Robert A. Weinberg
Whitehead Institute
MIT Biology Department
Cambridge MA

How does an individual tumor develop?

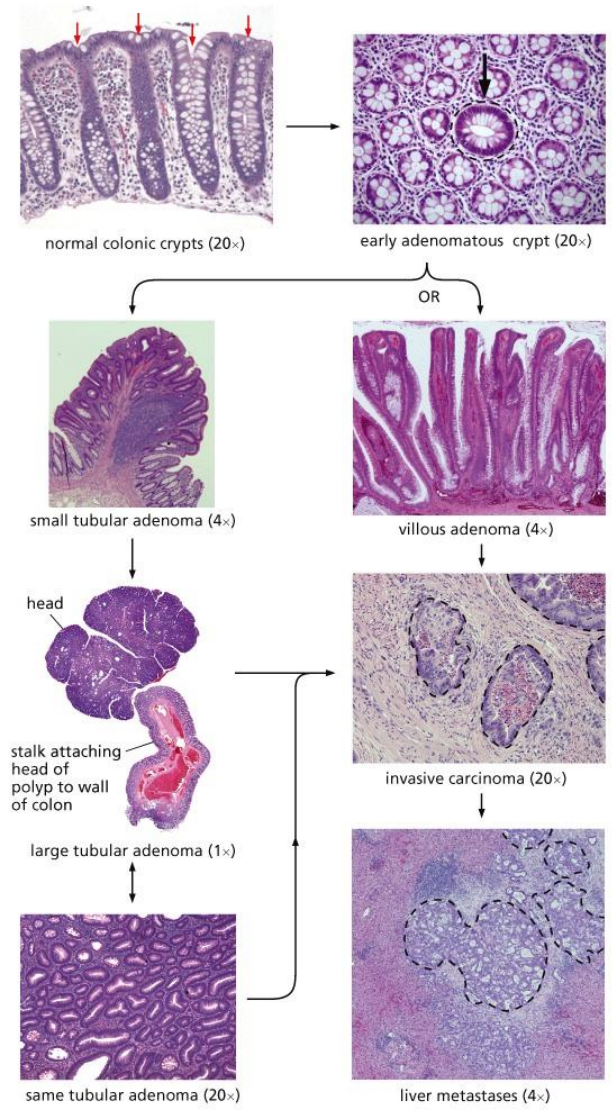


Figure 11.7 The Biology of Cancer (© Garland Science 2014)

Tumor development is a multi-step process in multiple distinct epithelial tissues.

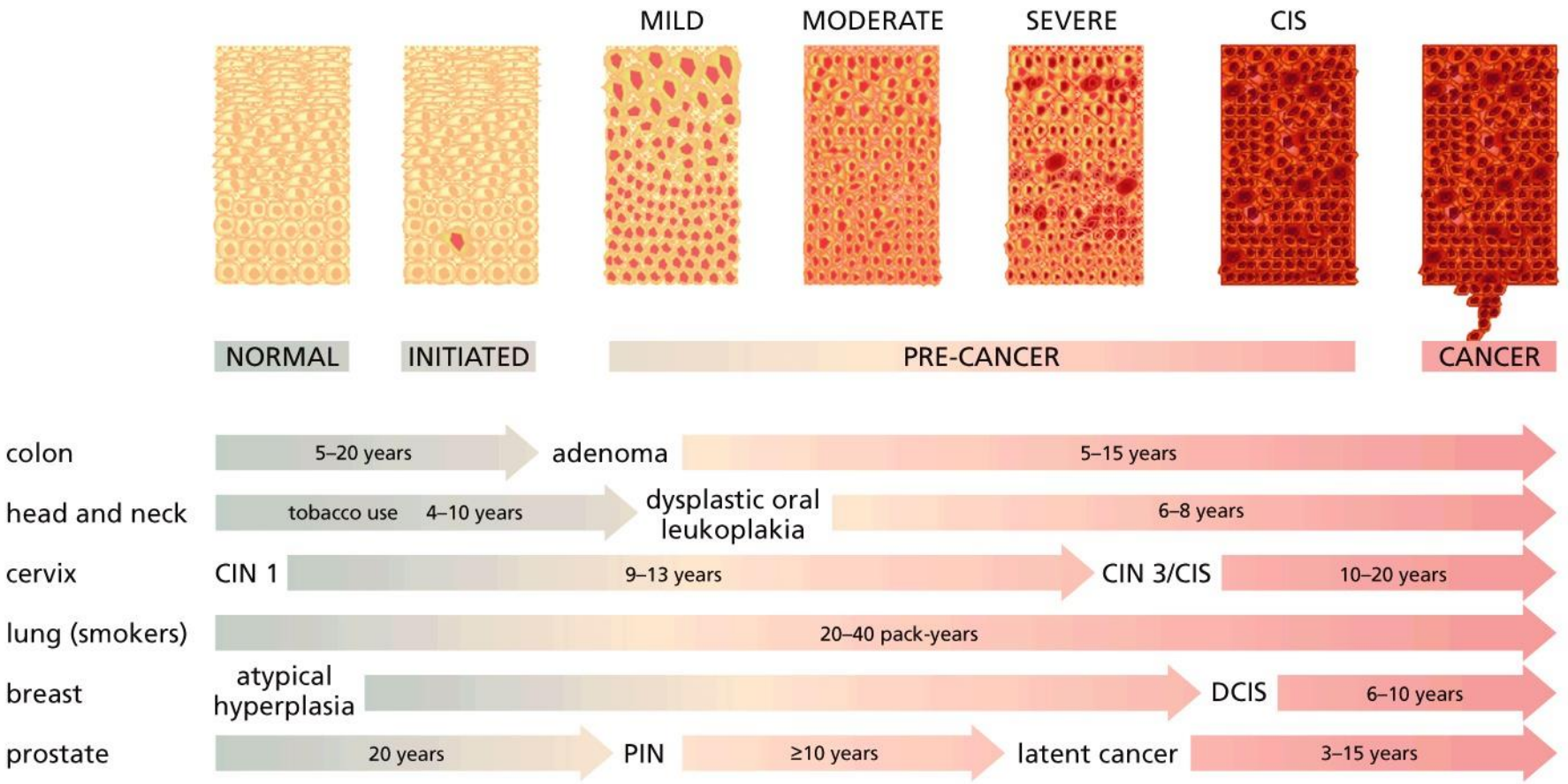


Figure 11.8a The Biology of Cancer (© Garland Science 2014)

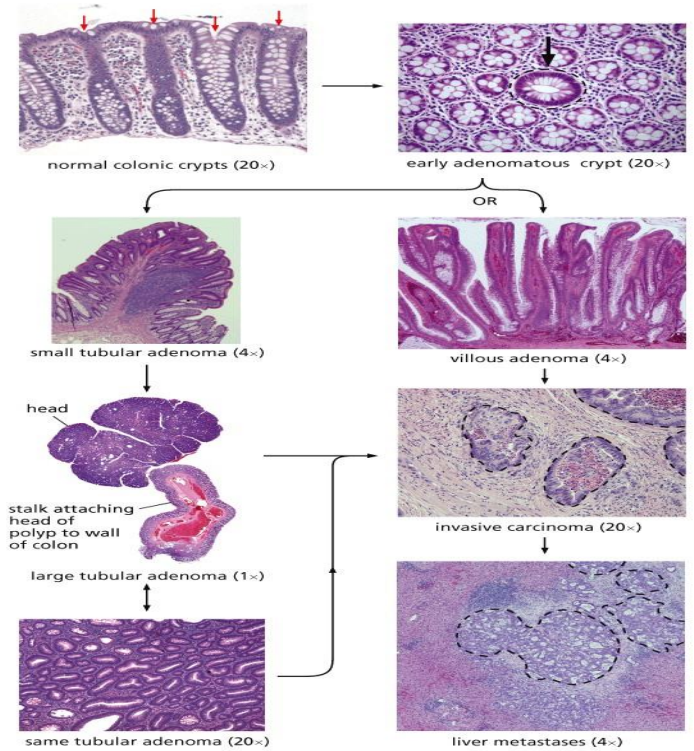


Figure 11.7 The Biology of Cancer (© Garland Science 2014)

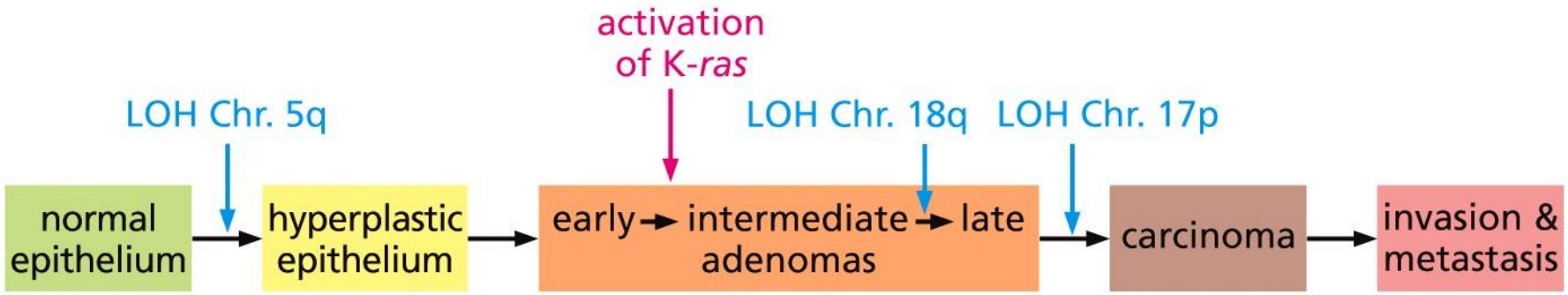
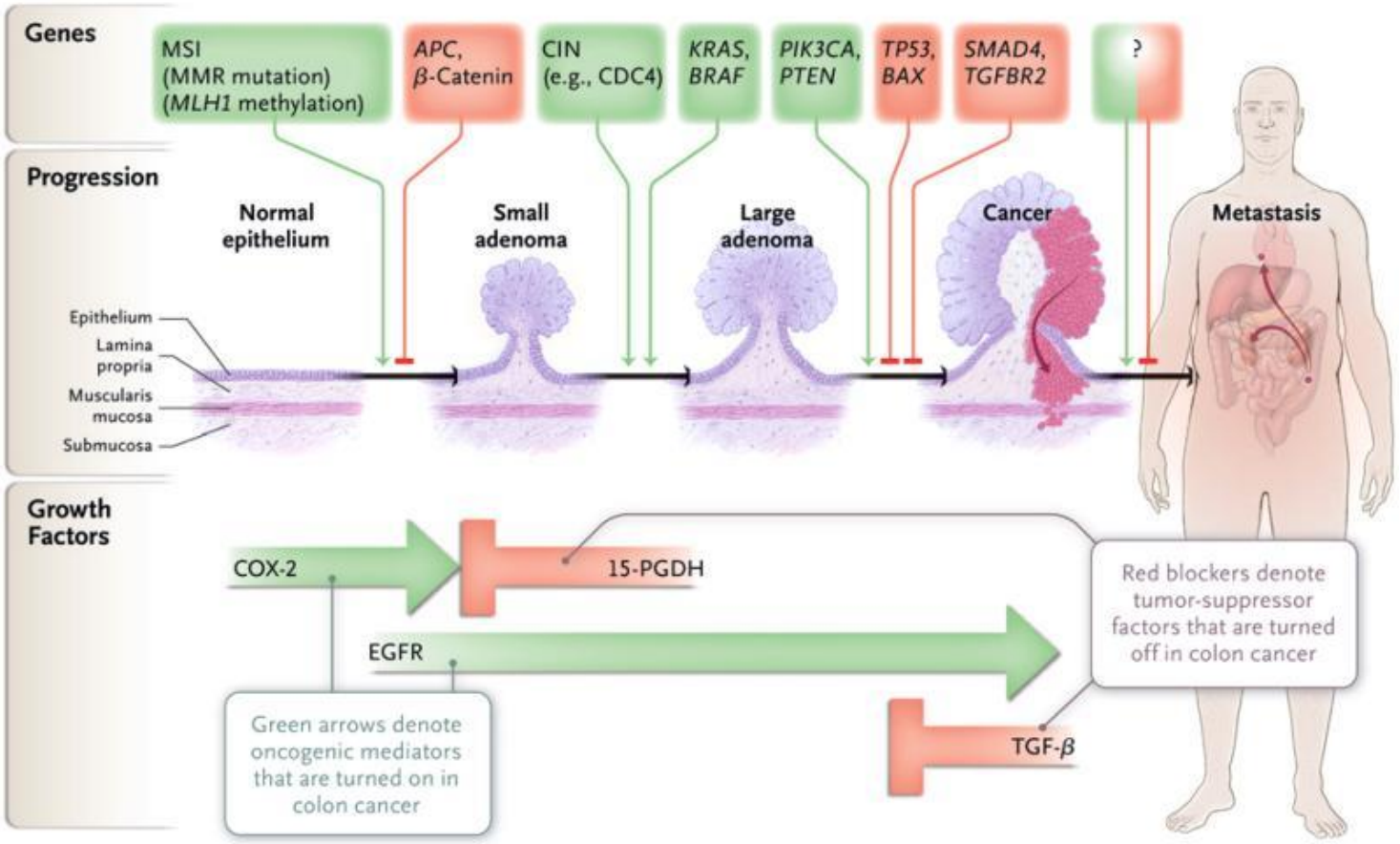


Figure 11.10 The Biology of Cancer (© Garland Science 2014)

Colorectal carcinoma. (Vogelstein et al 1989)

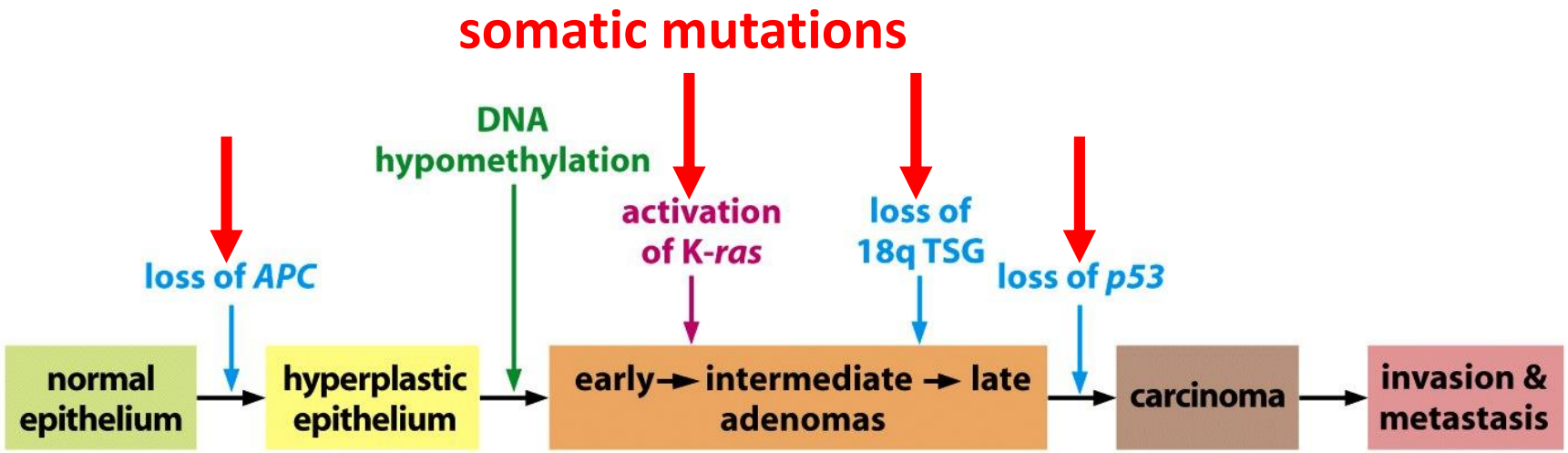


N Engl J Med. 2009 Dec 17;361(25):2449-60. doi: 10.1056/NEJMra0804588.

