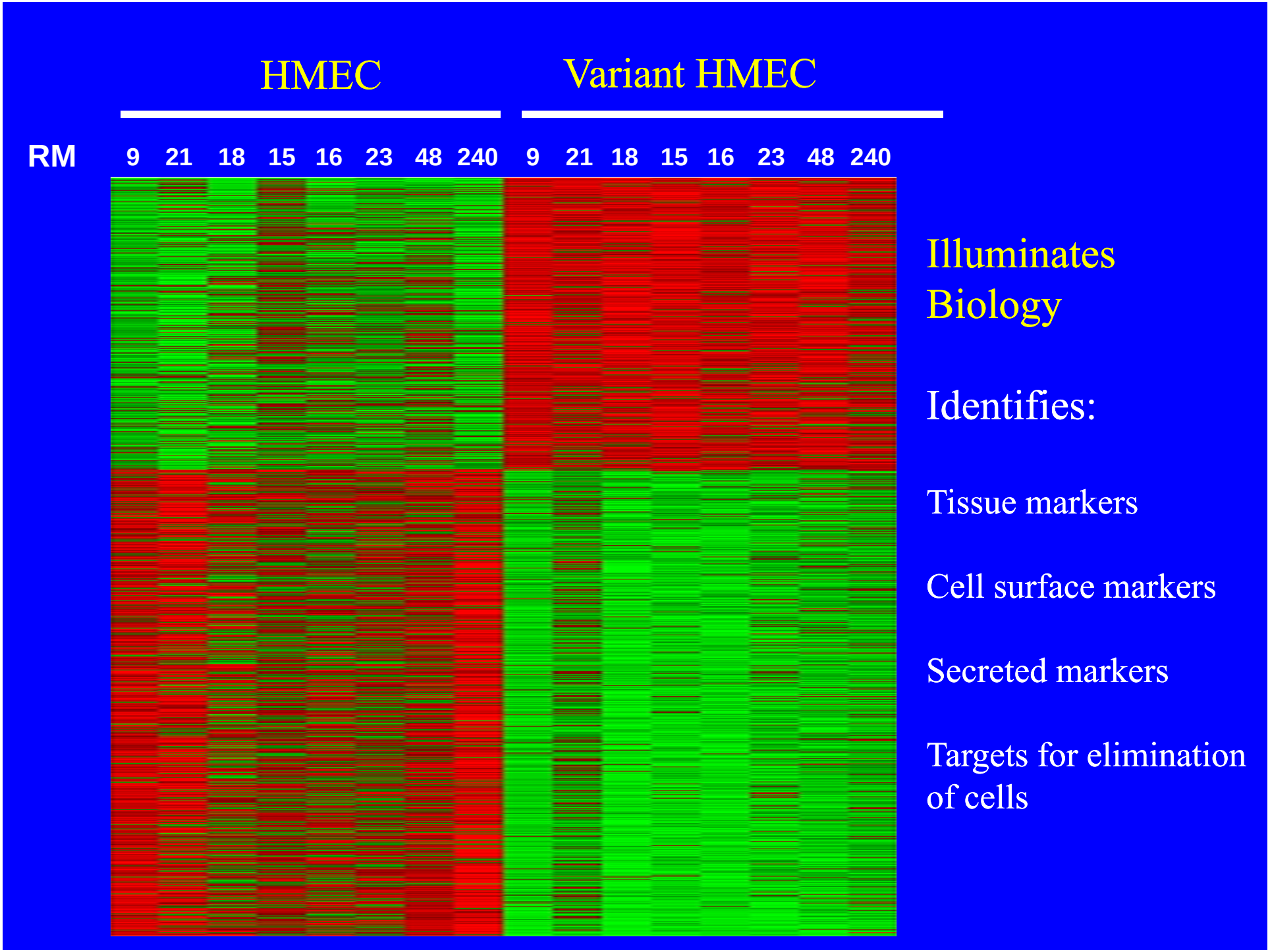
Holst et al., 2003
Crawford et al., 2004
Gauthier et al., 2007

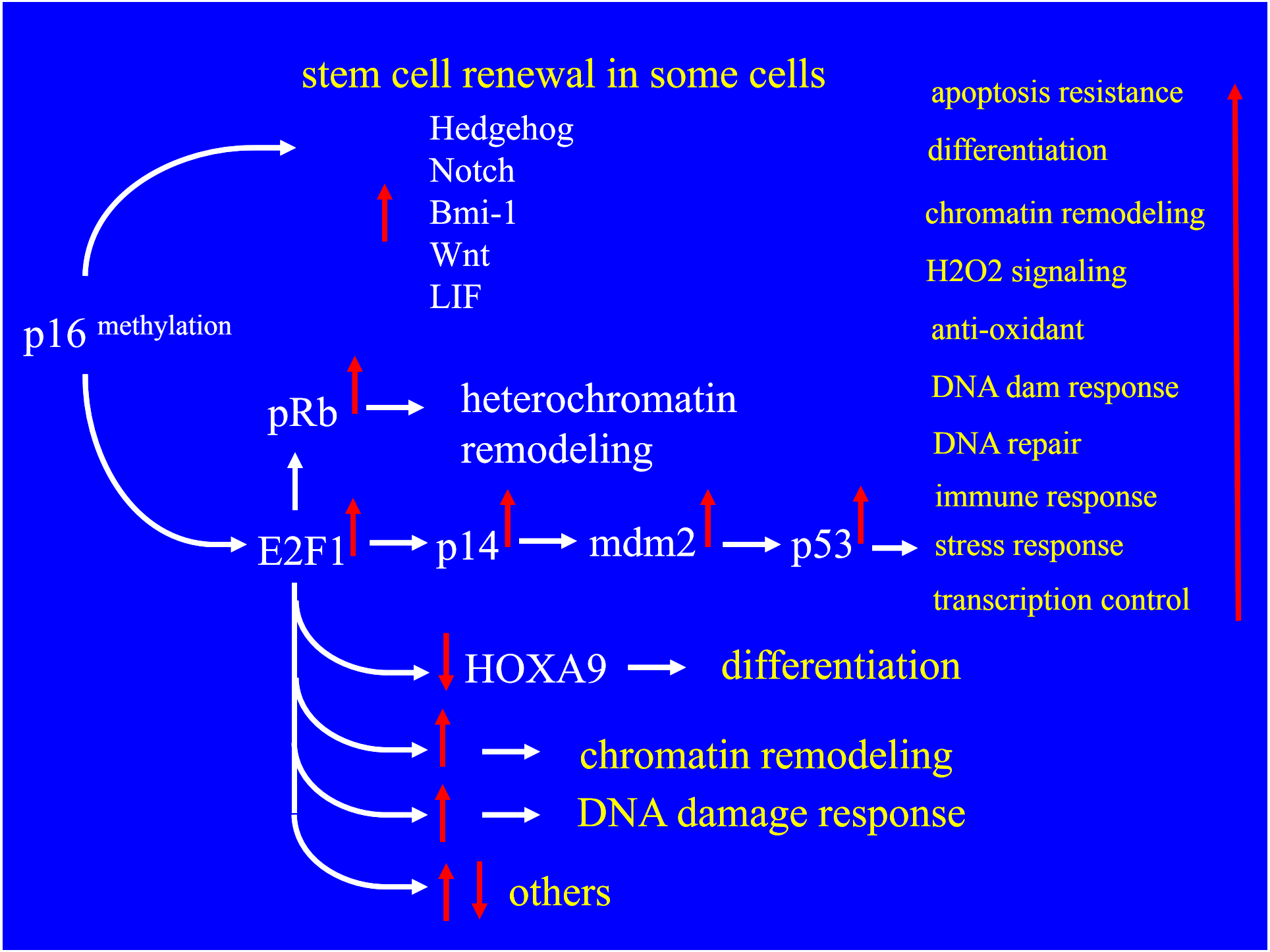
Clues to Early Cancer Phenotype: Use Expression Profiling



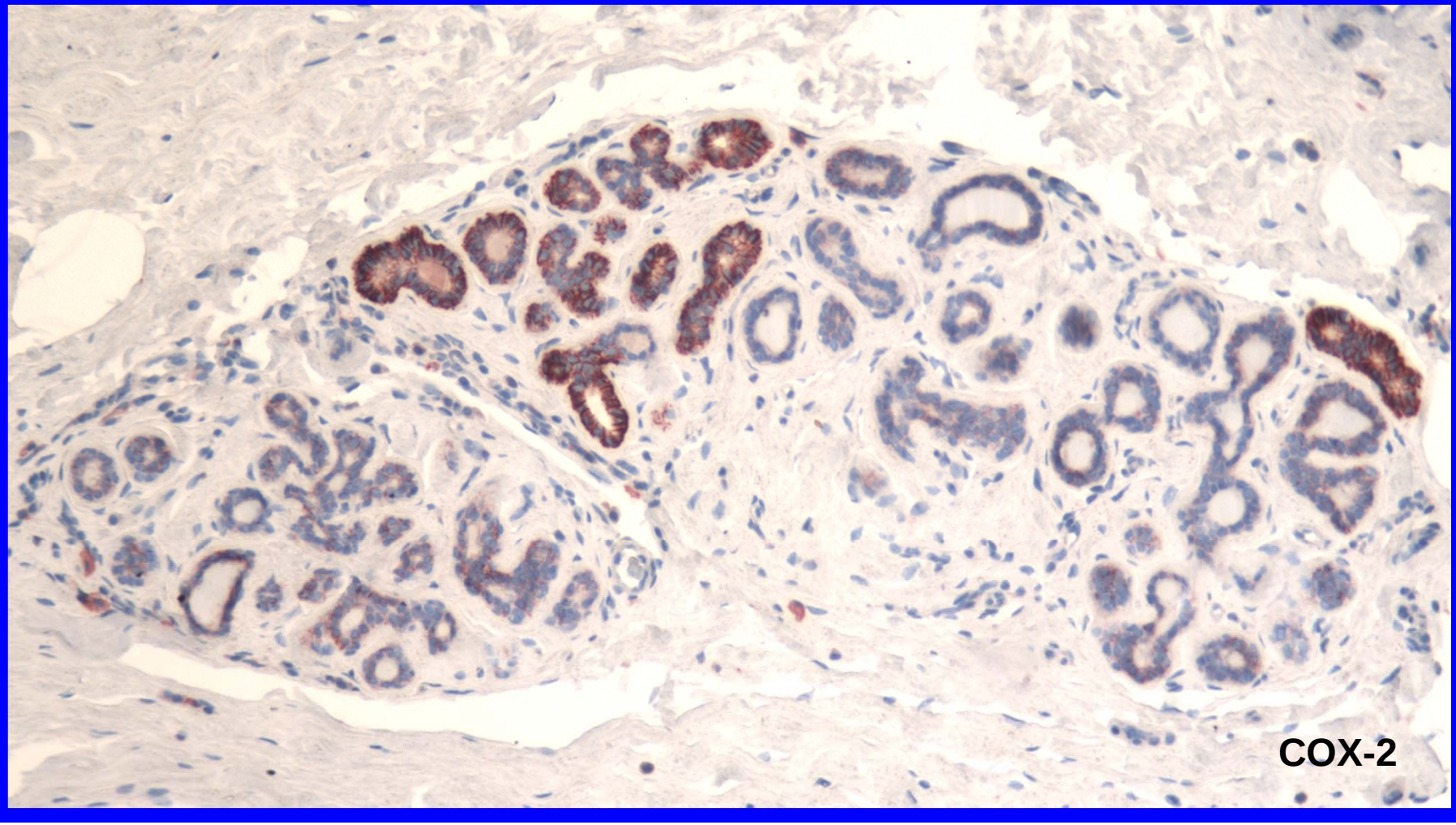
global analysis

candidate analysis

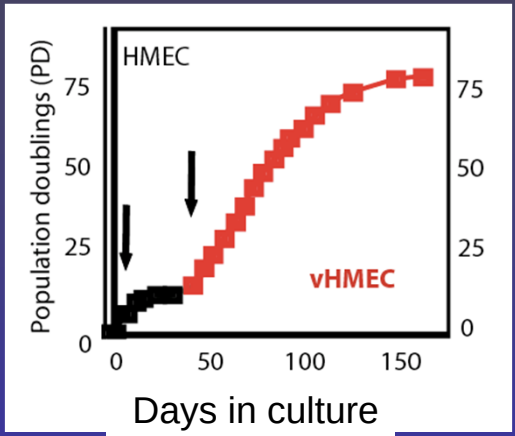

HMEC
Variant HMEC
RM
9 21 18 15 16 23 48 240
9 21 18 15 16 23 48 240
Illuminates Biology
Identifies:
Tissue markers
Cell surface markers
Secreted markers
Targets for elimination of cells