Molecular origins of cancer: Molecular basis of colorectal cancer.

Markowitz SD[1], Bertagnolli MM.

Can we understand the pathogenesis of a tumor in terms of the somatic mutations that it accumulates in the genome of its neoplastic cells? e.g., colorectal carcinomas



Colorectal carcinoma. (Vogelstein et al 1989)

How important are non-genetic programs in determining the biology of cancer cells and thus tumors?

Figure 11.10 *The Biology of Cancer* (© Garland Science 2007)

Can we understand the pathogenesis of a tumor in terms of the somatic mutations that it accumulates in the genome of its neoplastic cells? e.g., pancreatic adenocarcinomas

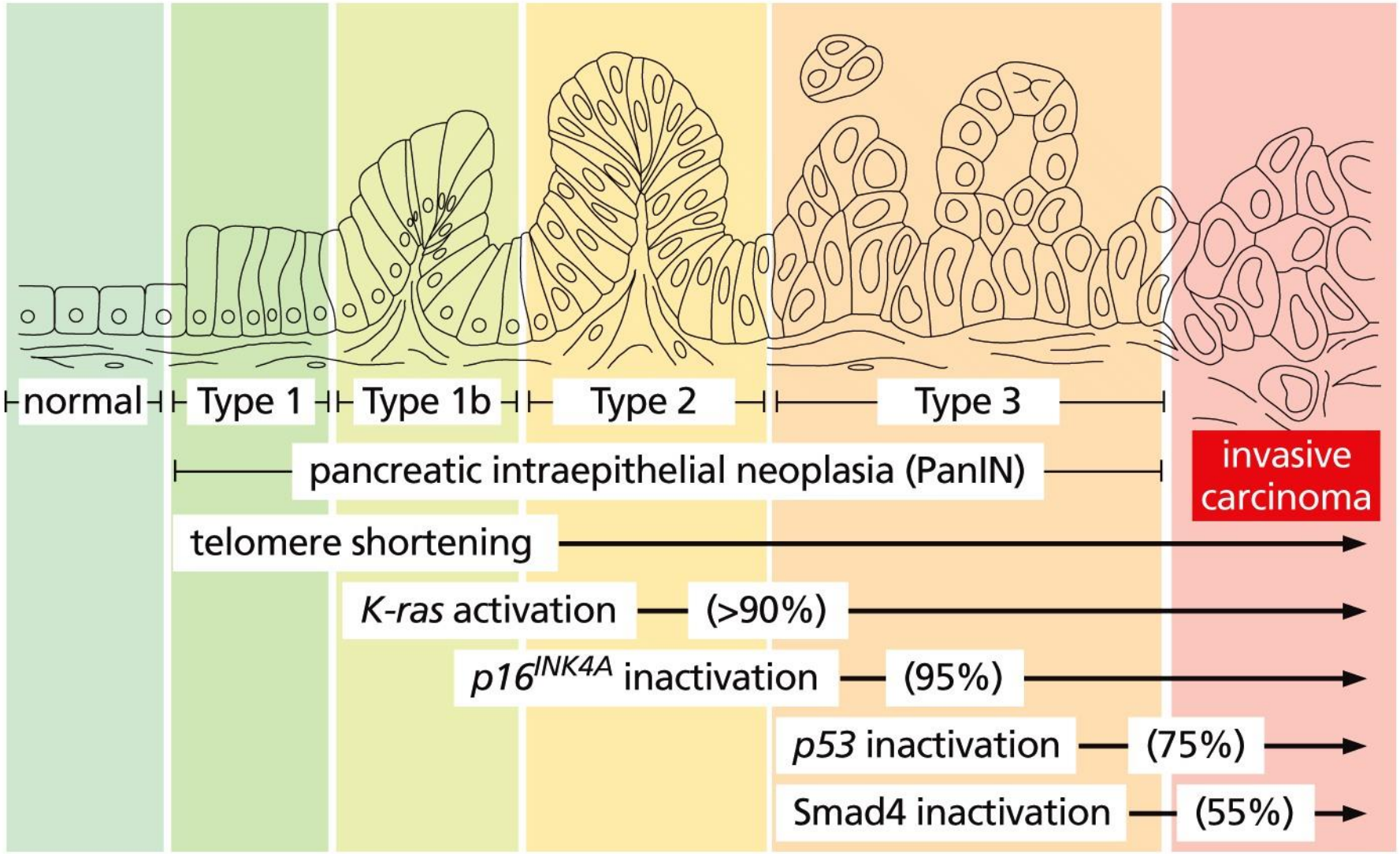


Figure 11.12b The Biology of Cancer (© Garland Science 2014)

Experimental transformation of a human cell. (1999)

pathway	Ras	pRb	p53	telomeres	PP2A
genes/agents used to deregulate pathway	*ras*, *MEK+ Akt/PKB*, *MEK+IKBKε*, *PAK1+ Akt/PKB*	SV40 *LT*, *CDK4 + D1*, HPV *E7*, *Rb shRNA*	SV40 *LT*, DN *p53*, HPV *E6*, *p53 shRNA*	*hTERT*, *myc* + SV40 *LT*	SV40 *sT* in some cells: *myc* *Akt/PKB+Rac1*, *PI3K*, *B56 shRNA*

Figure 11.27 The Biology of Cancer (© Garland Science 2014)

This scheme allows us to understand, at least in outline how a **primary tumor** can be formed.

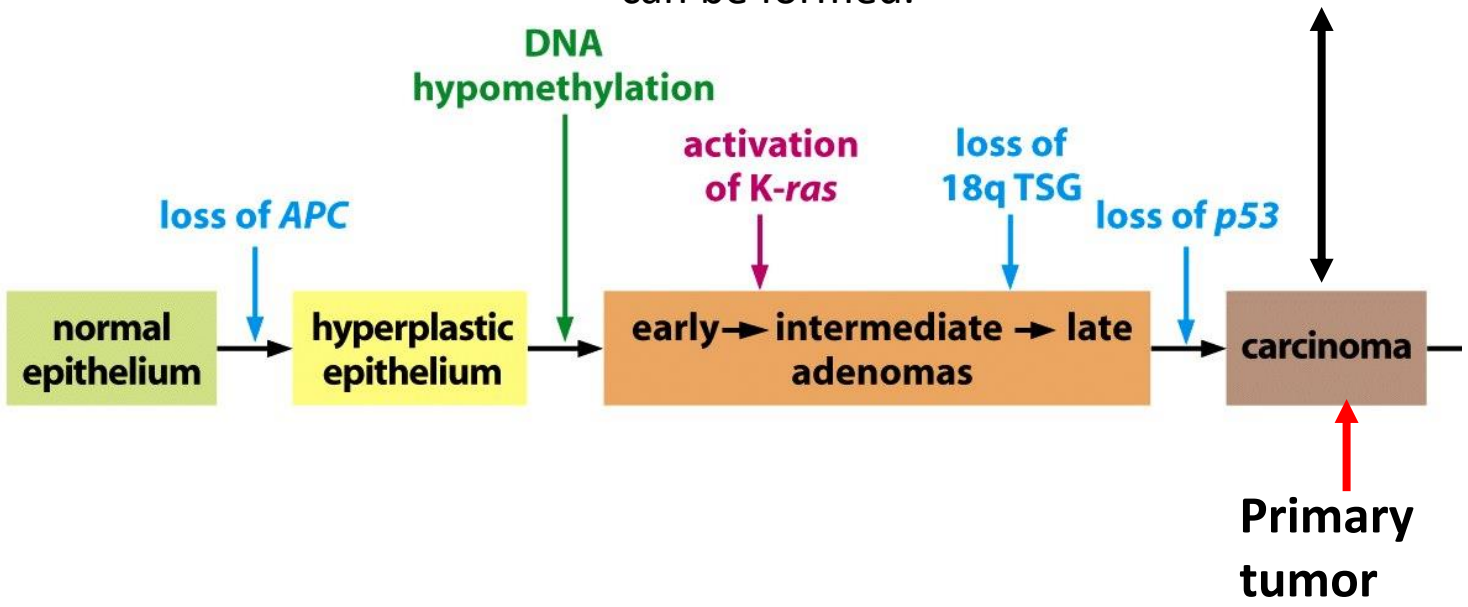


Figure 11.10 *The Biology of Cancer* (© Garland Science 2007)

Multiple subcircuits within a human cell must be perturbed before (experimental) transformation of human cells succeeds.

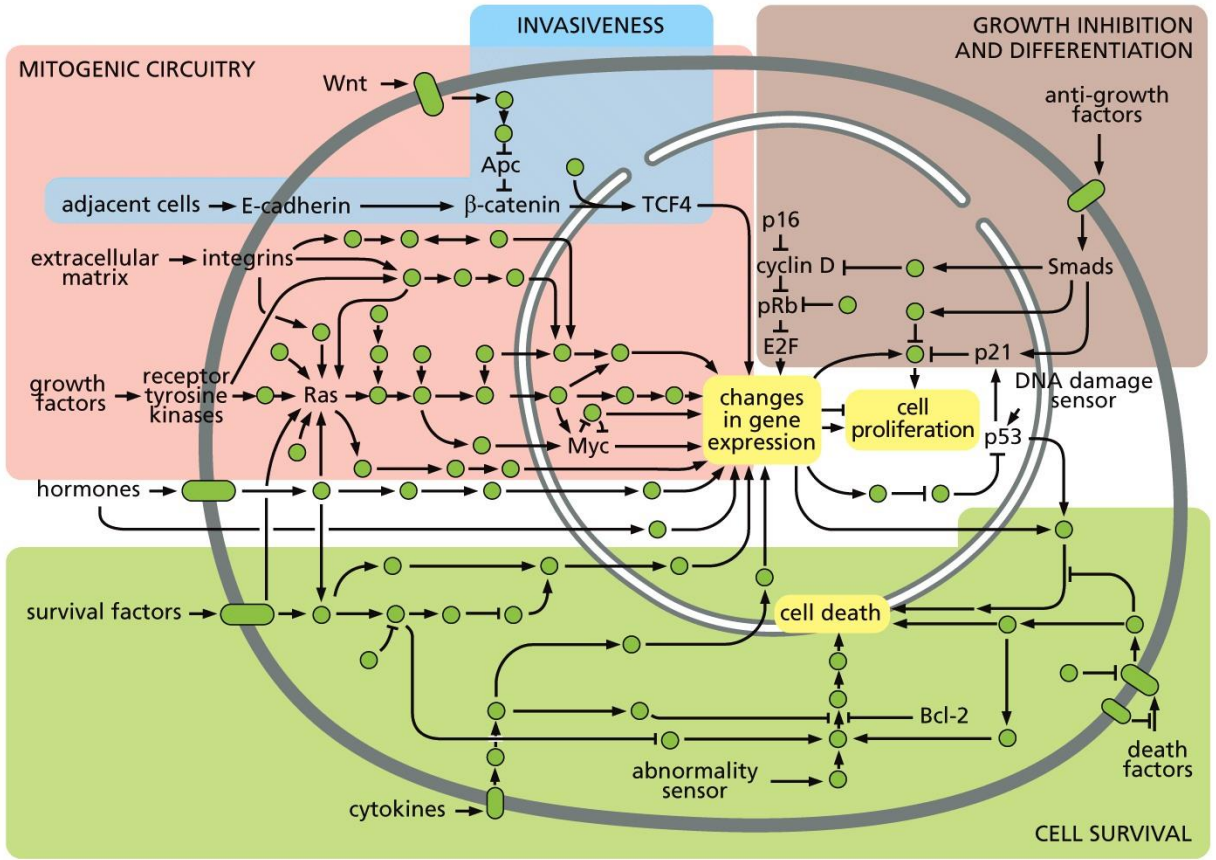


Figure 11.45 The Biology of Cancer (© Garland Science 2014)

Why is human cell transformation so complicated?

$\sim 10^{11}$ cell divisions

$\sim 10^{9}$ cell divisions?

bumble bee bat

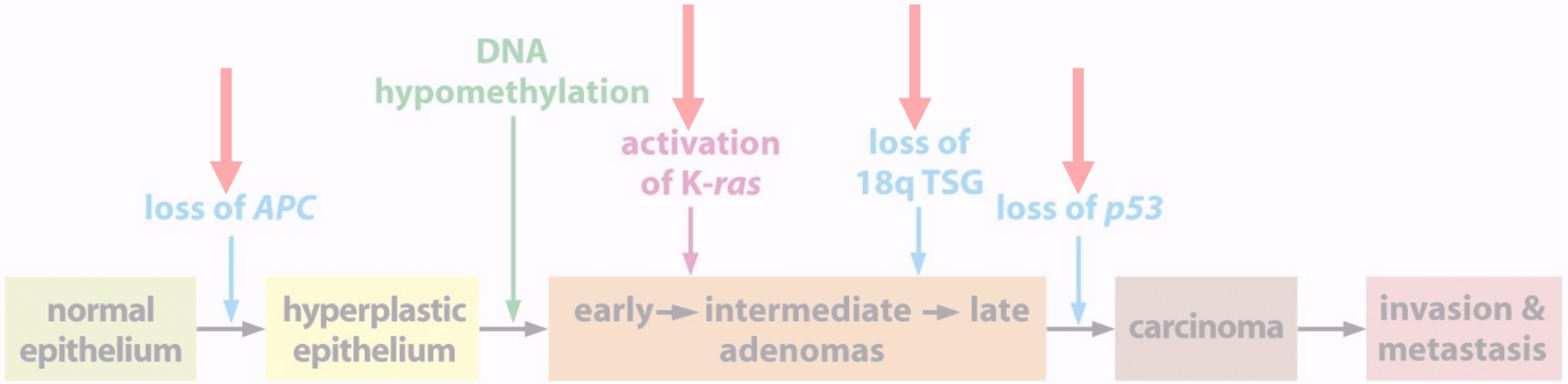
$\sim 10^{19}$ cell divisions ?

we

10^{16} cell divisions

If the risk of somatic mutations is proportional to the
the cumulative number of cell divisions in a lifespan
then the cells of larger, long-lived mammals must have
acquired proportionally increased numbers of anti-neoplastic defenses.

Can we understand the pathogenesis of a tumor in terms of the somatic mutations that it accumulates in the genome of its neoplastic cells?



Colorectal carcinoma. (Vogelstein et al 1989)

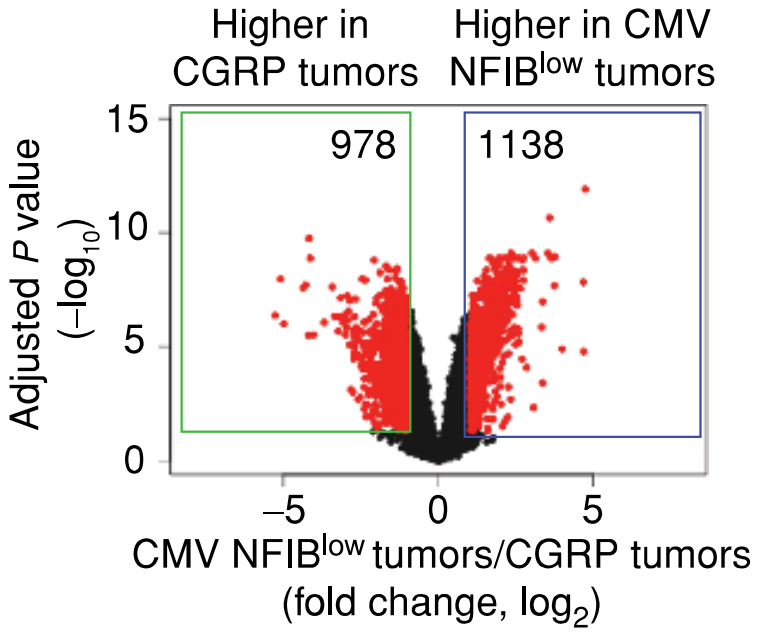
→ **How important are non-genetic programs in determining the biology of cancer cells and thus tumors?**

Figure 11.10 *The Biology of Cancer* (© Garland Science 2007)

A major factor: The continuing influence of the differentiation program of the cell-of-origin

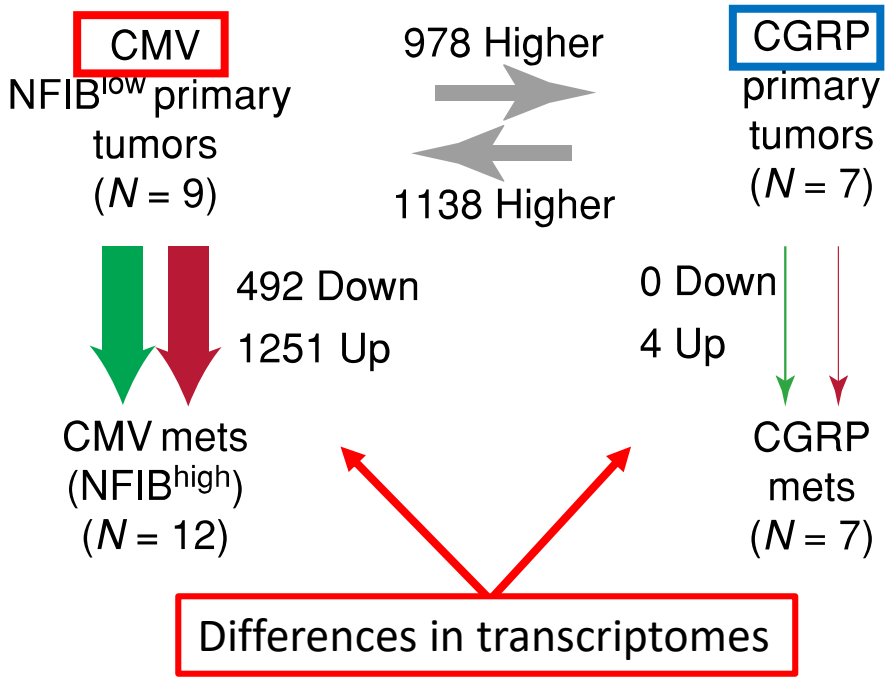
Two genetically identical tumors from closely related cell types

SCLC = small cell lung cancer



Differential gene expression in CMV TKO NFIB^{low} tumors and CGRP TKO tumors (|fold change| > 2, adj. $P < 0.05$

Different genes are up/down-regulated in SCLC cells originating from **two distinct subtypes** of neuroendocrine cells in the lungs.



Intertumoral Heterogeneity in SCLC Is Influenced by the Cell Type of Origin (2018) Dian Yang et al. *Cancer Discov; 8(10); 1316–31*.

The continuing influence on transcriptome of the differentiation program of the normal cell-of-origin

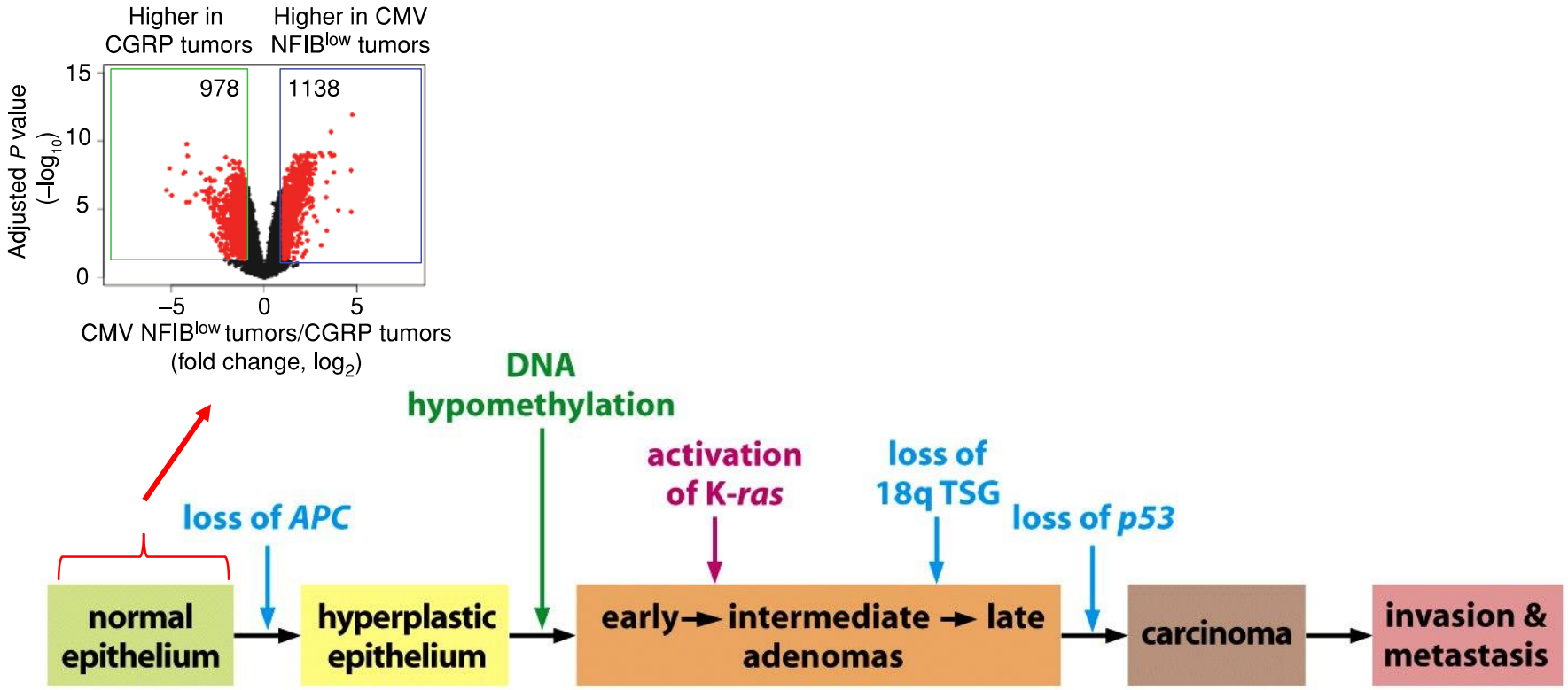
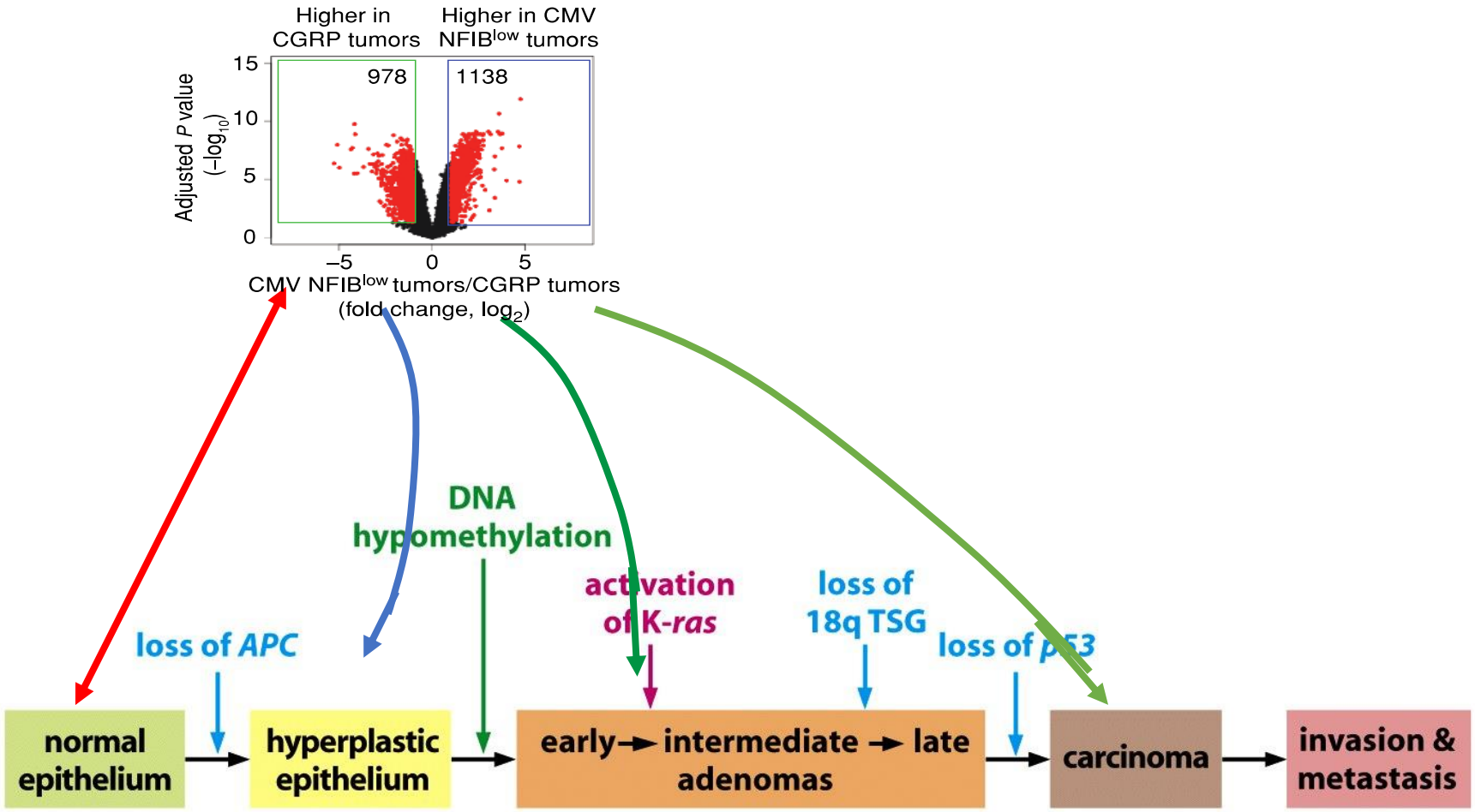


Figure 11.10 *The Biology of Cancer* (© Garland Science 2007)

The transcriptome of the normal cell-of-origin continues to imprint itself on the behavior of derived neoplastic cells.



The acquisition of somatic mutations does not eradicate the continuing influence of the normal cell-of-origin

Figure 11.10 *The Biology of Cancer* (© Garland Science 2007)