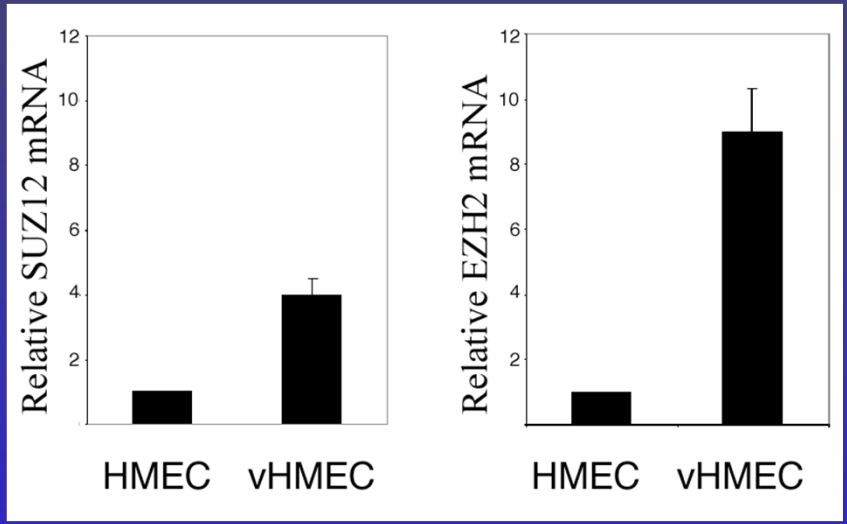

stem cell renewal in some cells
Hedgehog
Notch
Bmi-1
Wnt
LIF
p16 methylation
pRb
heterochromatin remodeling
E2F1
p14
mdm2
p53
HOXA9
differentiation
chromatin remodeling
DNA damage response
others
apoptosis resistance
differentiation
chromatin remodeling
H2O2 signaling
anti-oxidant
DNA dam response
DNA repair
immune response
stress response
transcription control

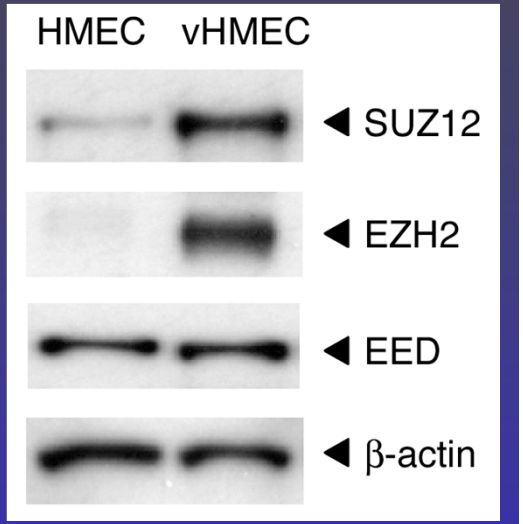
Normal tissue from healthy, cancer-free women contains foci that exhibit suppression of p16 and expression of a pre-malignant program

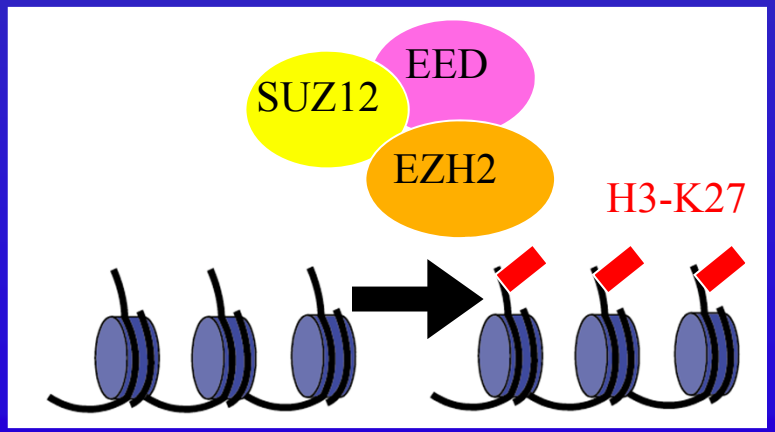


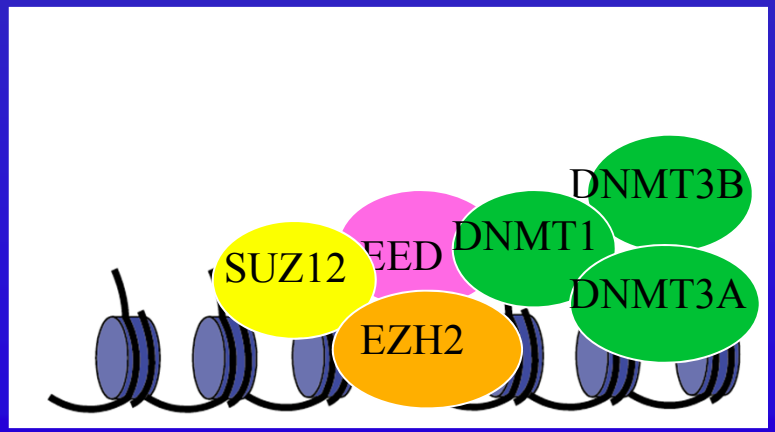
PcG proteins are upregulated in PRIMED HMEC



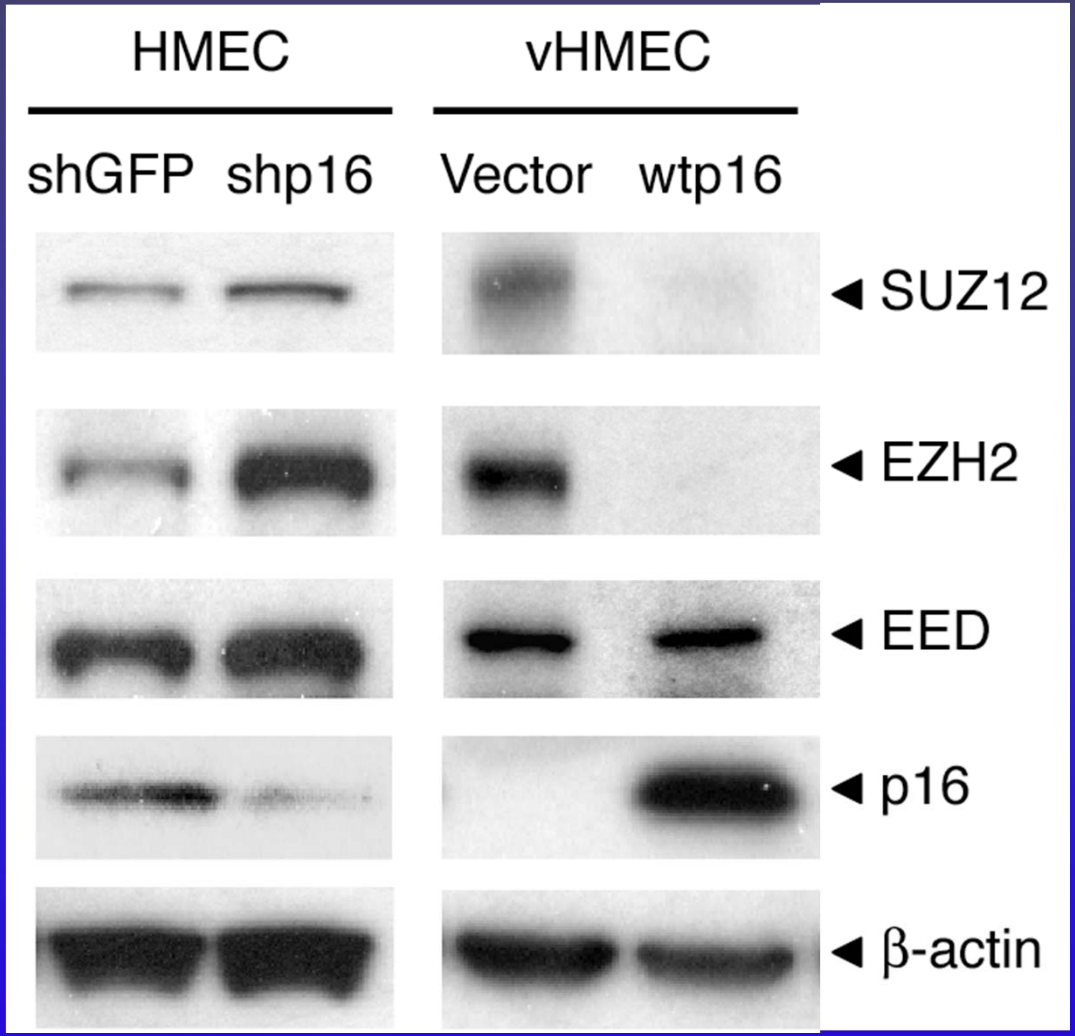








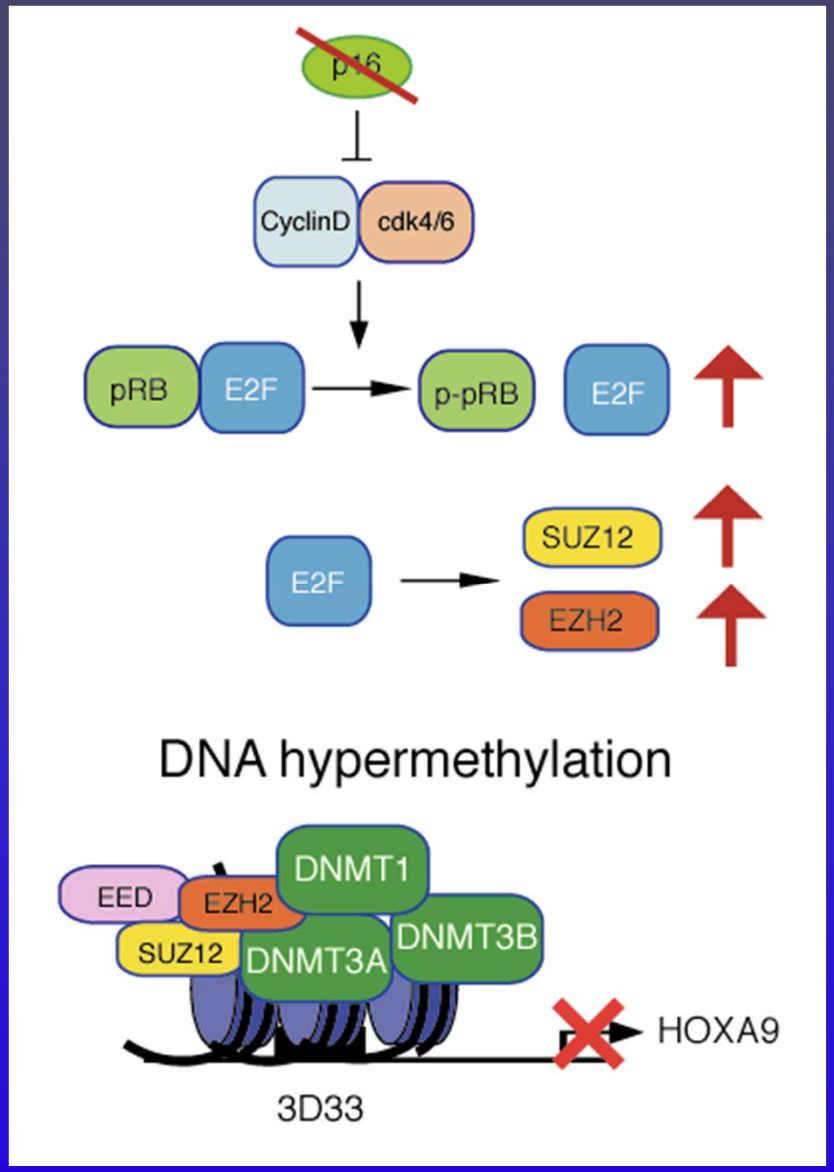
PcG protein expression is p16-dependent



How do stem cells and cancer cells acquire epigenetic plasticity?

(ability to methylate and demethylate gene sequences)

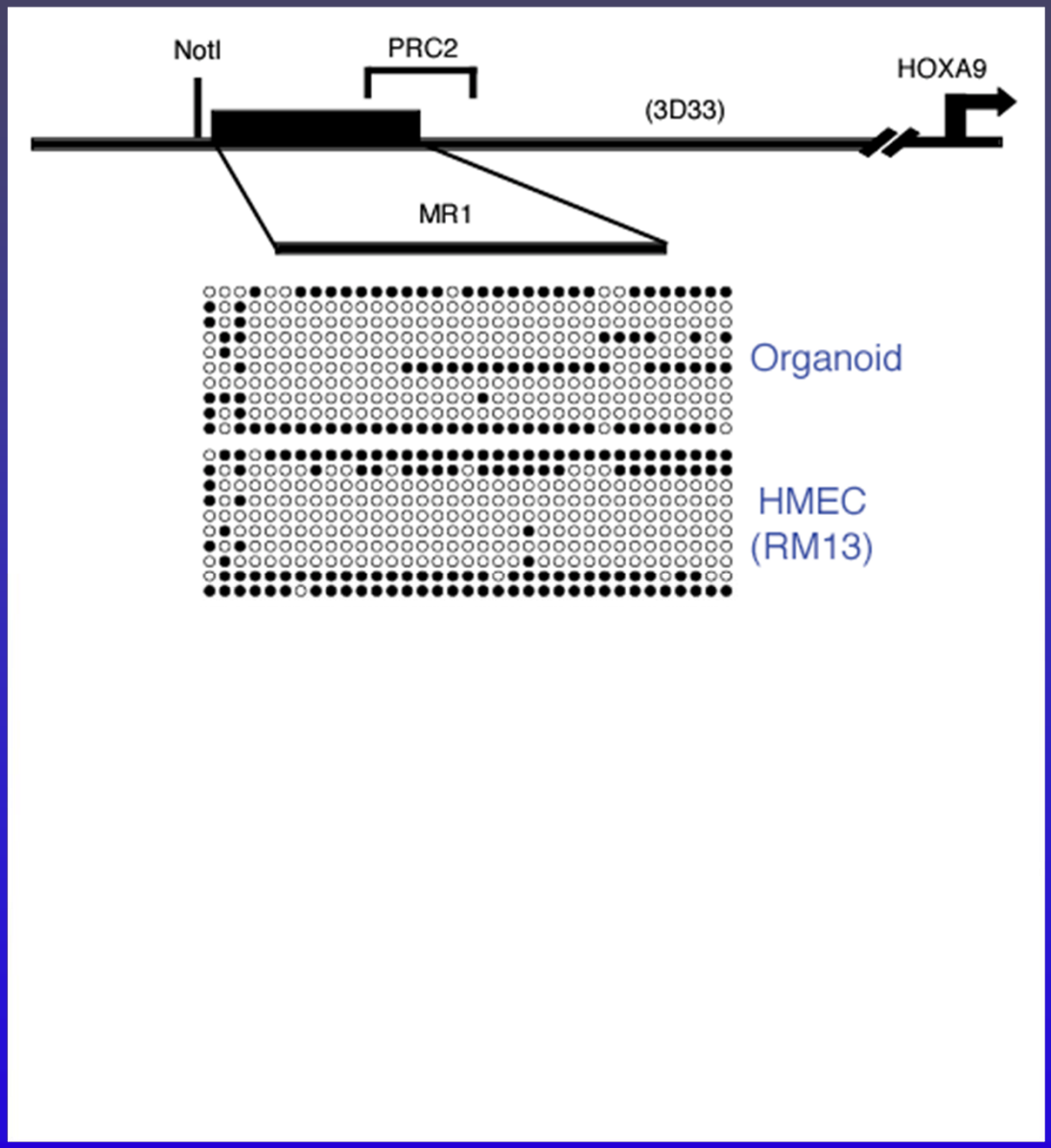
Loss of p16/pRb activity is necessary and sufficient for DNA hypermethylation at targeted loci

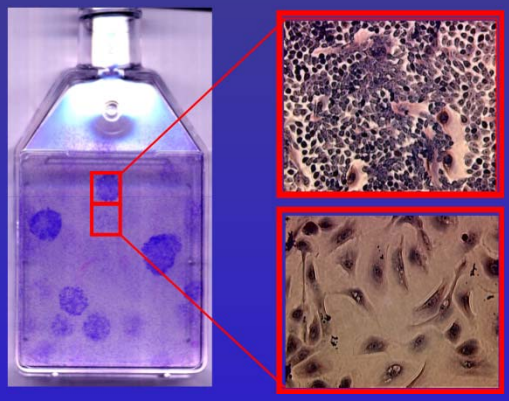


Repression of the p16/pRb pathway in human mammary epithelial cells activates an E2F-mediated increase in these proteins that remodel chromatin and causes targeted, *de novo* DNA methylation at a non-random collection of loci.

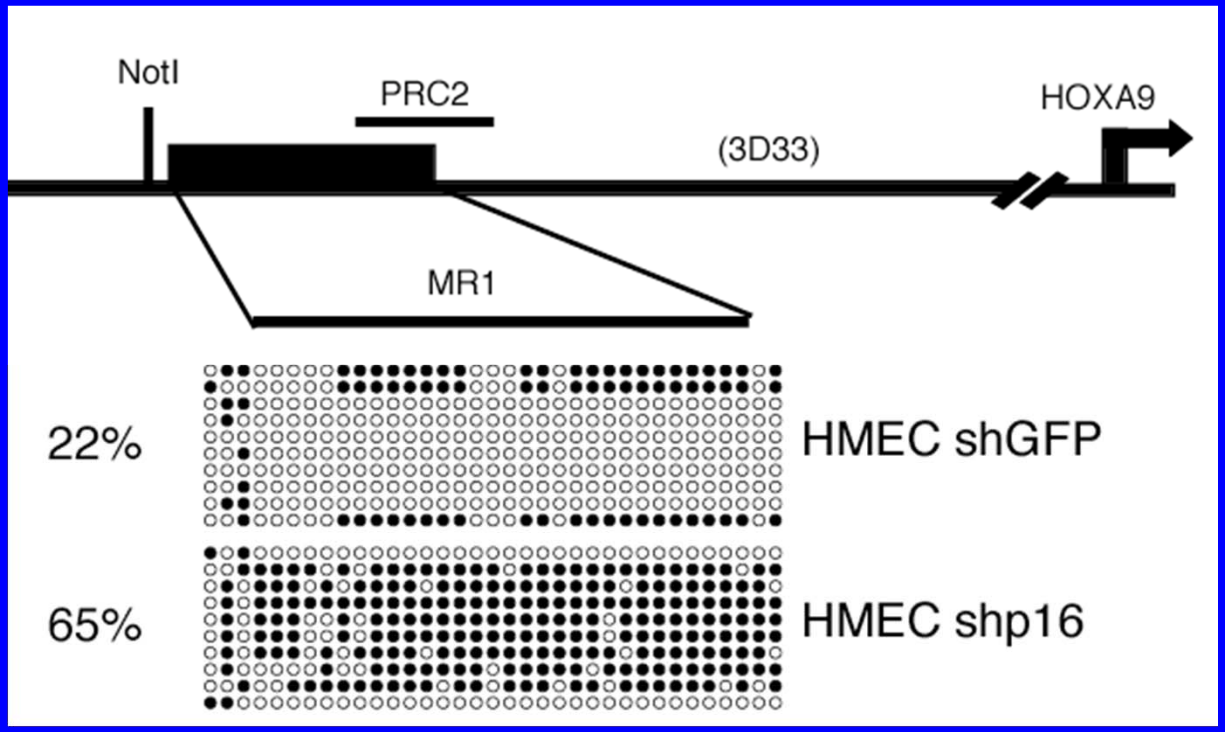
(Reynolds et al., J Biol Chem 281: 24790-24802, 2006).

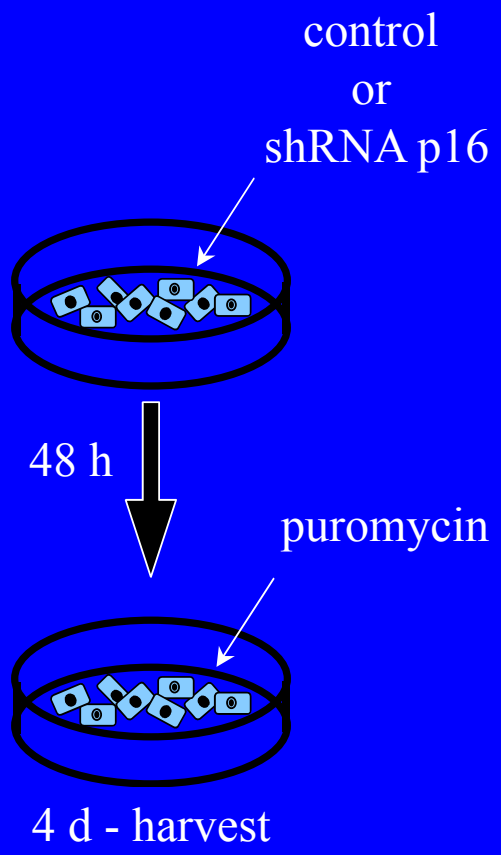
Bisulfite validation of HOXA9 DNA hypermethylation





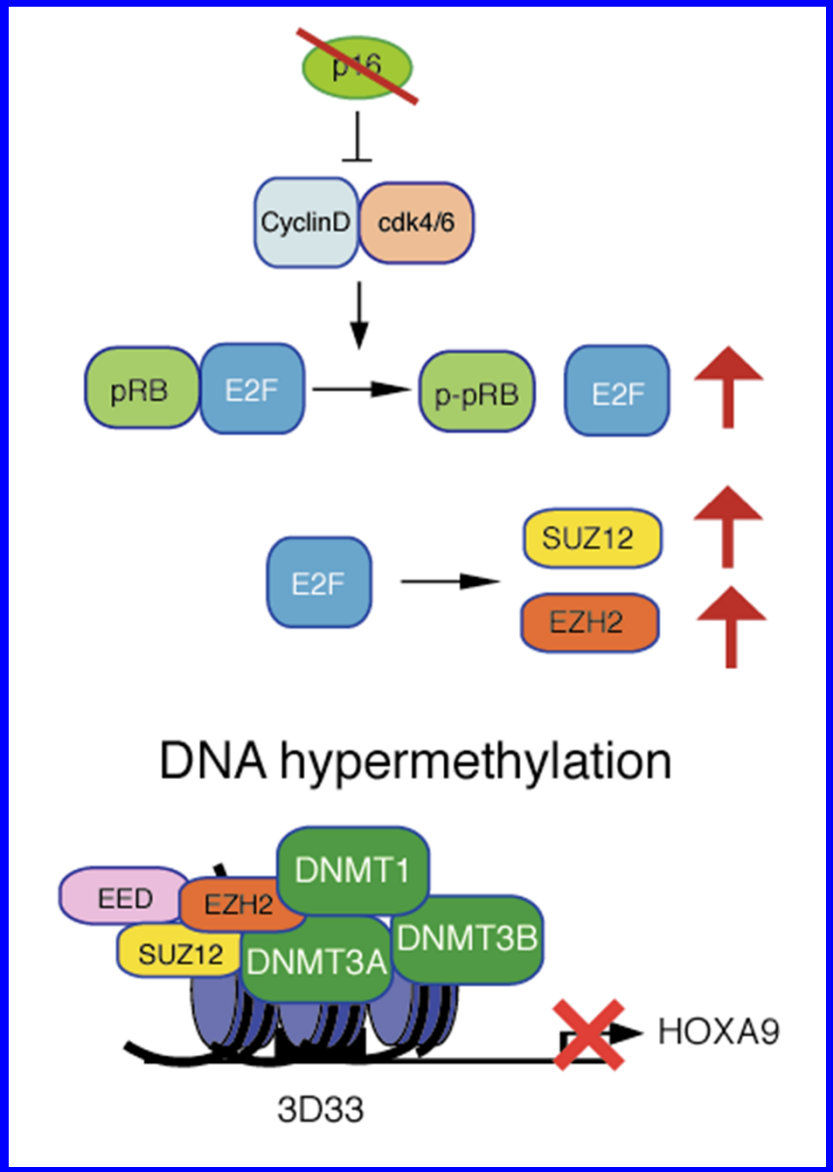
Removal of p16 expression in HMEC induces rapid DNA hypermethylation





Paul Reynolds

SUZ12/EZH2 are both necessary and sufficient for the activation of targeted DNA hypermethylation

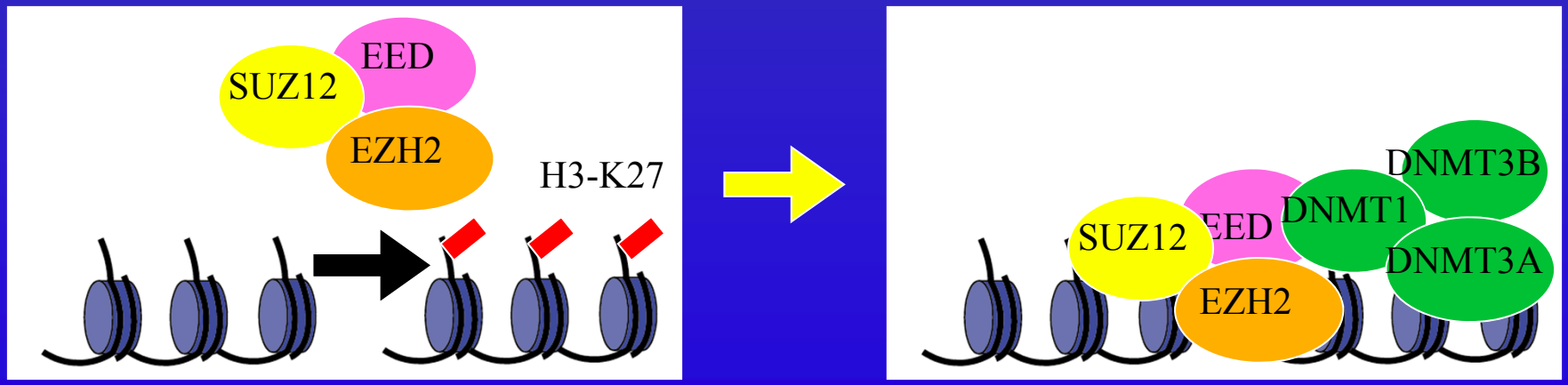


Removal of SUZ12/EZH2 prevents the methylation events.

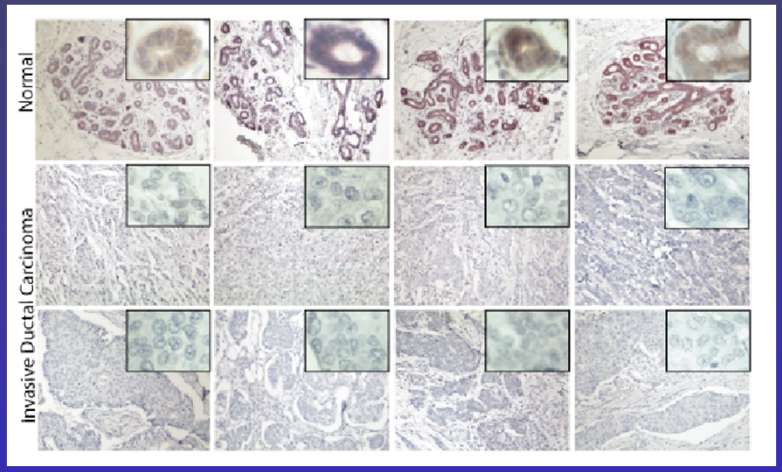
Exogenous addition of SUZ12/EZH2 activates the methylation events.