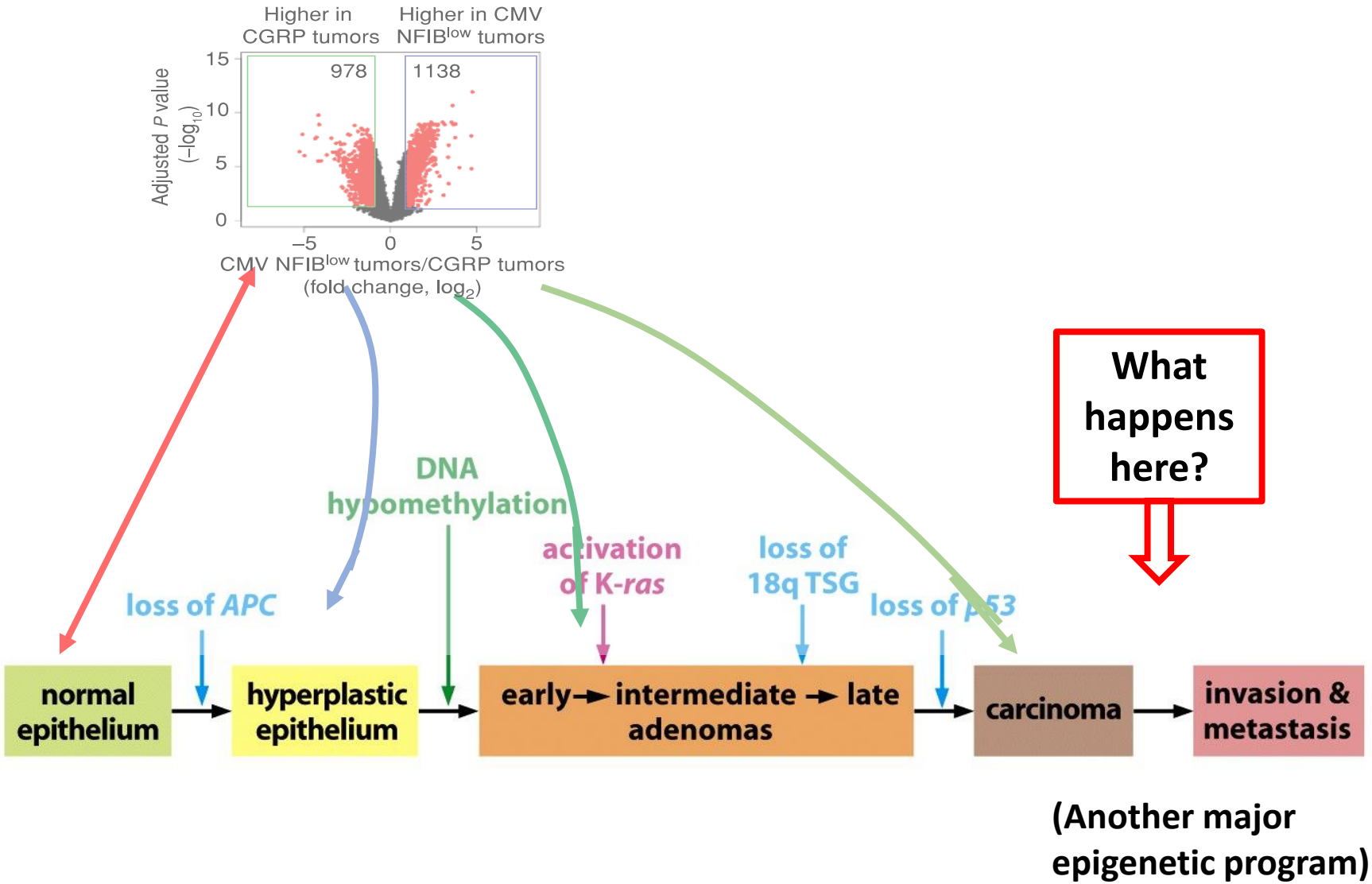
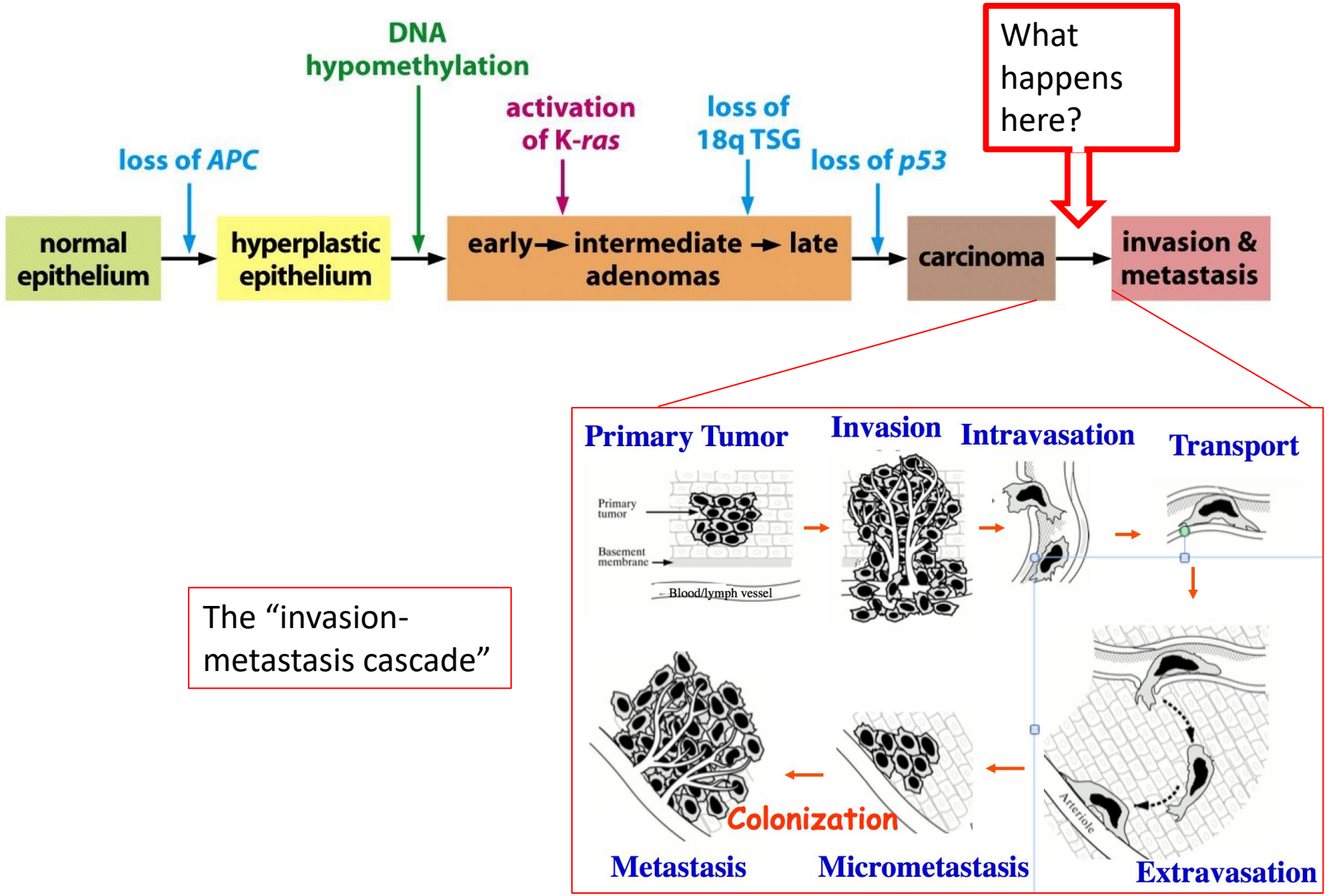
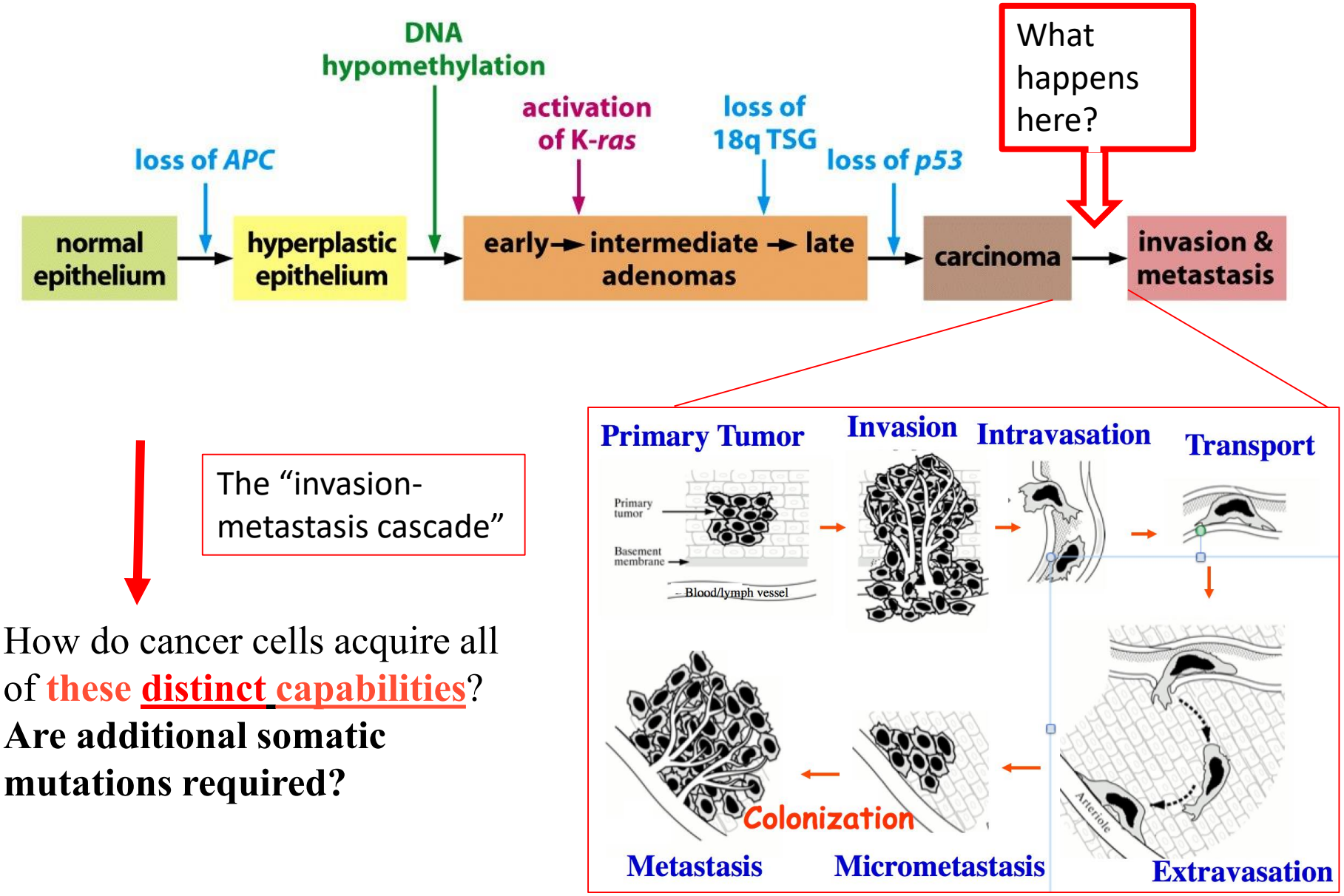


Figure 11.10 *The Biology of Cancer* (© Garland Science 2007)



The "invasion-metastasis cascade"

Figure 11.10 *The Biology of Cancer* (© Garland Science 2007)

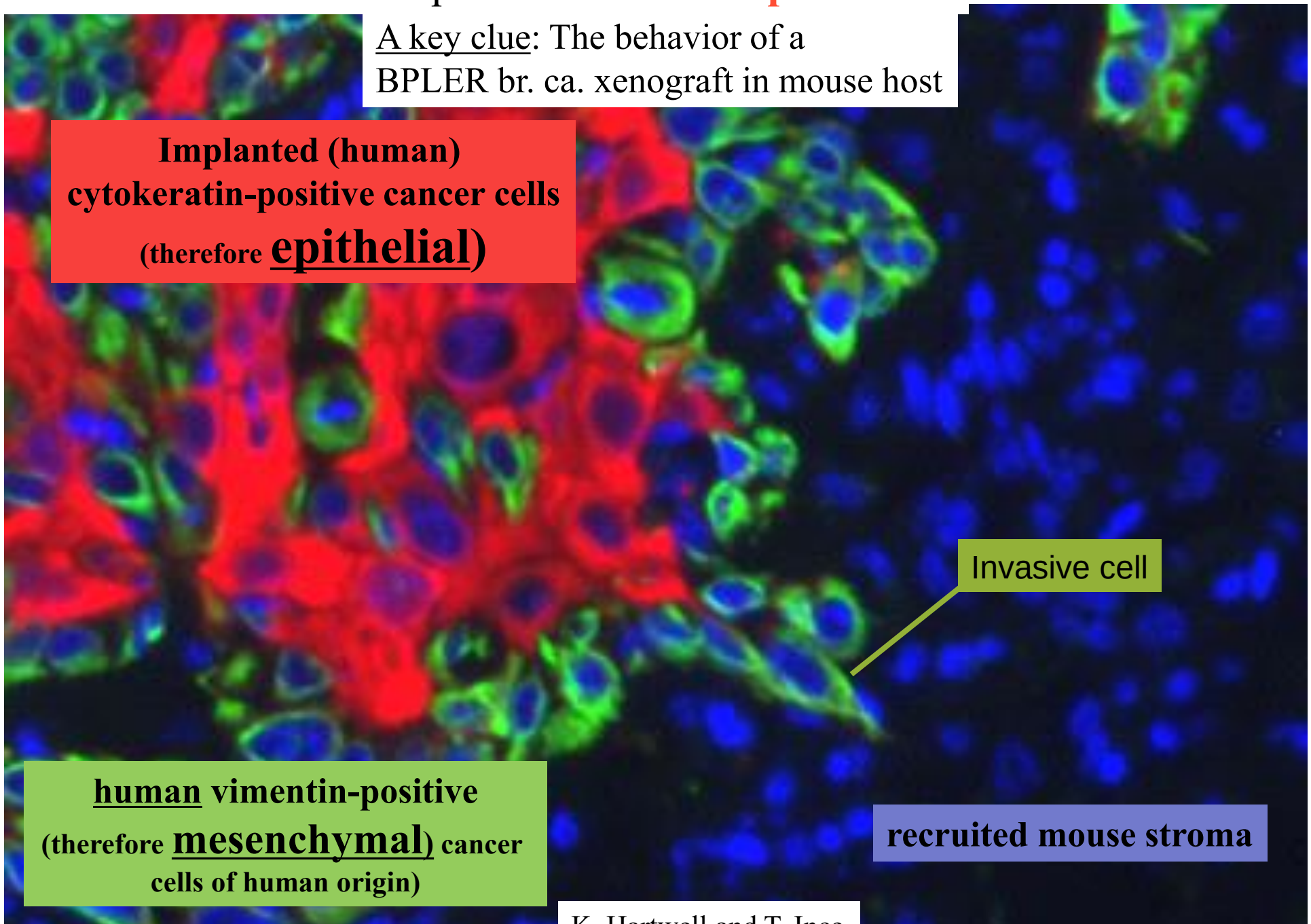


The "invasion-metastasis cascade"

How do cancer cells acquire all of these distinct capabilities?
Are additional somatic mutations required?

Figure 11.10 *The Biology of Cancer* (© Garland Science 2007)

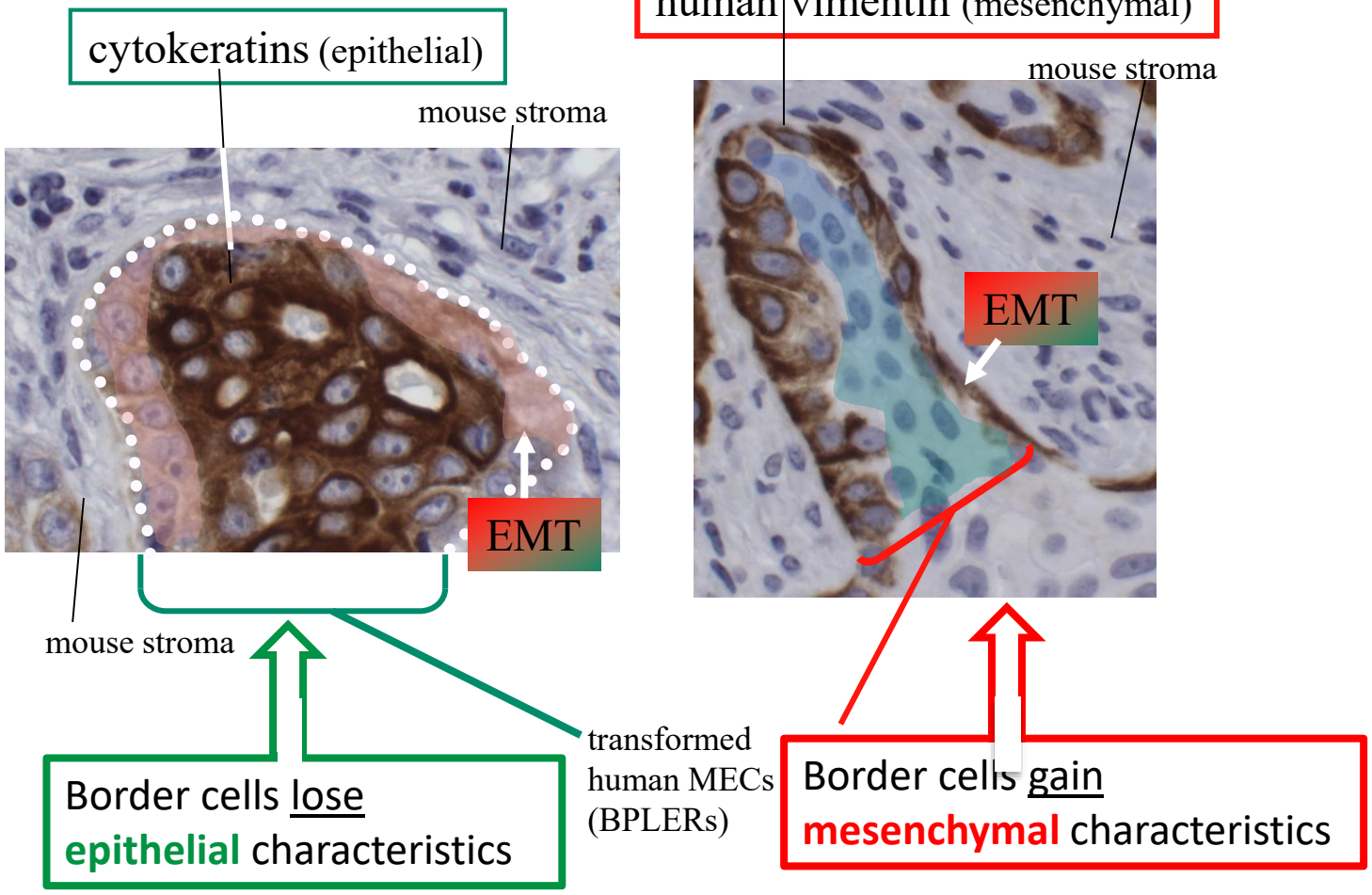
How do cancer cells acquire all of **these capabilities**?