Without histone methylation changes - DNA methylation changes cannot occur.

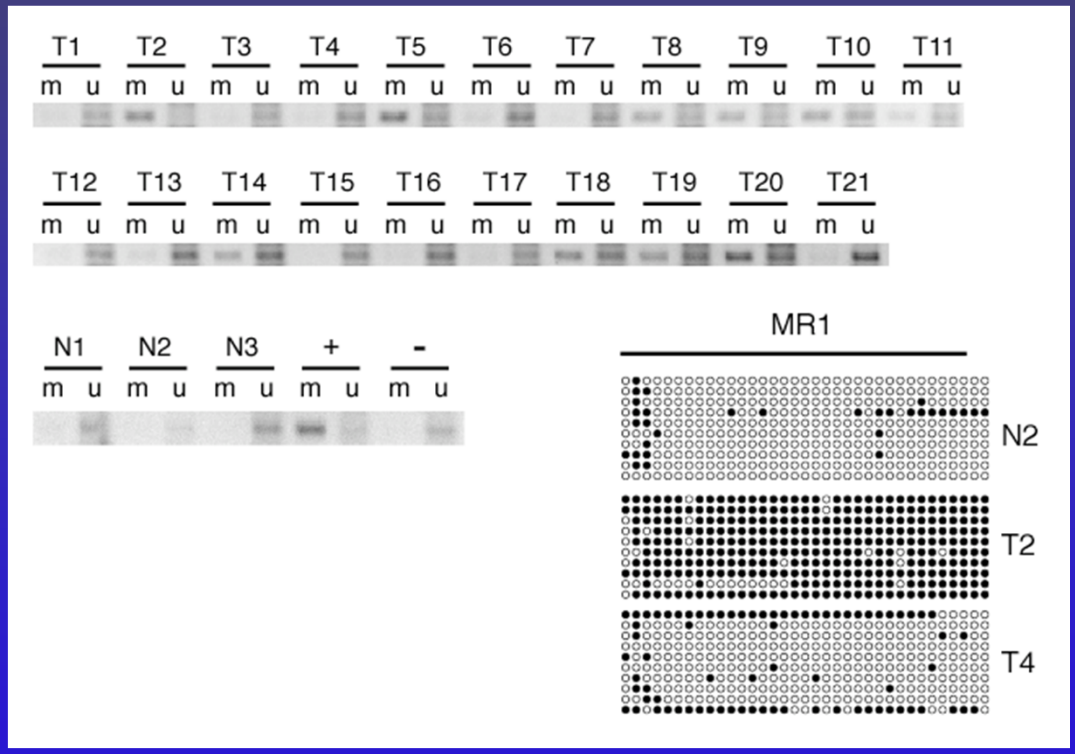
Transcriptional repression precedes
DNA hypermethylaton



HOXA9 is silenced and undergoes DNA hypermethylation in a large proportion of primary breast tumors



93%

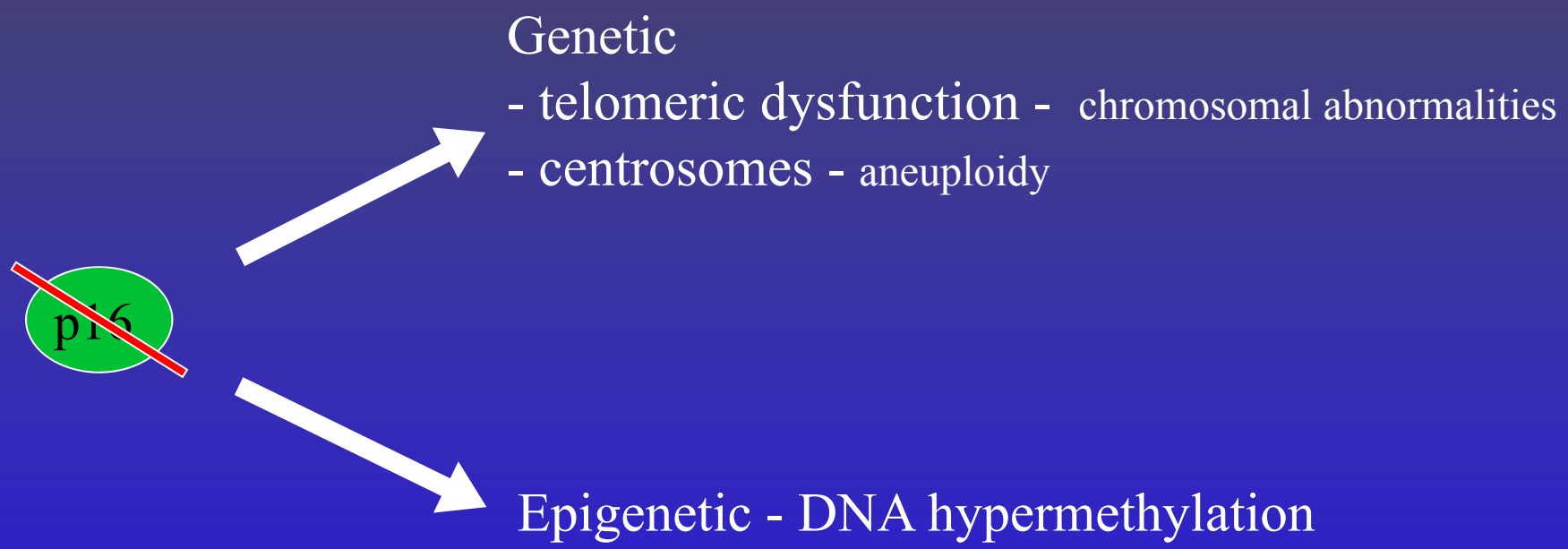


44%

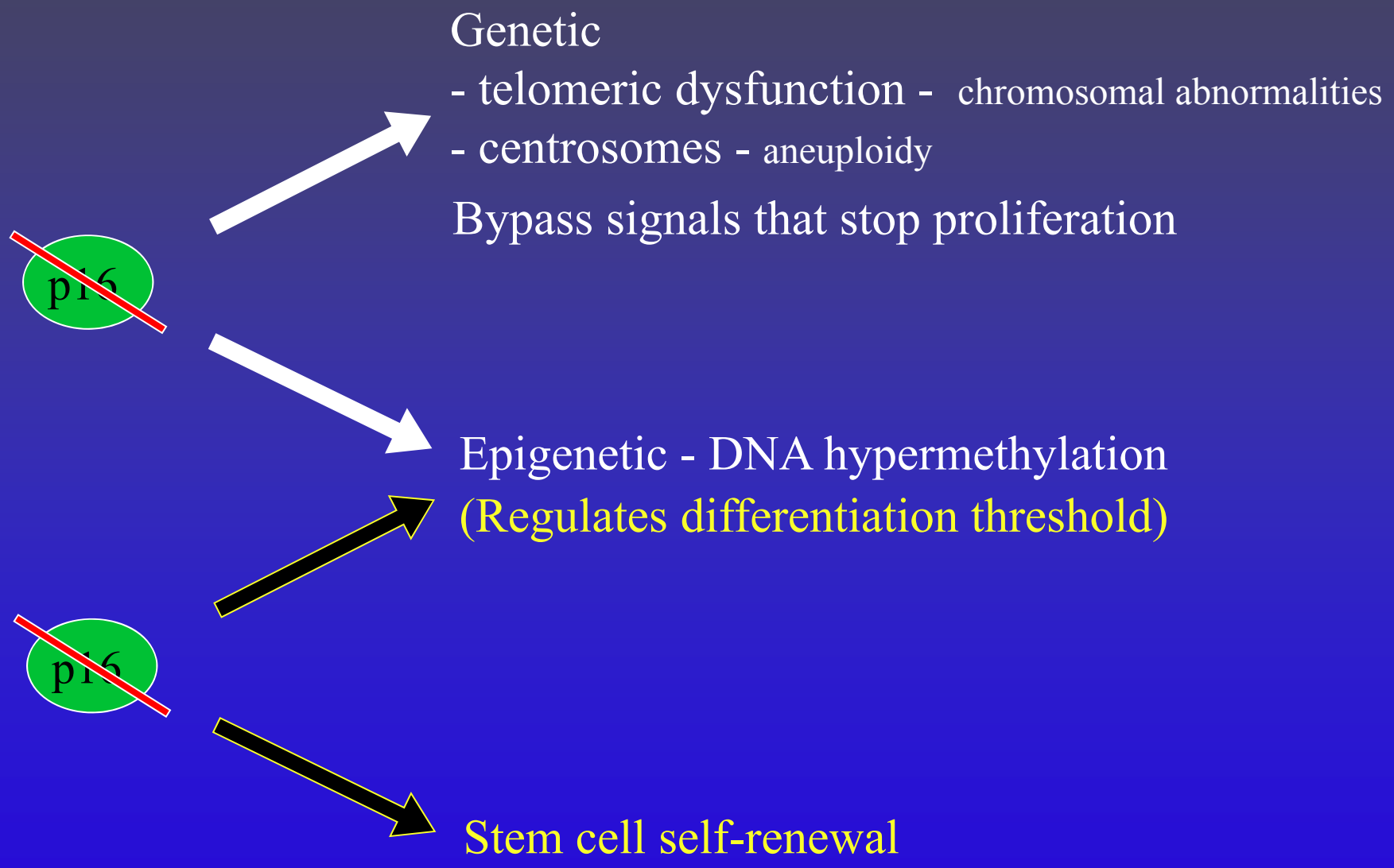
Summary and Conclusions - Part I

- A commonly inactivated tumor suppressor, p16, regulates DNA methylation
  (epigenetic changes occur in preneoplastic cells)
- DNA methylation is an active and dynamic process (targeted) - chromatin remodeling and repression precedes and is necessary for subsequent DNA hypermethylation
- Loss of p16 confers epigenetic plasticity - silencing genes important in differentiation
  (holds cells in a progenitor state)

p16 controls genetic and epigenetic plasticity



p16 controls genetic and epigenetic plasticity - and specific stem cell characteristics



Targeting of Epigenetic Silencing

Locus	Expressed HMEC	Expressed vHMEC	Reactivated by 5-AZA	Epigentically Silenced
HOXA9	+	-	+	+
HOXD9	+	-	+	+
BARX2	+	-	+	+
SFRP5	+	-	+	+
FABP3	+	-	+	+
E-Cadherin	+	+		-
MLH1	+	+		-
BRCA-1	+	+		-
IGFBP3	+	+		-

<u>methylated in vHMEC</u>

Polycomb-assoc genes involved in differentiation

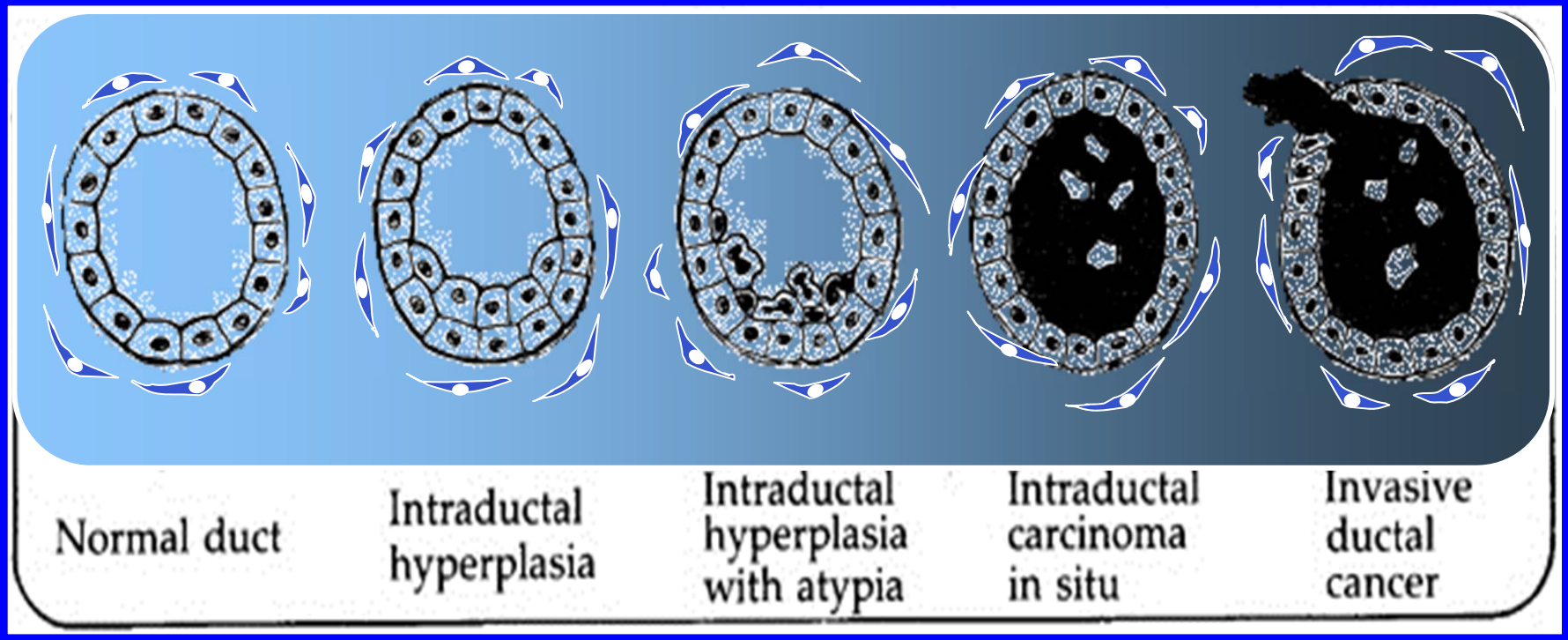
<u>unmethylated in vHMEC</u>

But often methylated in tumors

Matt Wilson and Paul Reynolds

How do Cells Acquire Methylation of Genes Shown to be Silenced in Invasive Cancers?

(random methylation events followed by selection or events programmed by signals that promote tumor progression)



Methylated: HOXA9, HOXD9, BARX2, SFRP5 …

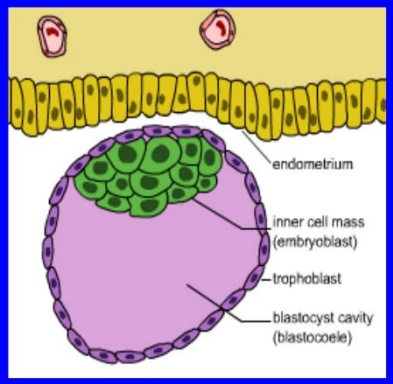
unMethylated: HOXA5, E-cadherin, Estrogen receptor, BRCA1 …

Programming Stem Cell Units

Tissue renewal

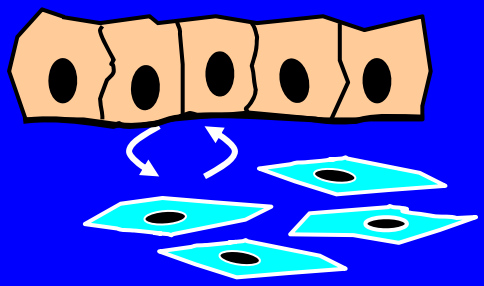
whole organism - zygote (totipotent)

embryonic stem cell (pluripotent)



specialized parts of the organism -

somatic stem cell



<u>Condition</u>:

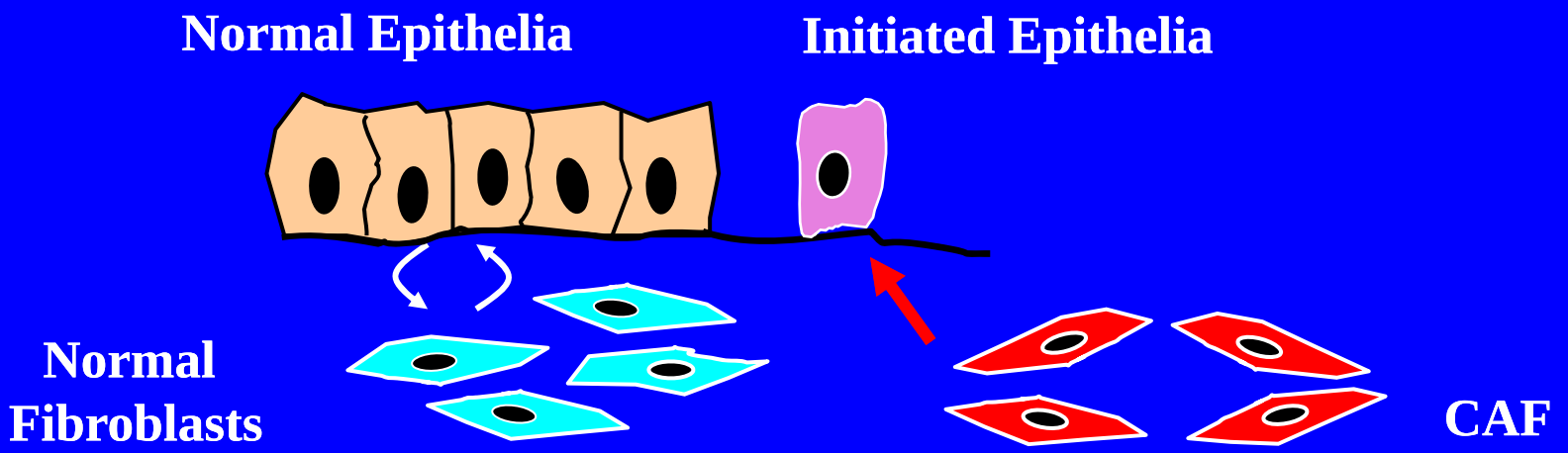
stem cell

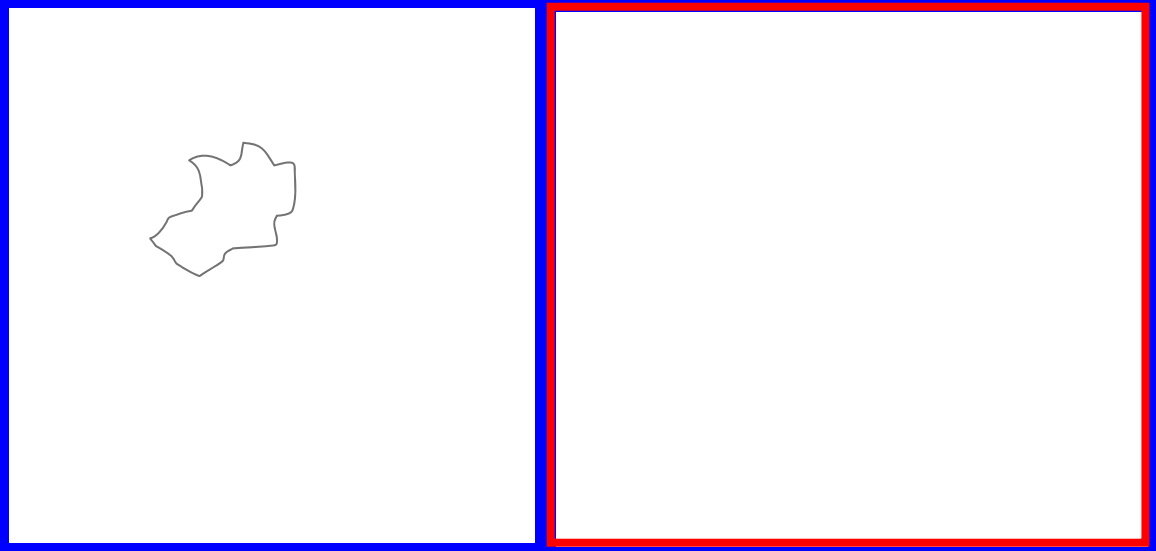
A

B

C

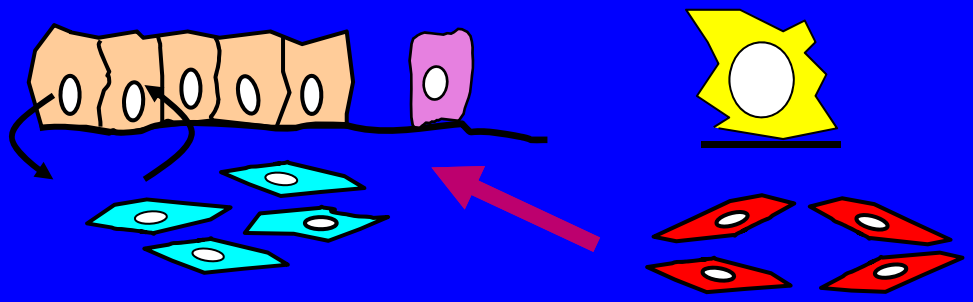
Carcinoma-associated Fibroblasts (Reactive Stroma) Promote Epithelial Tumor Progression





Olumi, et.al., *Cancer Res.* (1999)

Carcinoma-associated Fibroblasts influence epithelial cells by causing:



Increased Cell Proliferation

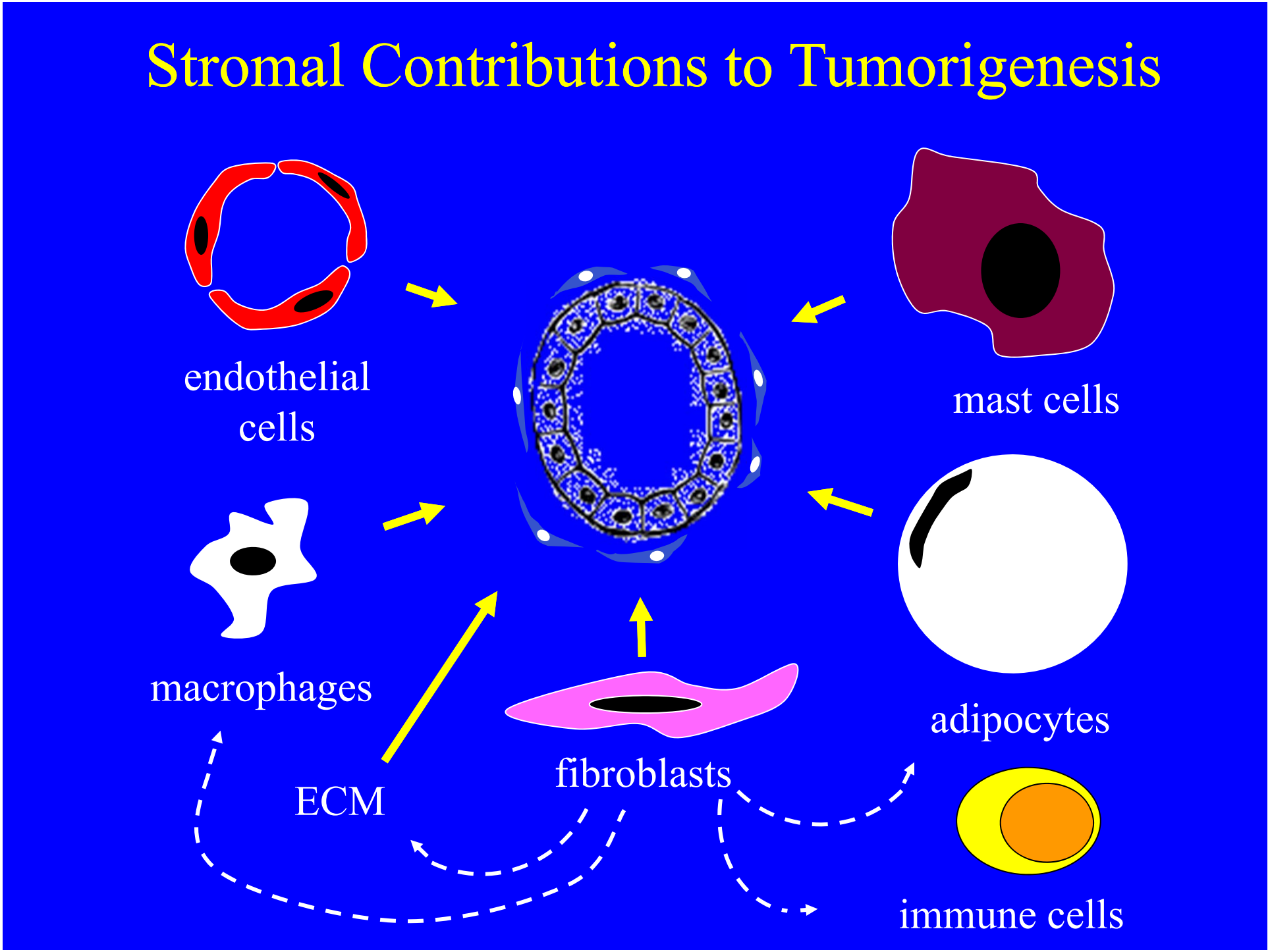
Decreased Cell Death

Stimulation of Angiogenesis

Alteration of Cell Adhesion

Decreased genomic integrity

Stromal Contributions to Tumorigenesis



HYPOTHESIS

We hypothesize that cells in an epigenetically-plastic state can be programmed by the microenvironment to acquire epigenetic changes that promote tumorigenesis.