Contextual signals influence the induction of EMT programs

EMT = epithelial-mesenchymal transition

BPLER tx human mammary epithelial cells in mouse host

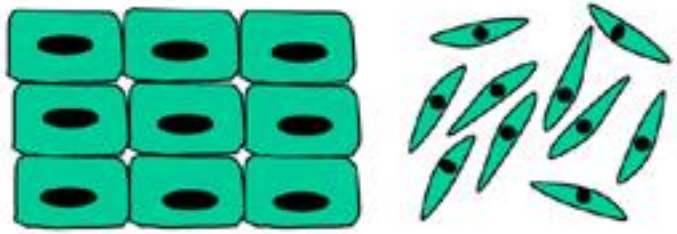


K. Hartwell & T. Ince

How do carcinoma cells acquire traits needed to metastasize?: **One possible solution**:

The epithelial-mesenchymal transition (**EMT**) is a complex, multi-faceted **program** involving multiple coordinated changes in cell-biological properties.

epithelial → mesenchymal



For both normal and neoplastic epithelial cells:

Cobblestone	Elongated
Non-motile	Motile
Non-invasive	Invasive
Polarised	Non-polarised
Epithelial markers:	Mesenchymal markers:
• E-cadherin	• N-cadherin
• Desmoplakin	• Vimentin
• Keratin	• Fibronectin

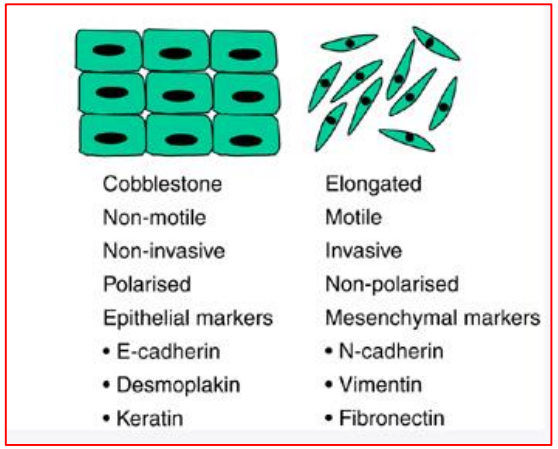
Elizabeth Hay

epithelial → mesenchymal

EMT

There are **many <u>alternative</u> EMT programs** that share in common a relatively small set of cell-biological changes.

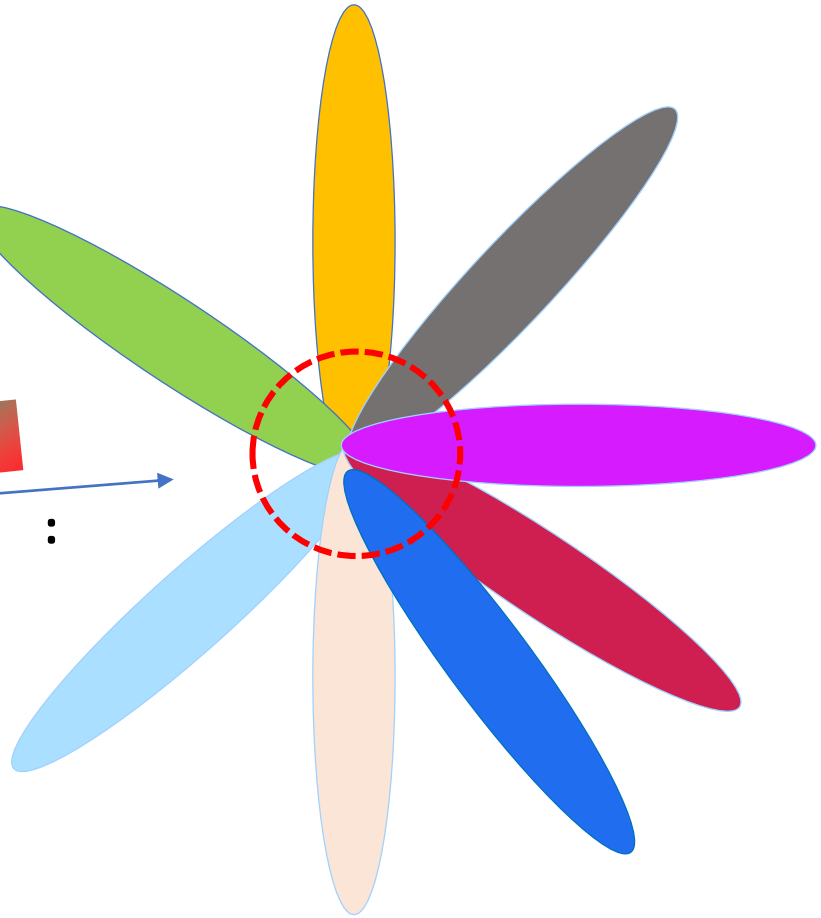
The canonical EMT program



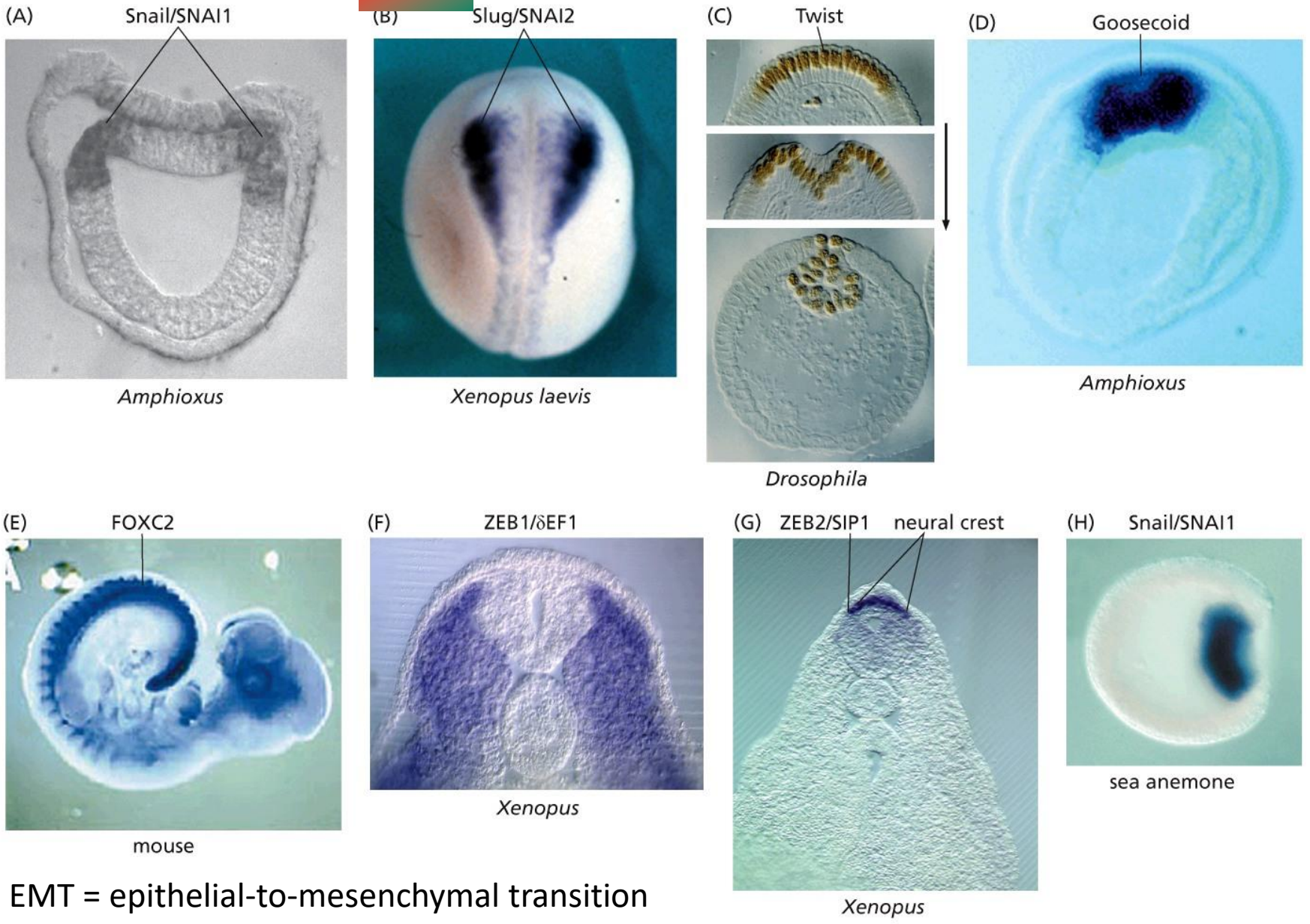
Cobblestone	Elongated
Non-motile	Motile
Non-invasive	Invasive
Polarised	Non-polarised
Epithelial markers	Mesenchymal markers
• E-cadherin	• N-cadherin
• Desmoplakin	• Vimentin
• Keratin	• Fibronectin

EMT

:



A group of pleiotropically acting **transcription factors** (**EMT-TFs**) that induce EMT at various stages of metazoan embryogenesis

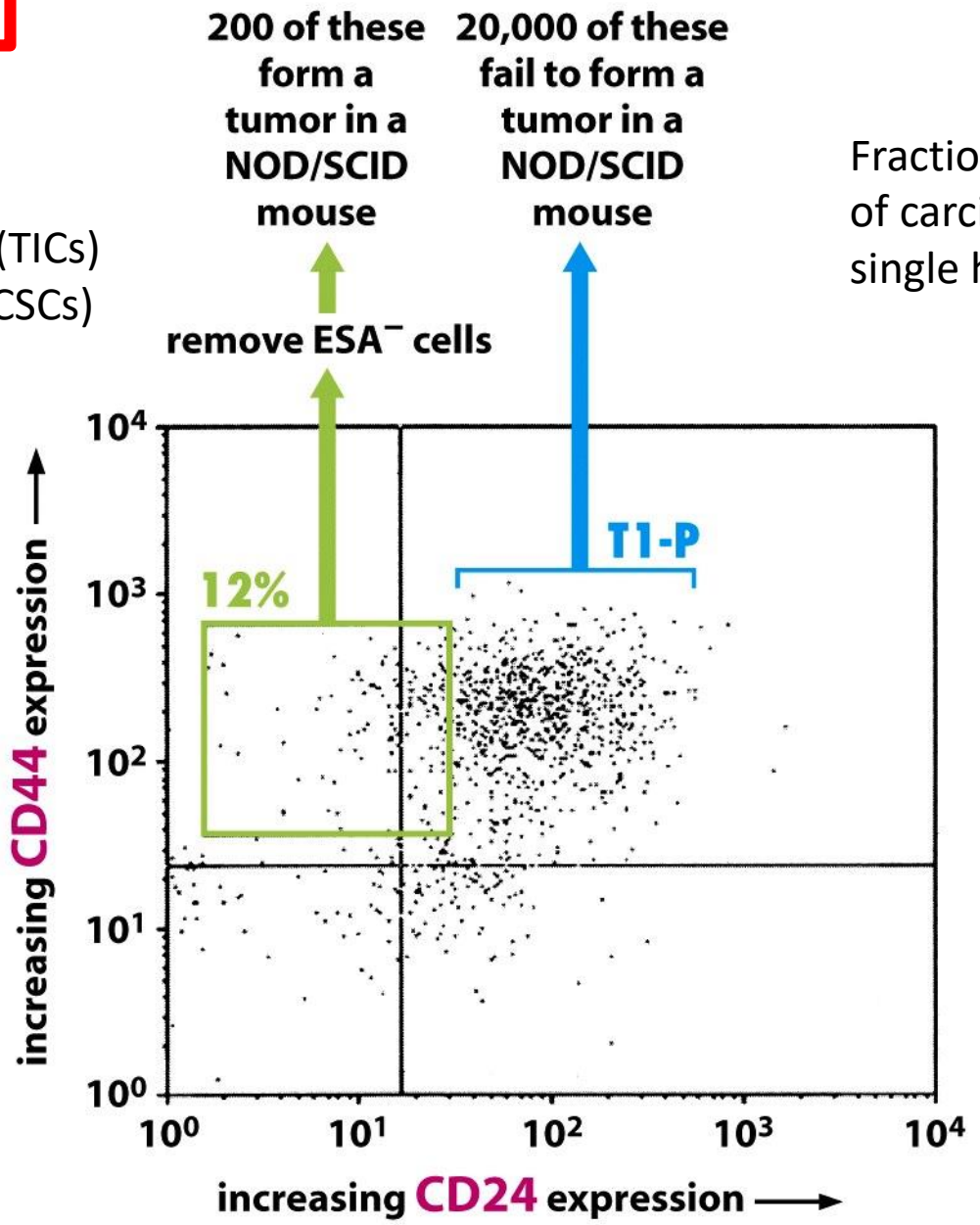


EMT = epithelial-to-mesenchymal transition

Figure 14.25 The Biology of Cancer (© Garland Science 2014)

Shifting gears

Discovery of
tumor-initiating cells (TICs)
(=cancer stem cells) (CSCs)