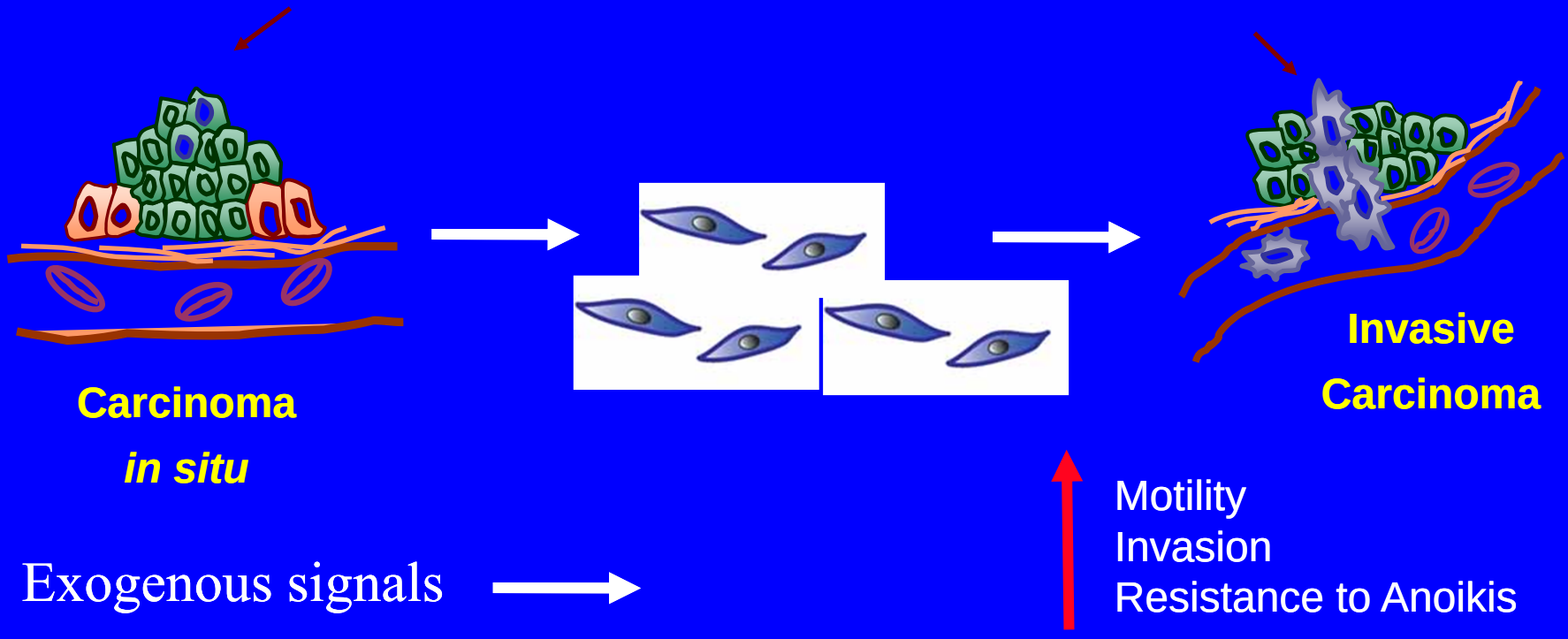
We use an *in vitro* model system where epigenetically-plastic cells are placed in an environment that induces a malignant program.

Epithelial to Mesenchymal Transition (EMT)

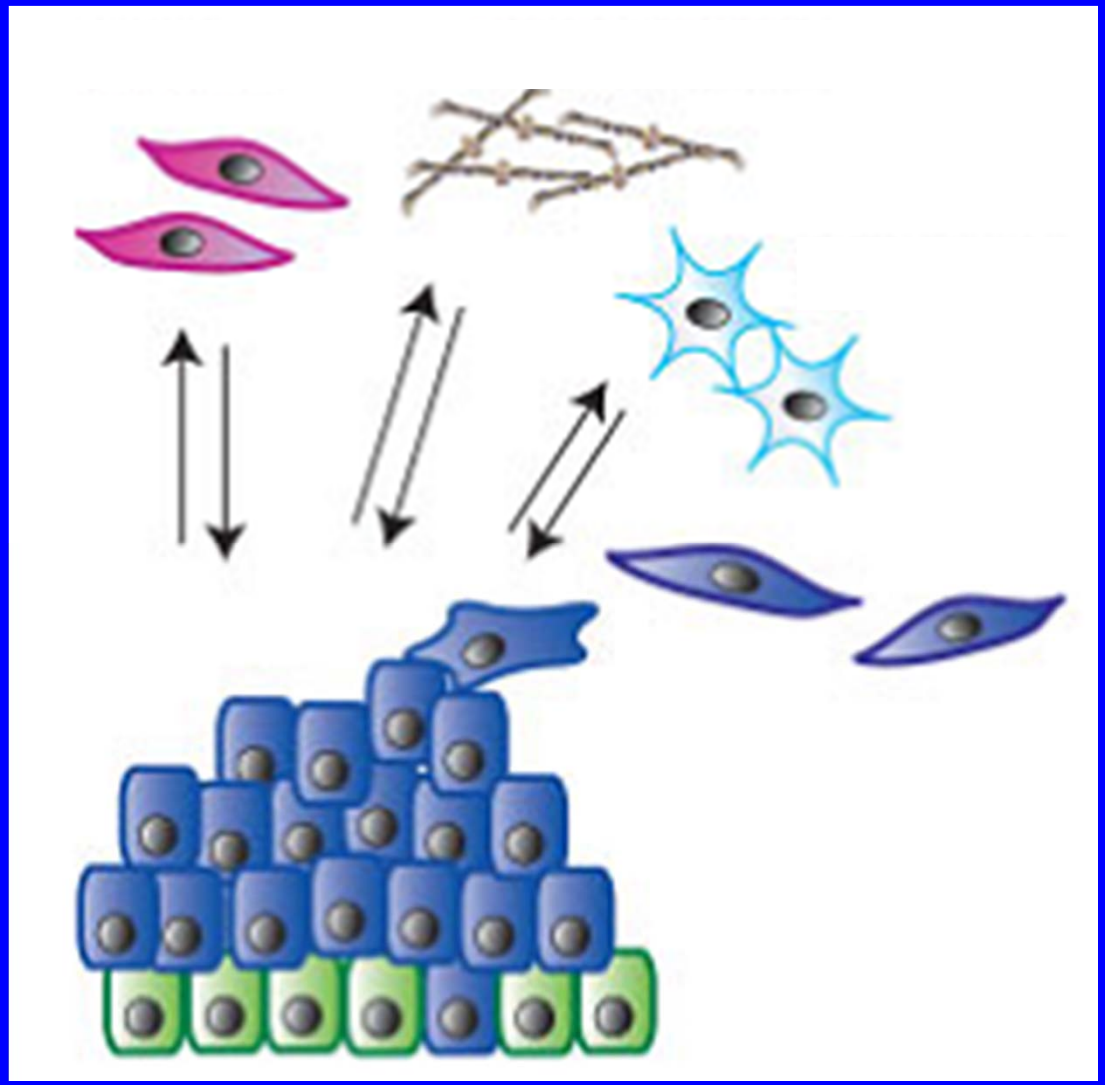
Used during:

(1) development to position cells for individual fates

(2) cancer progression

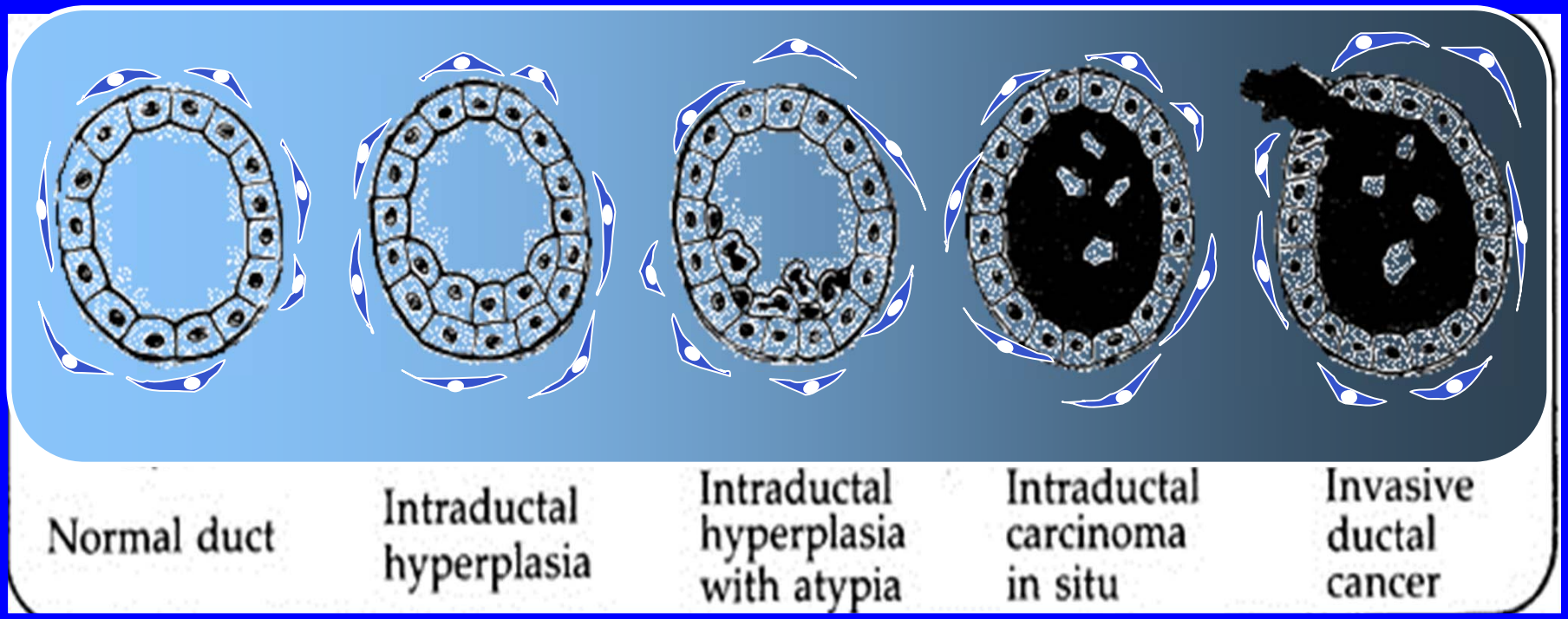


Oncogenic ras can cooperate with factors in serum to induce an Epithelial to Mesenchymal Transition and promote tumorigenesis in murine cells



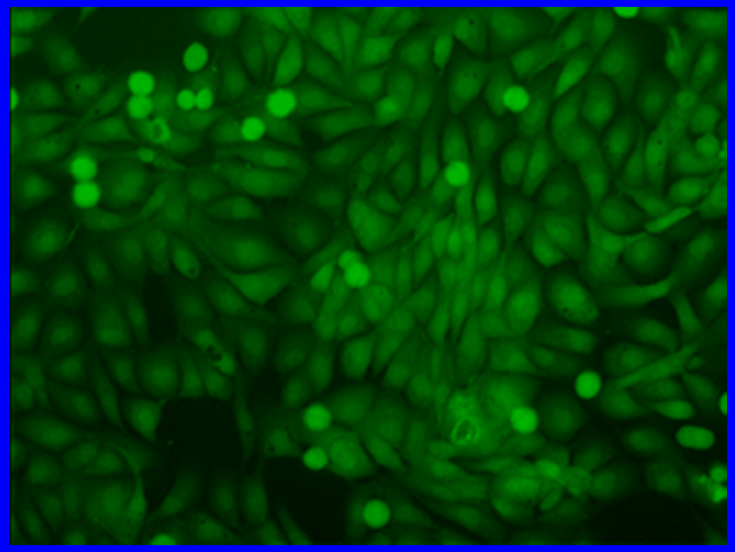
(Oft et al., Genes Dev 10: 2462-2477, 1996)

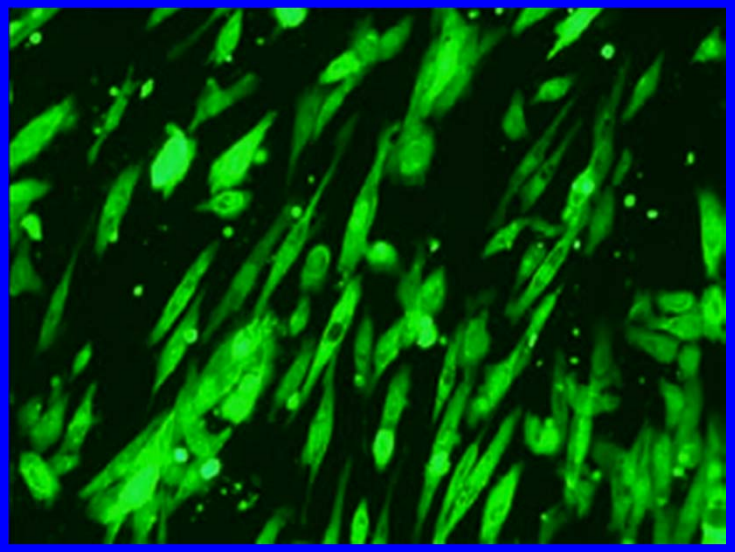
Acquistion of Epithelial to Mesenchymal Transition

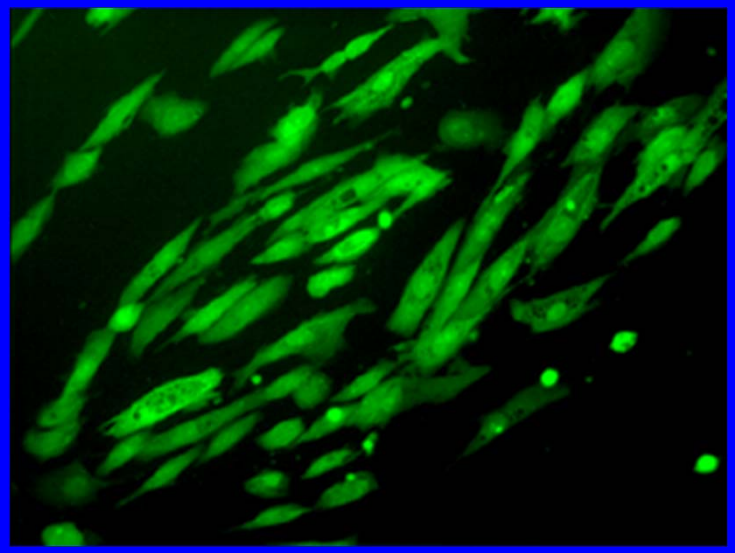


abnormal Ras signaling
+
immortalization
→ + serum →
EMT?

Epithelial to Mesenchymal Transition in Human Cells







Nancy Dumont

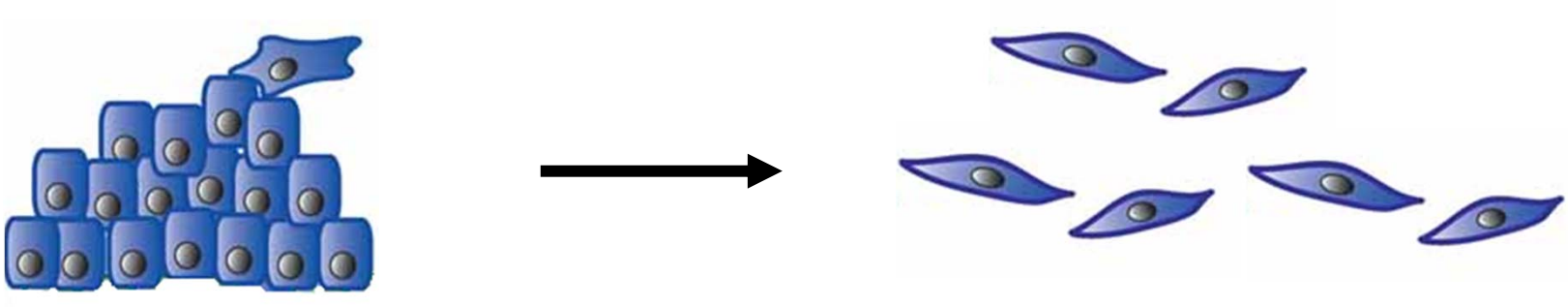
Epithelial Mesenchymal Transition (EMT)

Epithelial Markers

E-cadherin
ZO-1

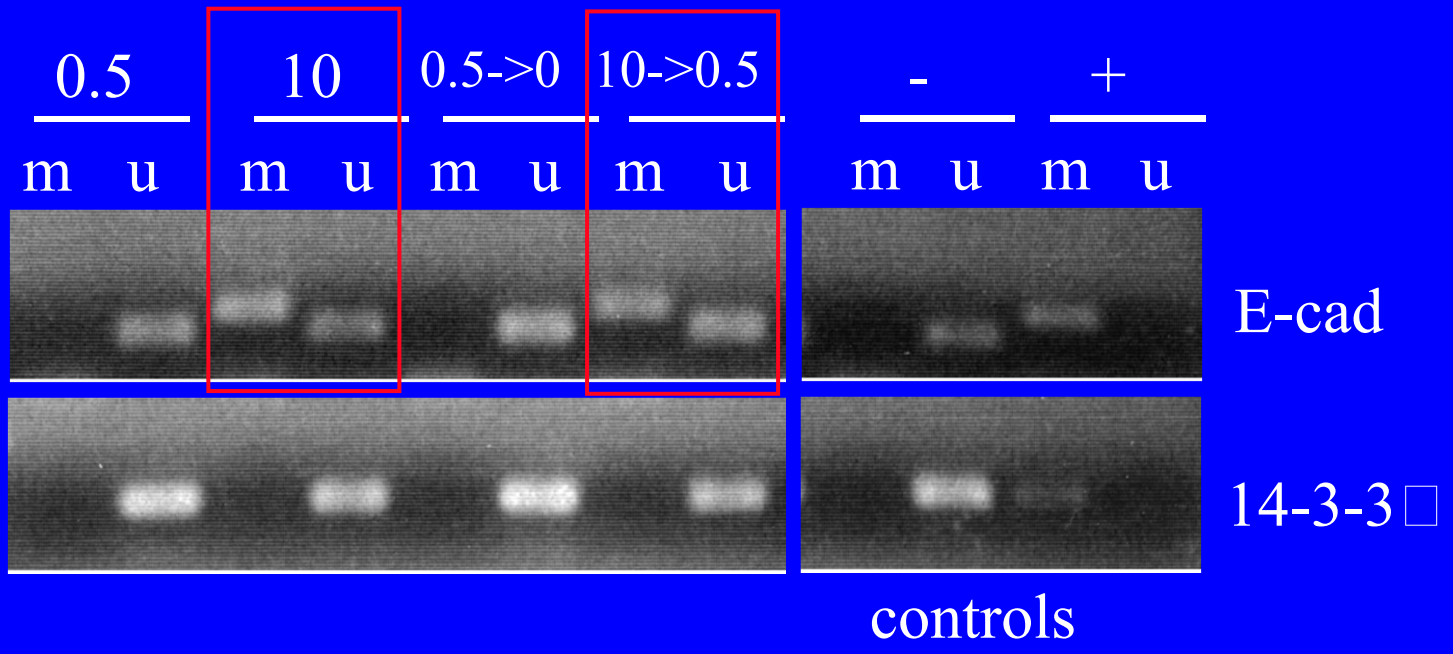
Mesenchymal Markers

N-cadherin
Fibronectin
Vimentin



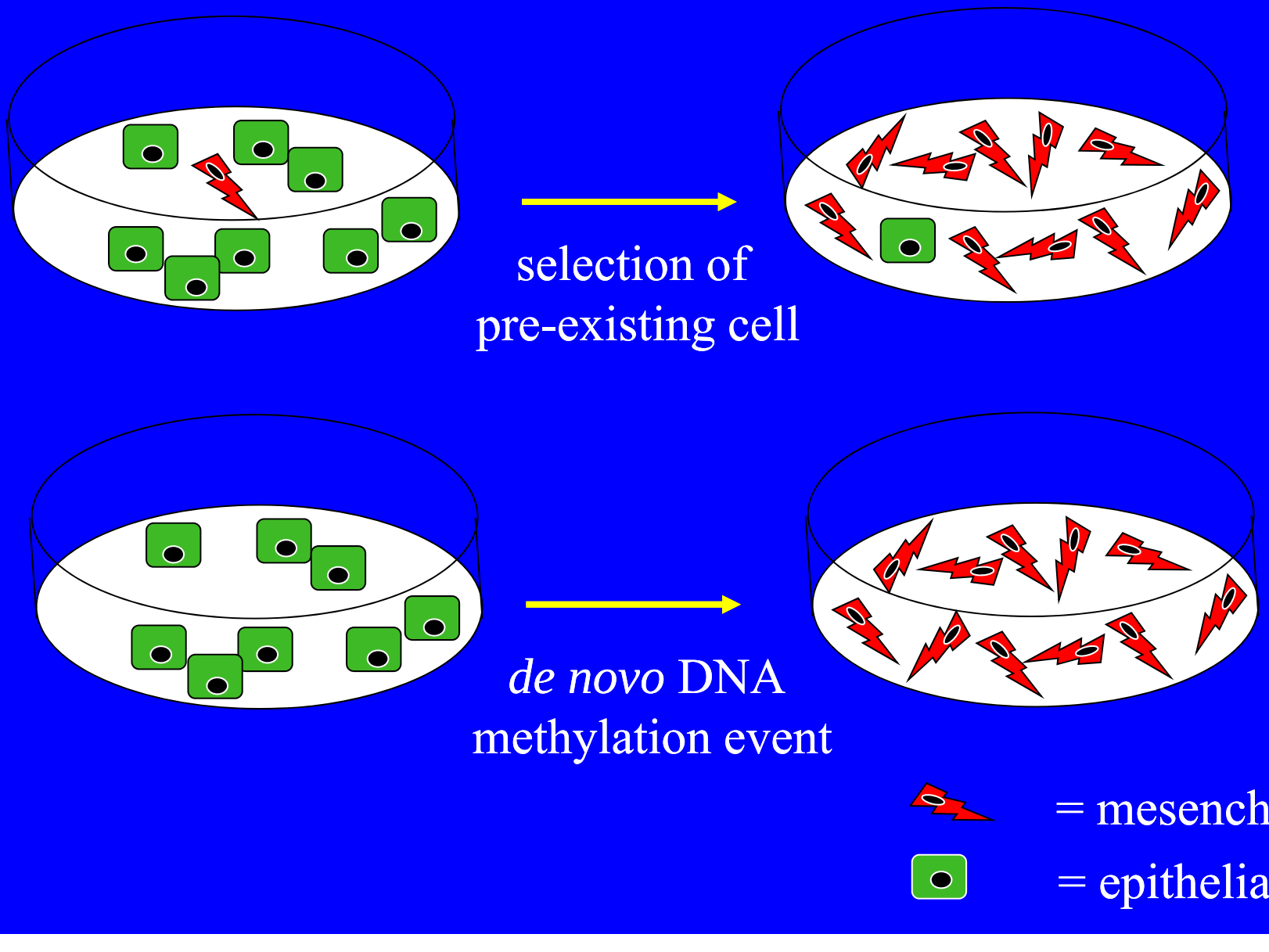
Phenotypes - increased motility

Using MS-PCR, we found that E-cadherin is silenced via Promoter DNA Methylation in vHMEC-ras with a Mesenchymal Morphology



Paul Reynolds

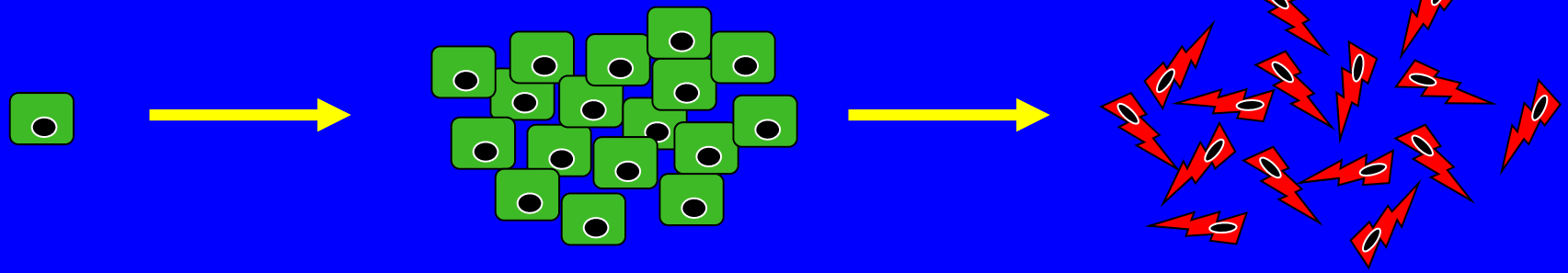
Selection or *de novo* DNA Methylation Event?



propagated in serum

Early

Late



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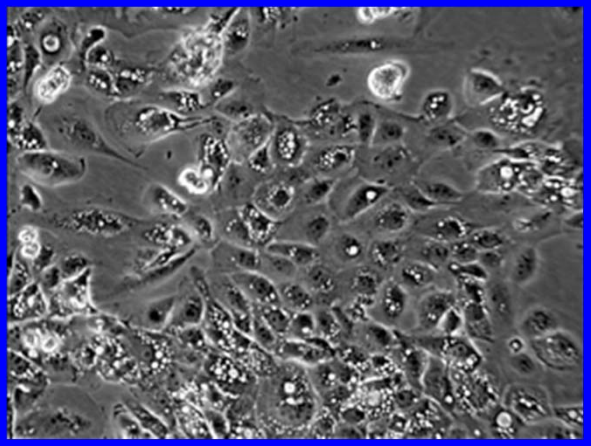