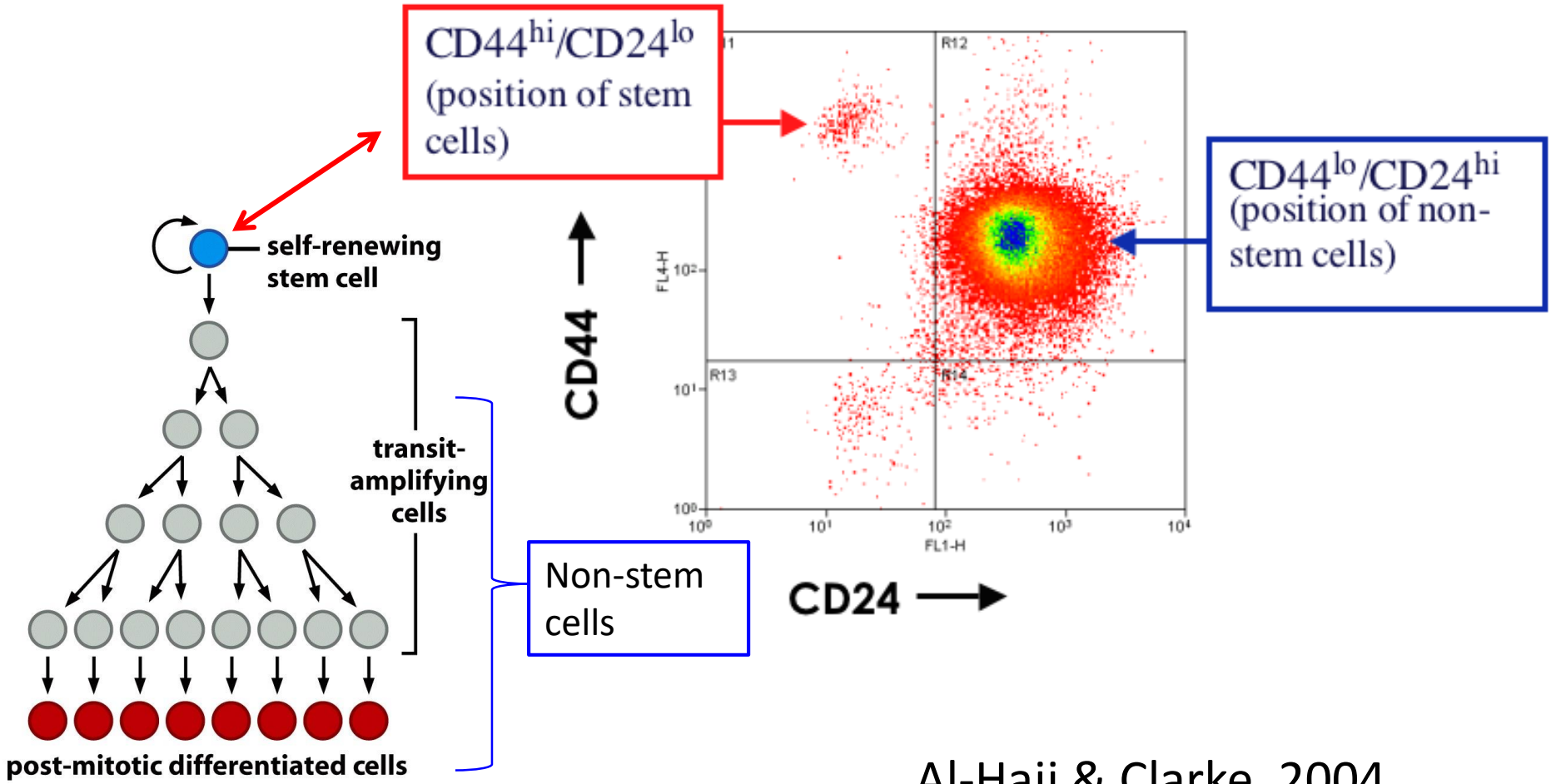
Fractionate subpopulations
of carcinoma cells from a
single human breast cancer

al-Hajj et al. 2004

Figure 11.14a *The Biology of Cancer* (© Garland Science 2007)

Shifting gears: Is there any connection between the EMT & SC programs??

Immortalized human mammary epithelial cells

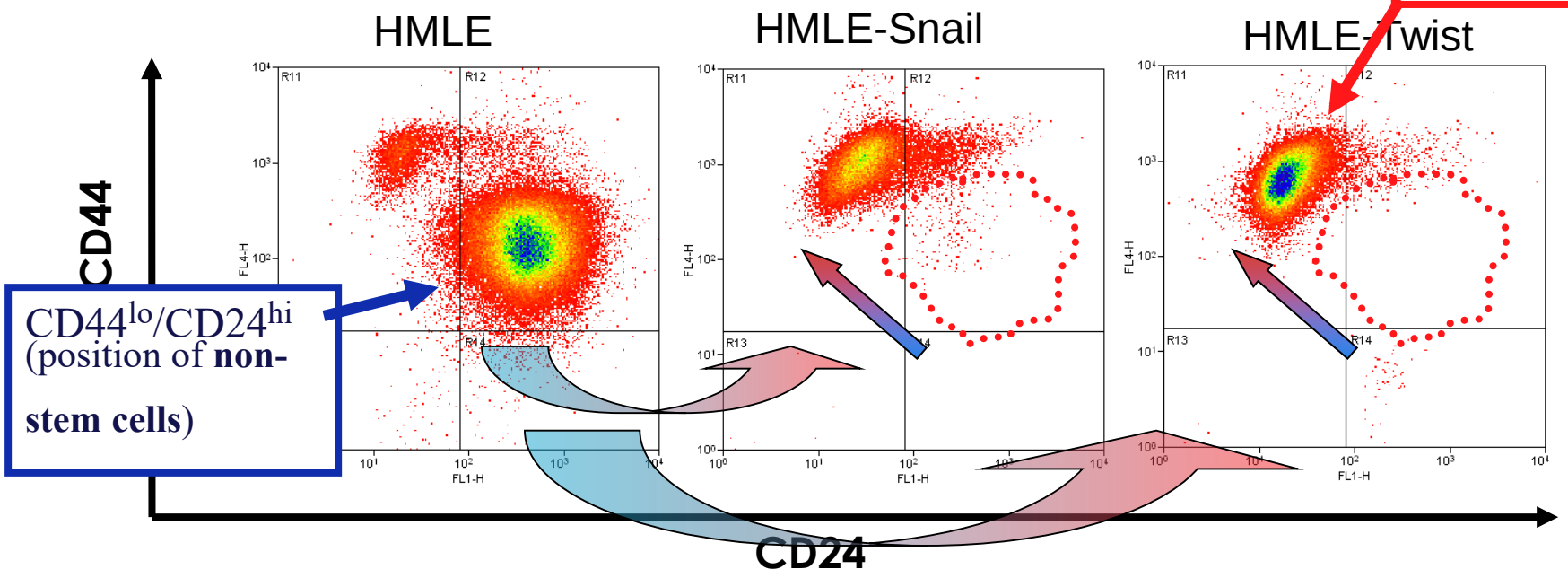


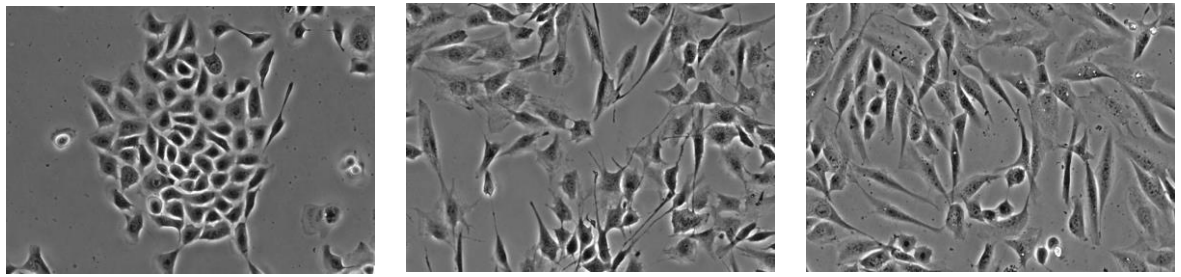
Al-Hajj & Clarke, 2004
(in breast cancer cells)

Induction of EMT by Snail and Twist EMT-inducing TFs generates CD44^{hi} CD24^{lo} cells including CSCs

CD44^{hi}/CD24^{lo} (position of **stem cells**)

CD44^{lo}/CD24^{hi} (position of **non-stem cells**)





Morphological shift in monolayer culture

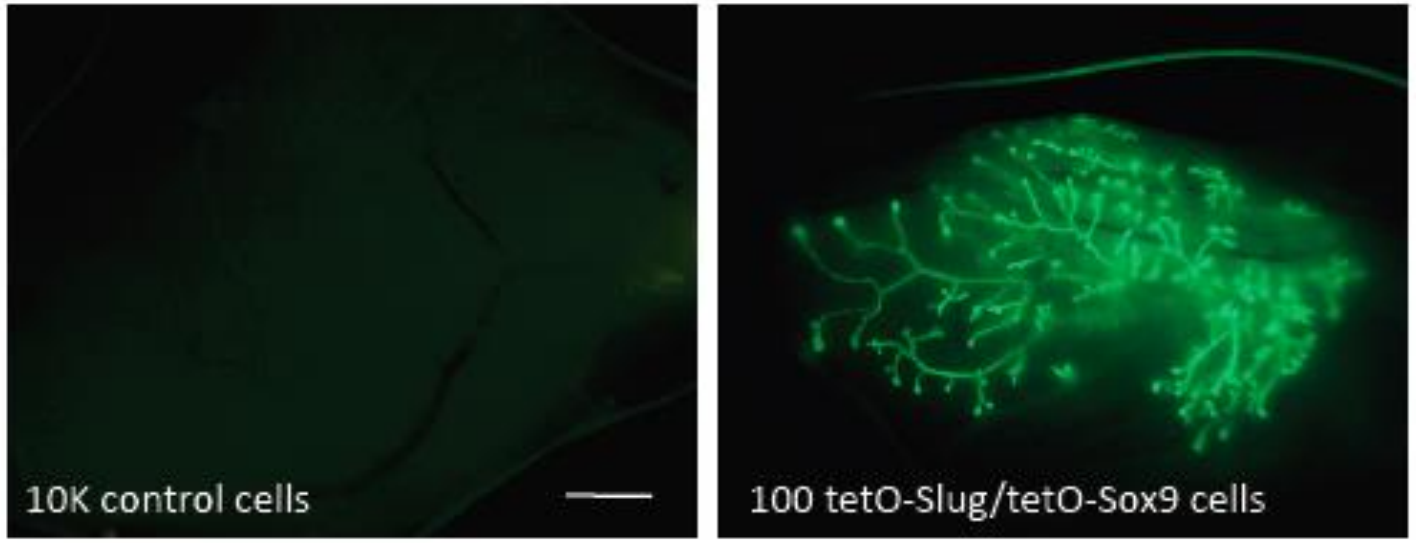
S.A.Mani & W. Guo

epithelial → mesenchymal

Can expression of EMT-TFs in fully normal mammary epithelial cells induce the formation of normal mammary stem cells?

Transient Expression (4-5 days) of two EMT-inducing transcription factors (Slug + Sox9) prior to fat pad implantation induces a >100-fold excess of <u>normal</u> mammary stem cells (visualized 6 weeks later)