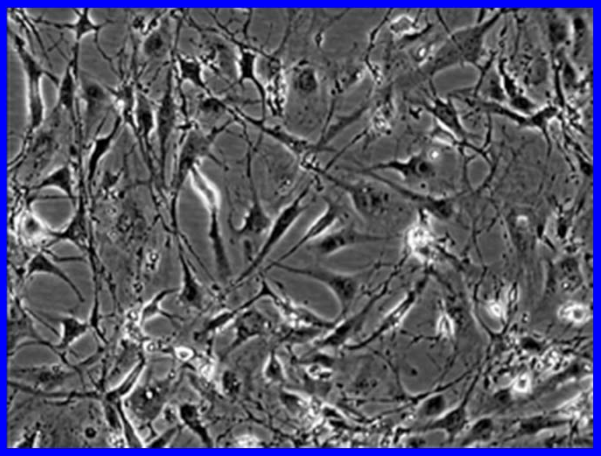

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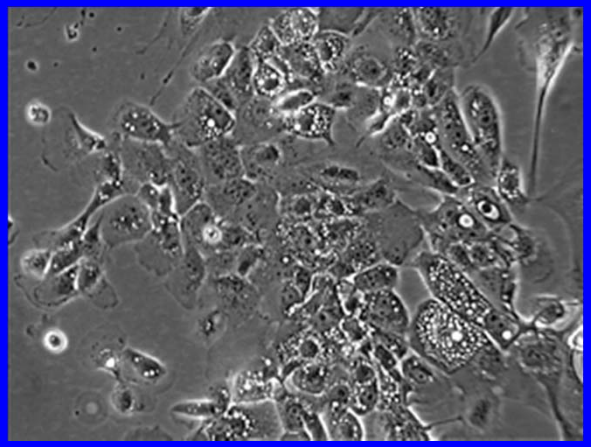
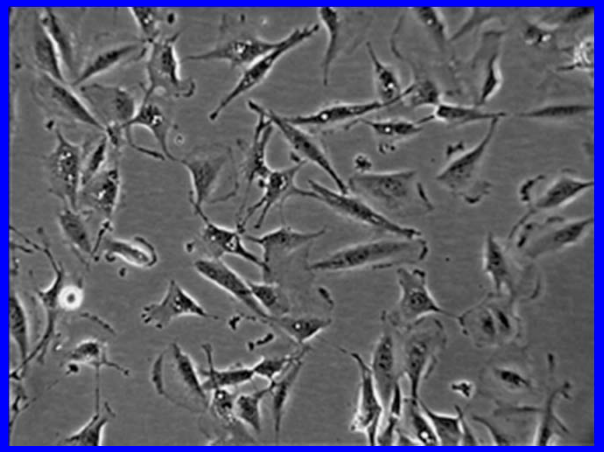
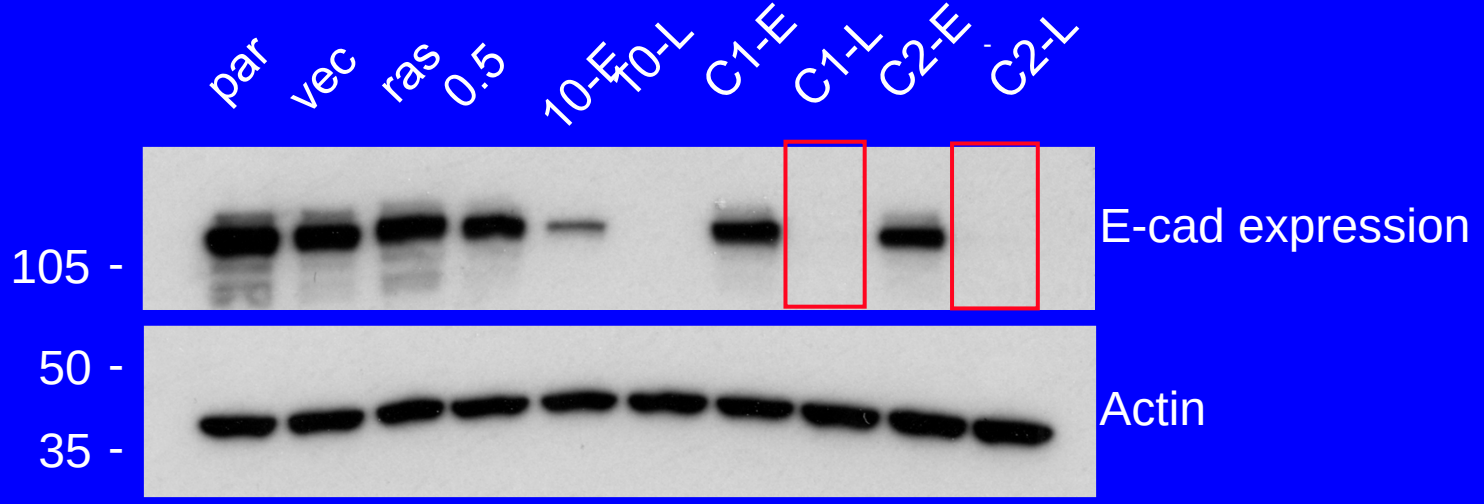
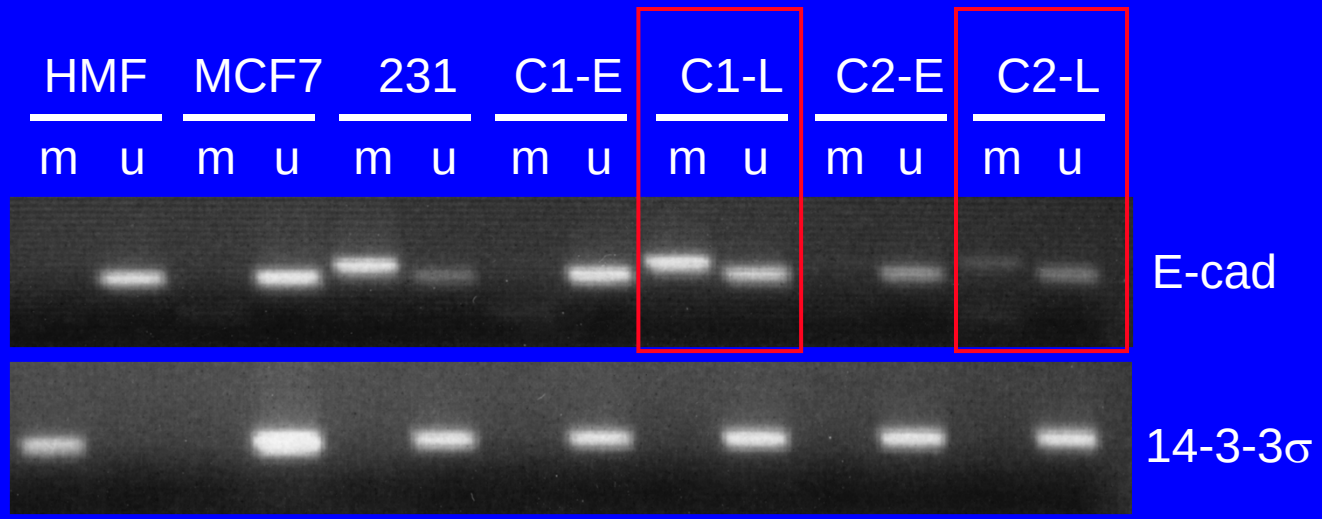




Nancy Dumont

Methylation of the E-cadherin Promoter is a *de novo* Event





Nancy Dumont and Matt Wilson

Panel of loci become hypermethylated:

E-cadherin
Estrogen receptor α
Twist
CST6

This group of loci is part of a signature of loci that are only hypermethylated in basal-like breast tumors that exhibit mesenchymal phenotypes and poor prognosis.

DNA Methylation Events in Cancer can be Deterministic Rather than Stocastic

In this system, methylation is not a random event.

Raises questions about common methylation events in cancer…

the methylation profile of a cell tells a story of where it's been and the cell's capacity to interact with the incoming information.

Summary and Conclusions - Part II

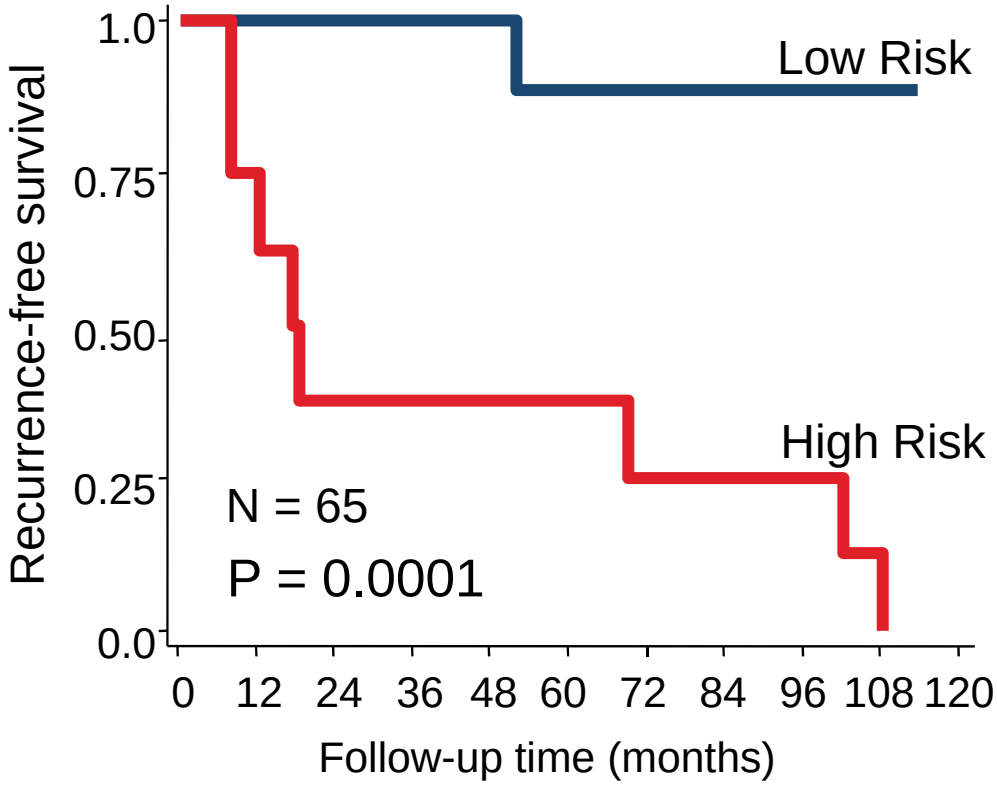
- Cells can be programmed by their microenvironment to undergo phenotypic and gene expression changes associated with targeted *de novo* epigenetic alterations important in tumor progression (and stem cell function?).

  other phenotypes - immortality, etc

- We can use this information to create tools that address clinical questions.
  Prognostic biomarkers, novel therapeutic targets, etc.

New Assay Stratifies Risk for Subsequent Tumor Events in a Subset of DCIS Patients

Assay Signature



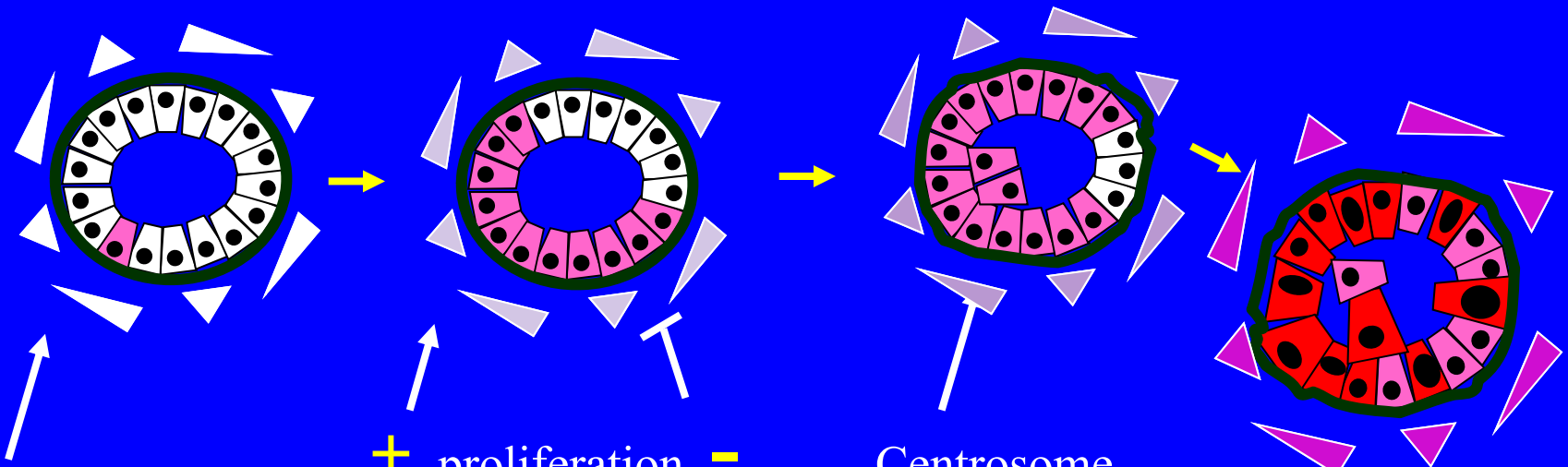
- 98% of biopsies that express the positive risk markers are associated with subsequent tumor events.
- 97% of biopsies that express negative risk markers are associated with NO subsequent tumor events.

How do cells acquire epigenetic plasticity (stemness)?
Alter expression of key tumor suppressor and oncogene pathways

What determines which genes become hypermethylated during cancer progression?
Process is deterministic - not random - guided by transcriptional co-regulators

Can we use this information to create tools that address clinical questions?
Prognostic biomarkers, novel therapeutic targets, etc.

Mutual Reprogramming by Epithelial cells and their Adjacent Neighbors Generate Malignancies

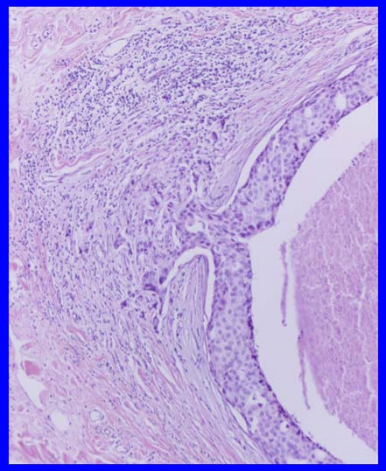


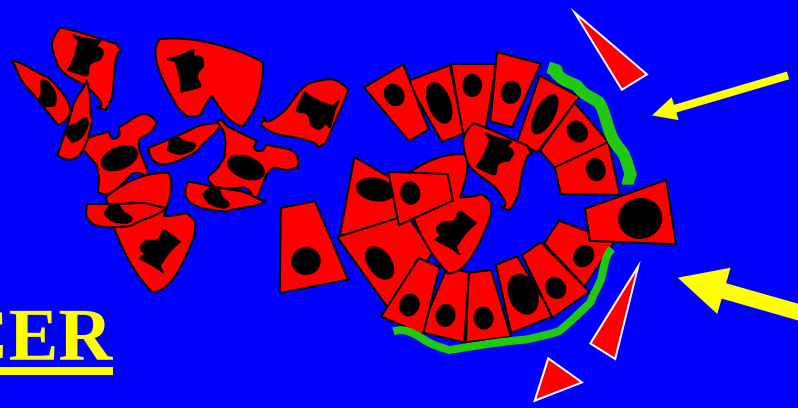
methylation (aging, damage, diet, ?)

\+ proliferation signals -

Centrosome abnormalities/ Methylation changes

telomeric dysfunction and altered gene expression





CANCER

Stroma (inflammation, aging, etc.)

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Collaborators

Joe Costello

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