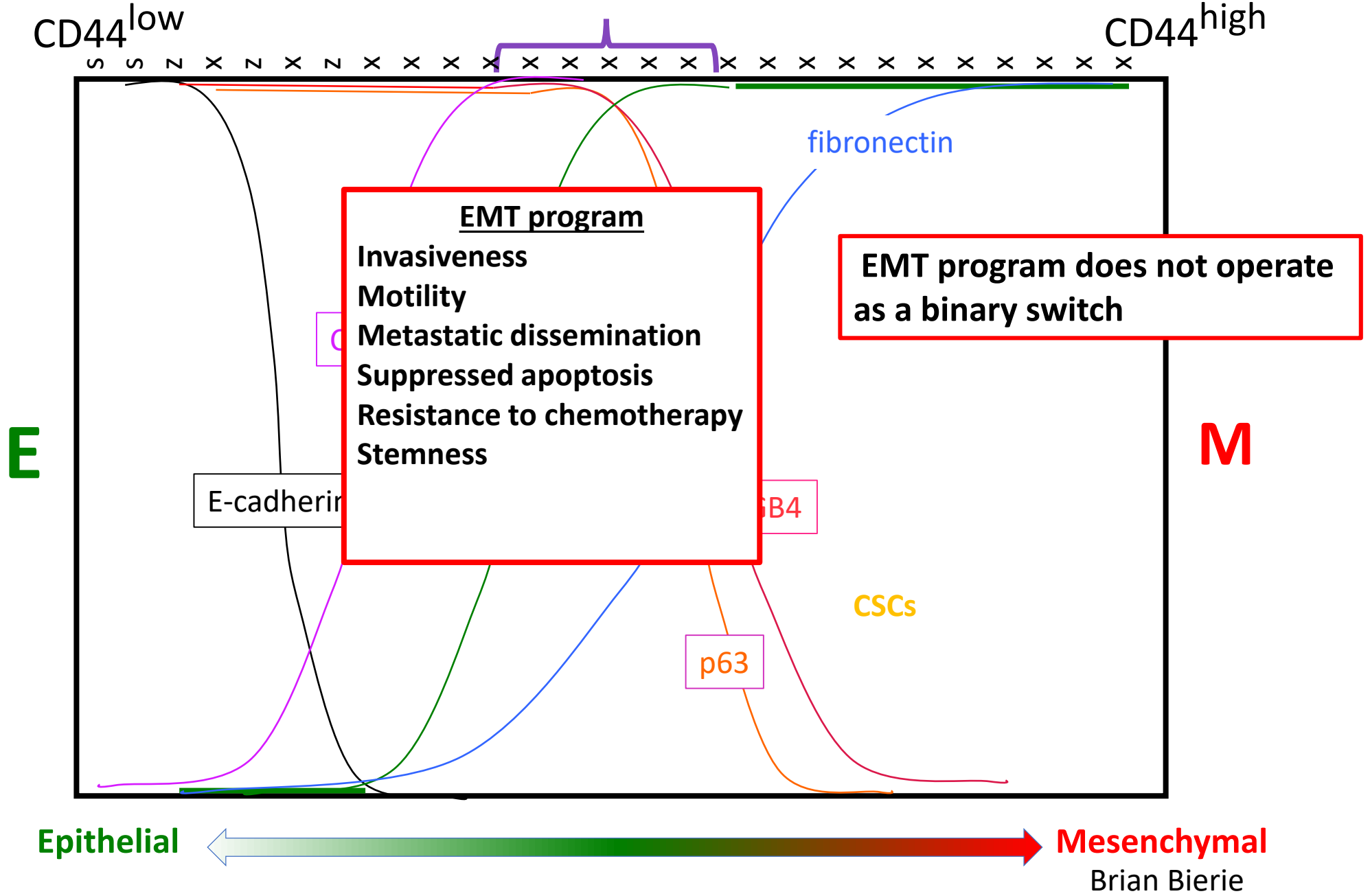
D

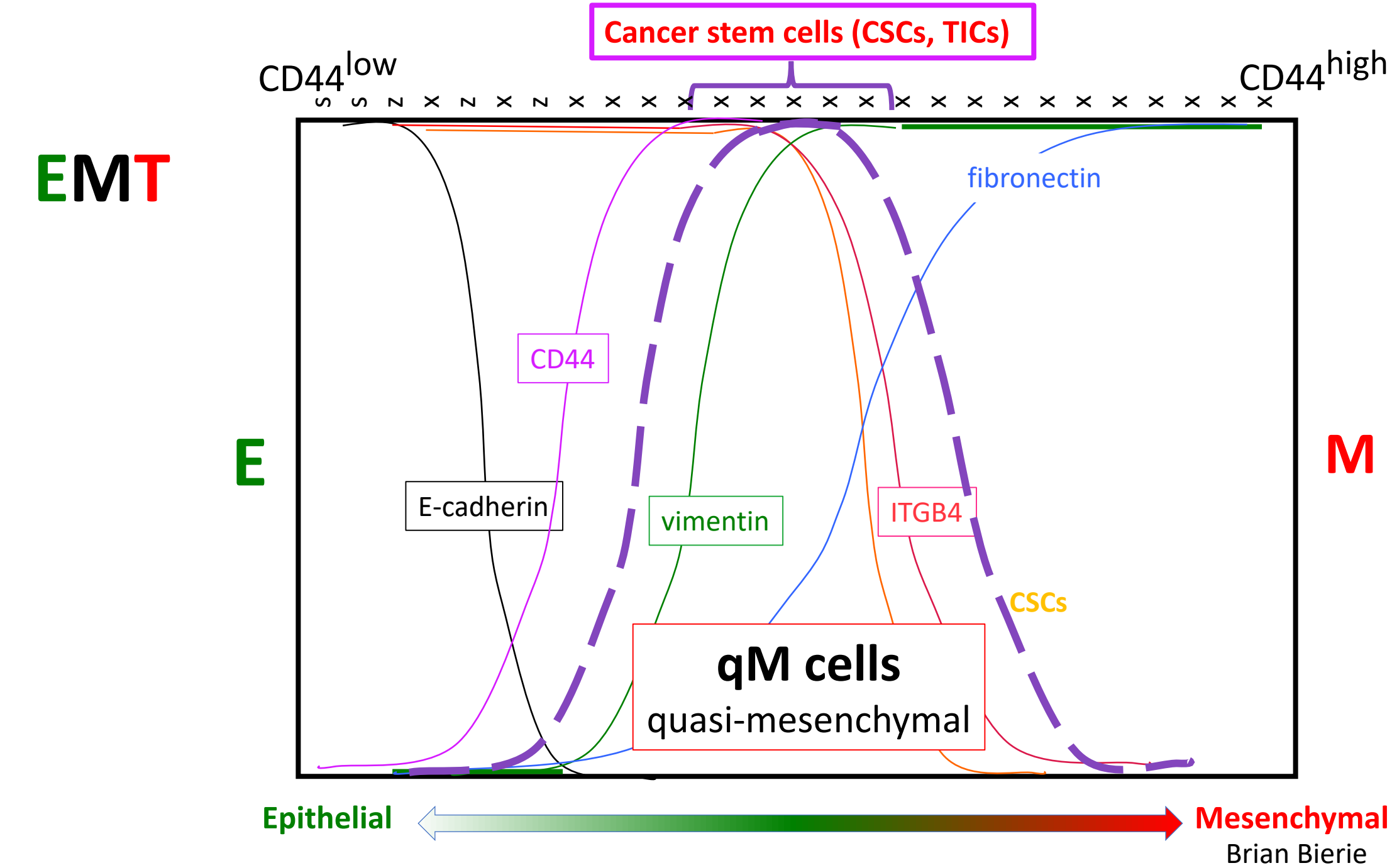


No EMT-TFs

Concomitant **<u>transiently induced</u>** expression of Slug + Sox9 EMT-TFs

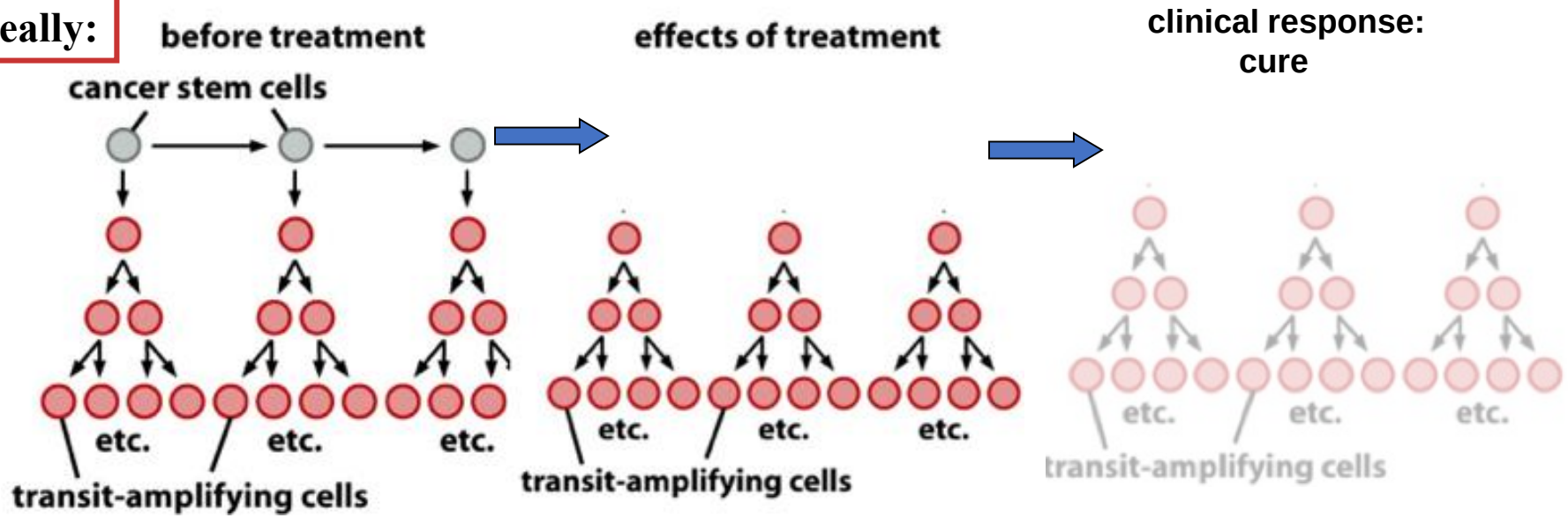
Wenjun Guo


CD44low
CD44high
fibronectin
EMT program
Invasiveness
Motility
Metastatic dissemination
Suppressed apoptosis
Resistance to chemotherapy
Stemness
EMT program does not operate as a binary switch
E
M
E-cadherin
CSCs
p63
Epithelial
Mesenchymal
Brian Bierie

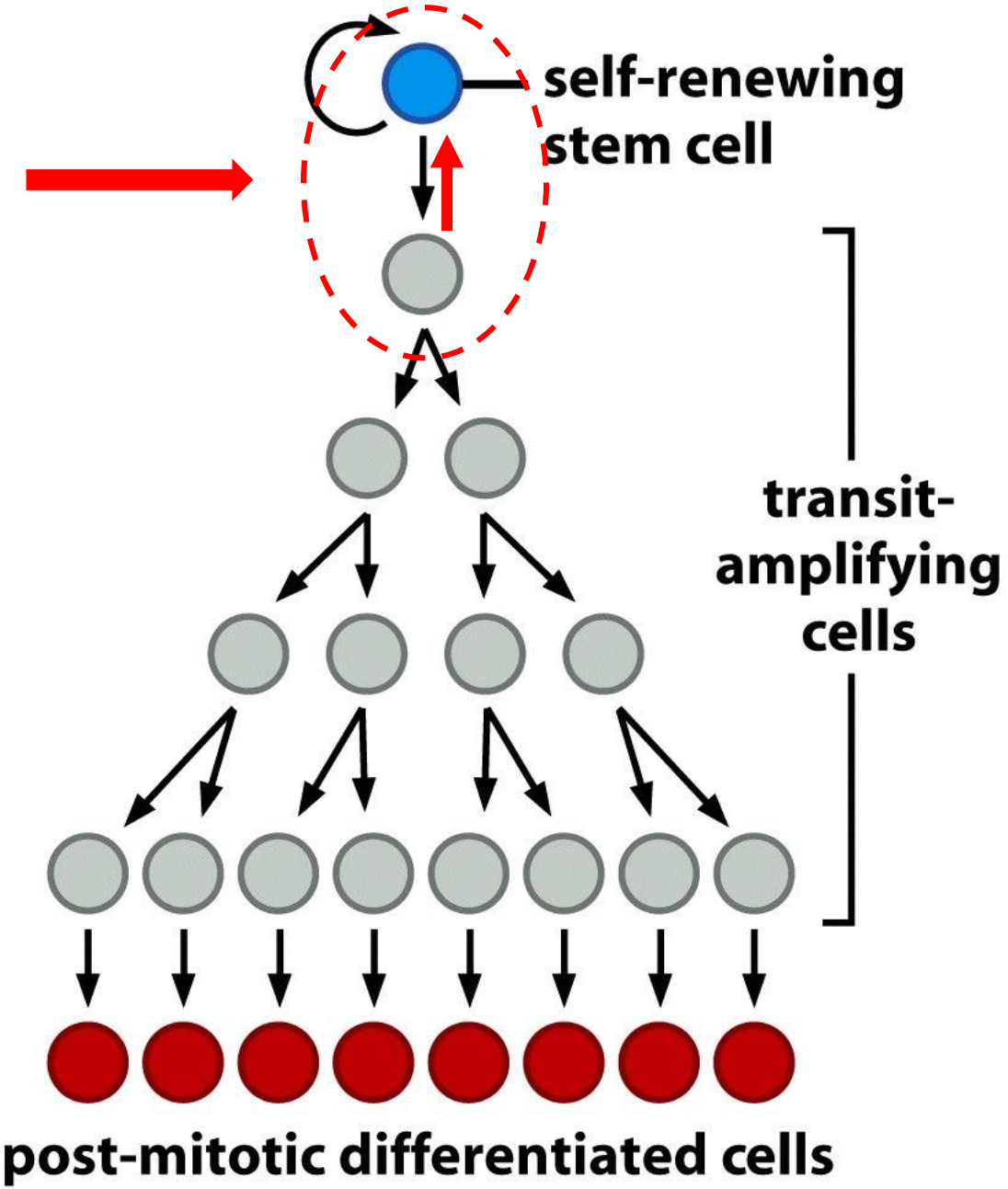

Cancer stem cells (CSCs, TICs)
CD44low
CD44high
EMT
E
M
fibronectin
CD44
E-cadherin
vimentin
ITGB4
CSCs
qM cells
quasi-mesenchymal
Epithelial
Mesenchymal
Brian Bierie

What if we developed an anti-CSC treatment?

Ideally:

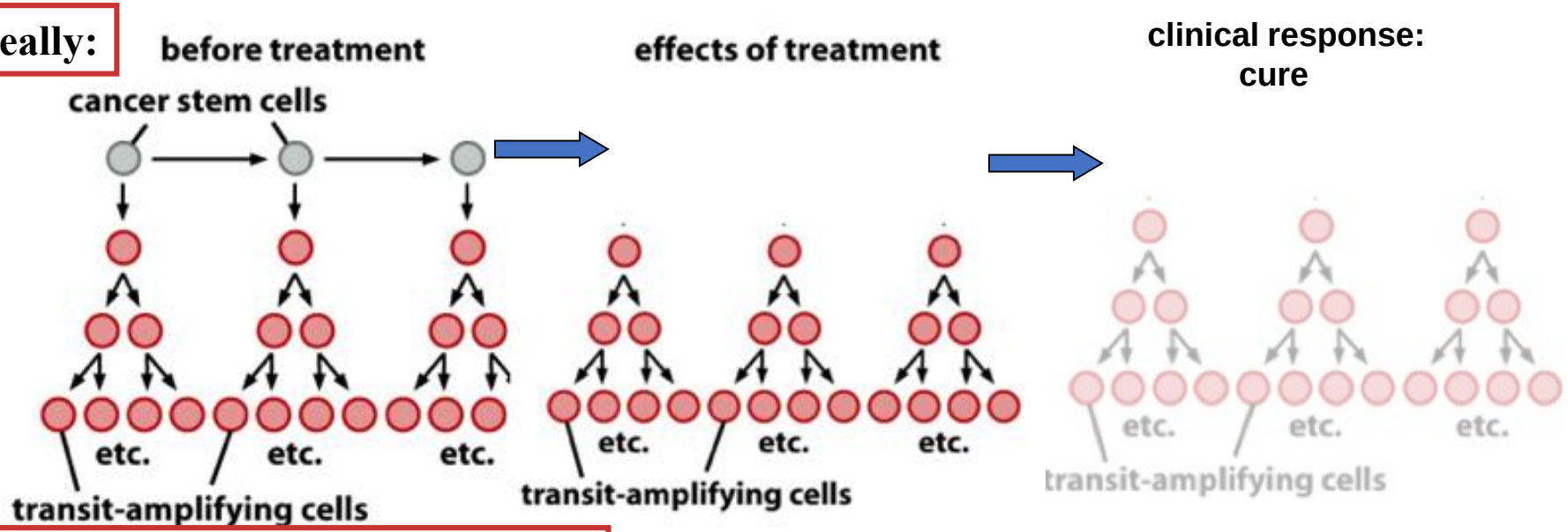


Reversibility of SC differentiation

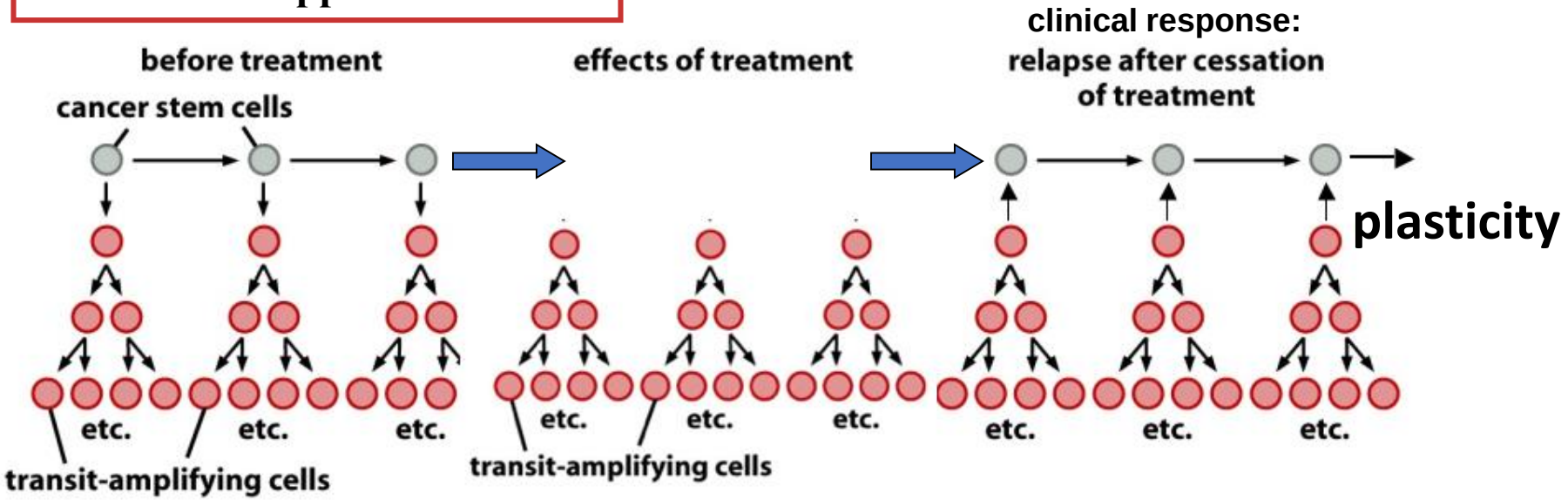


What if we developed an anti-CSC treatment, e.g., cAMP induction?

Ideally:



What if this happens instead?



Given all this, how does multi-step tumor progression actually proceed?

The Darwinian Model

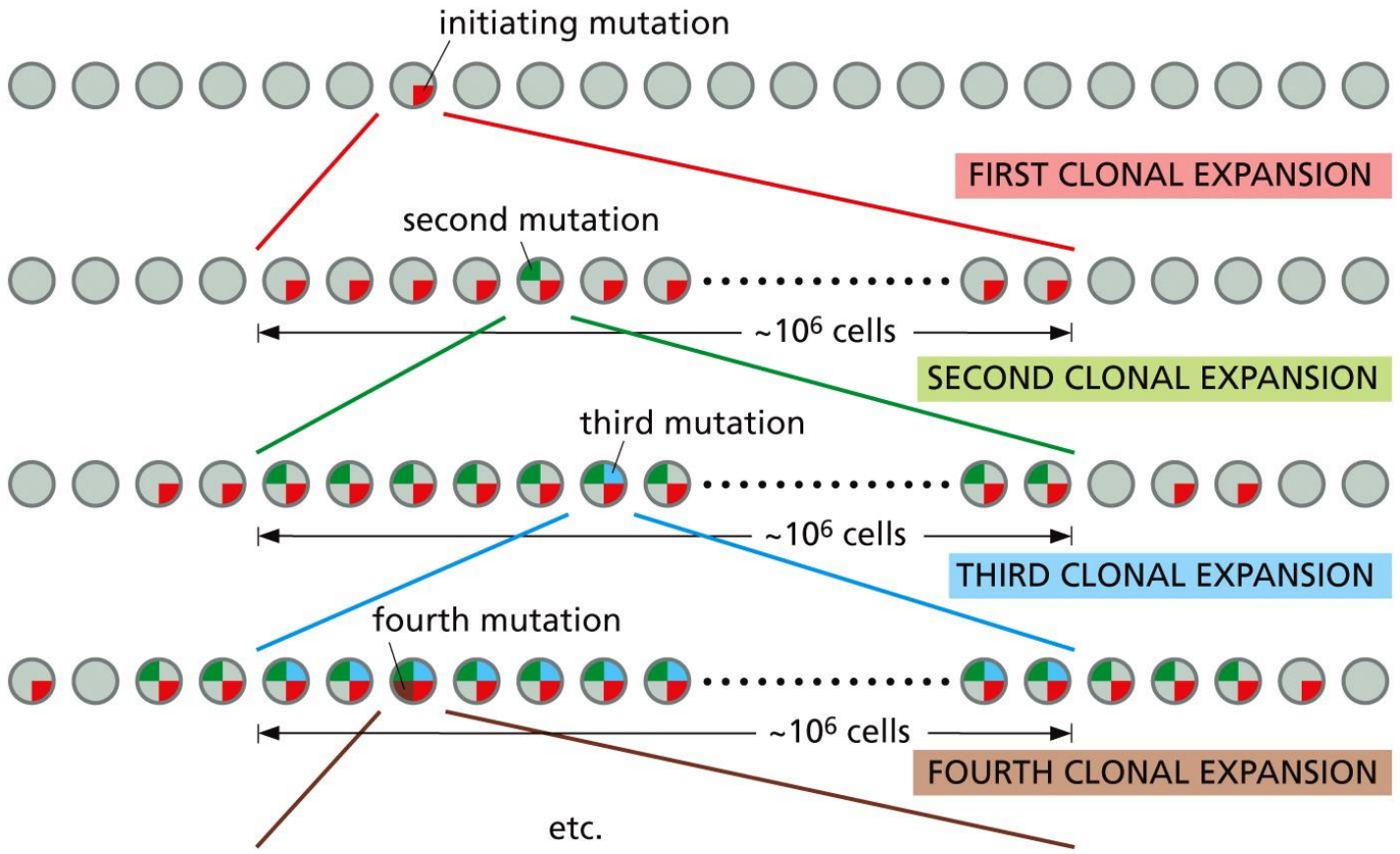


Figure 11.15 The Biology of Cancer (© Garland Science 2014)

Intra-tumoral diversification:

The Darwinian Model: Mutations spawn diverse clonal sub-populations more rapidly than selection eliminates them

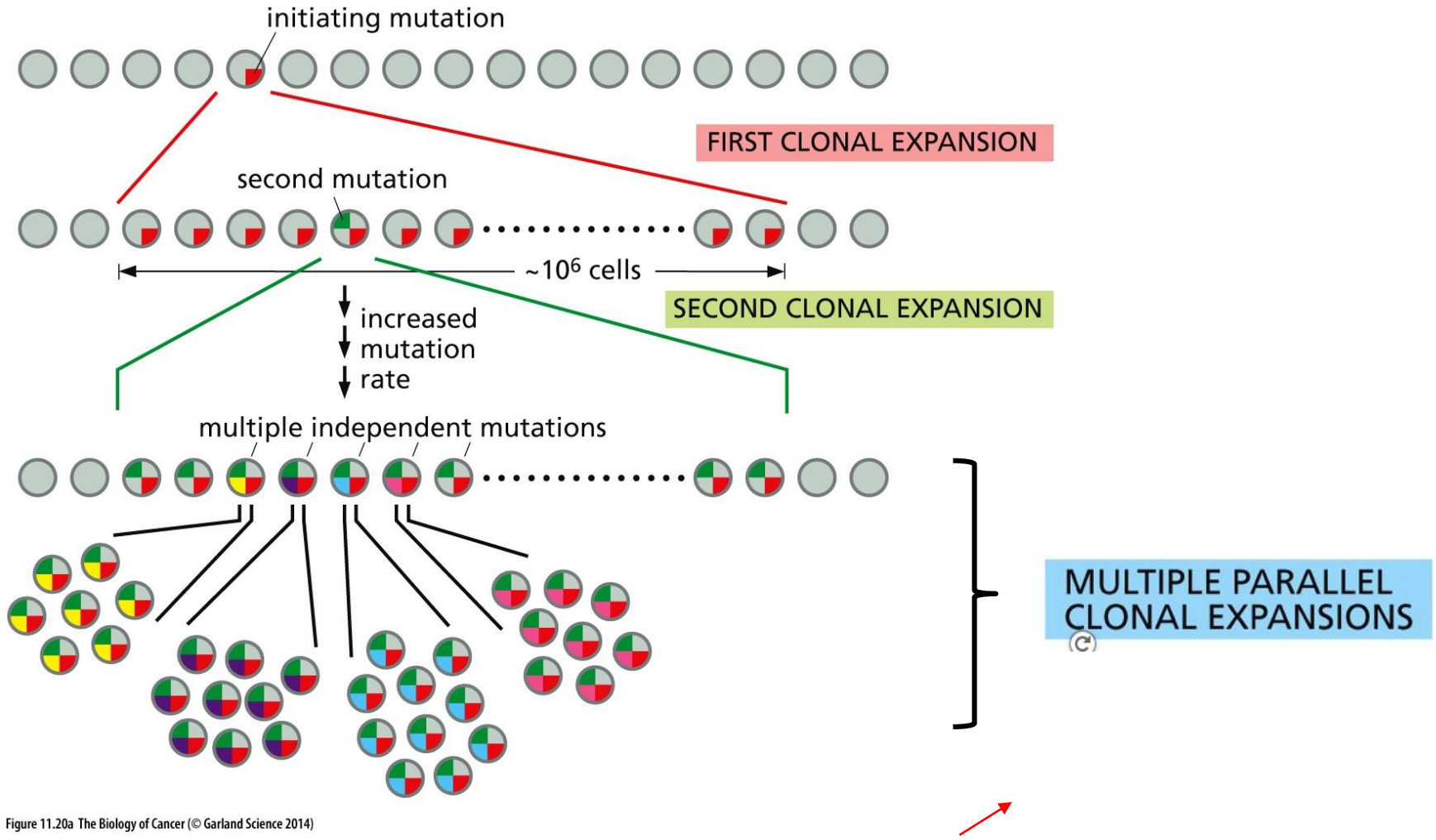
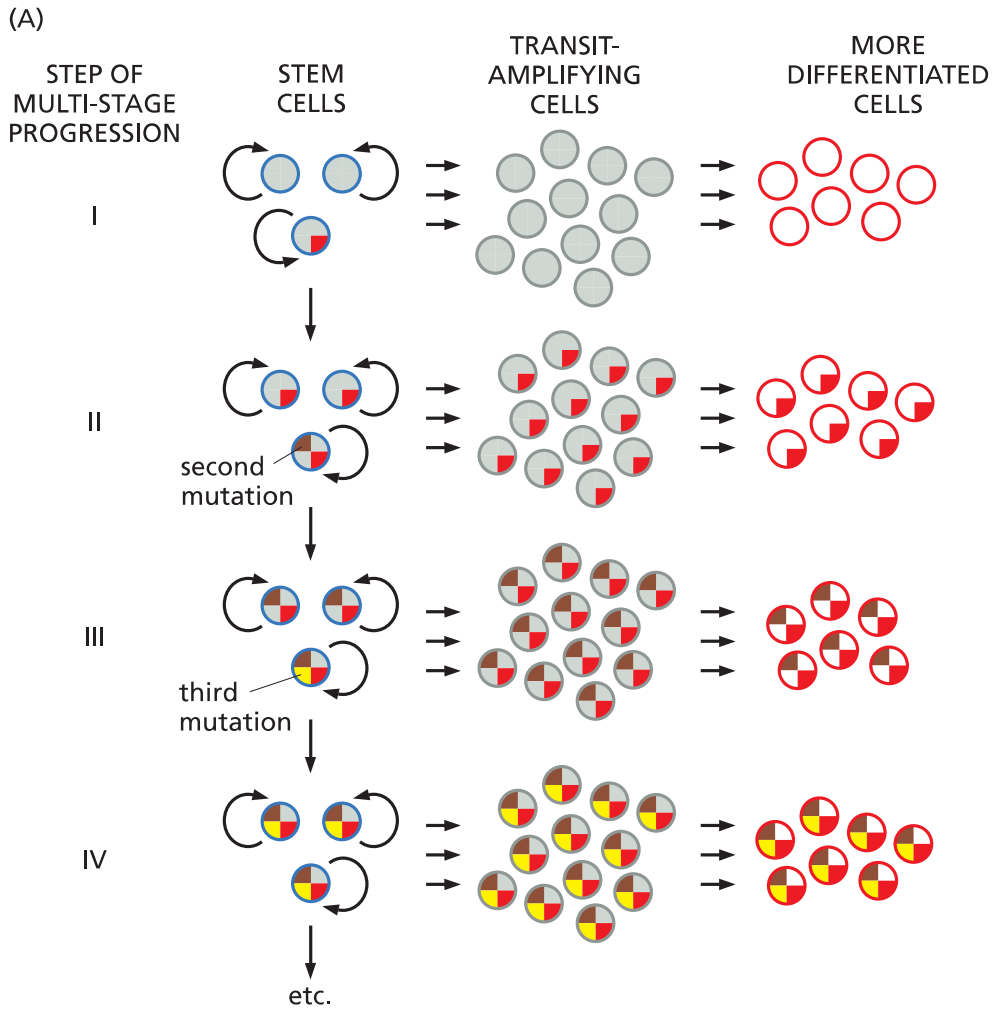
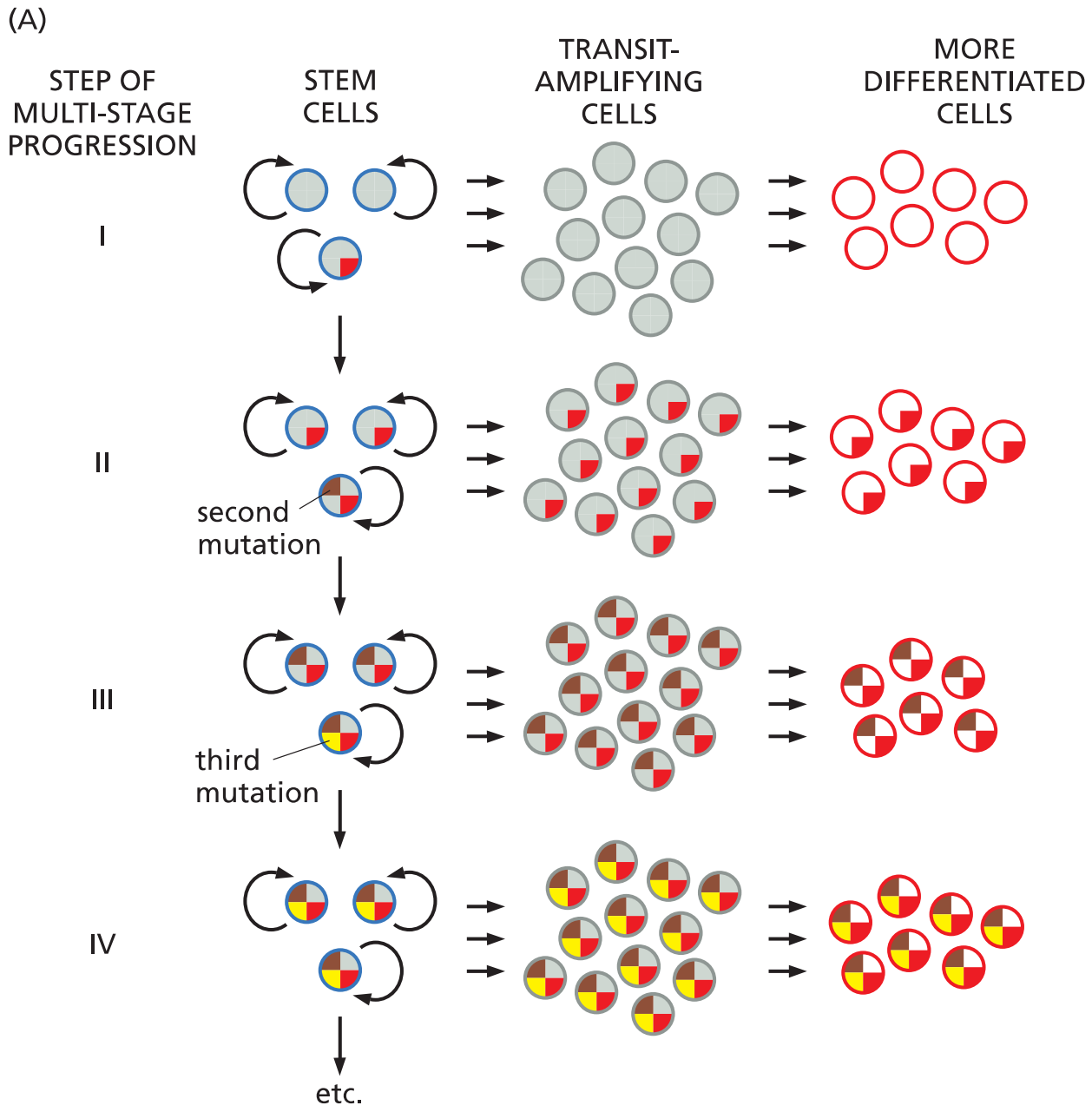


Figure 11.20a The Biology of Cancer (© Garland Science 2014)

However, the Darwinian model does not address the complexity of multiple <u>alternative phenotypic states</u> at each step of tumor progression.



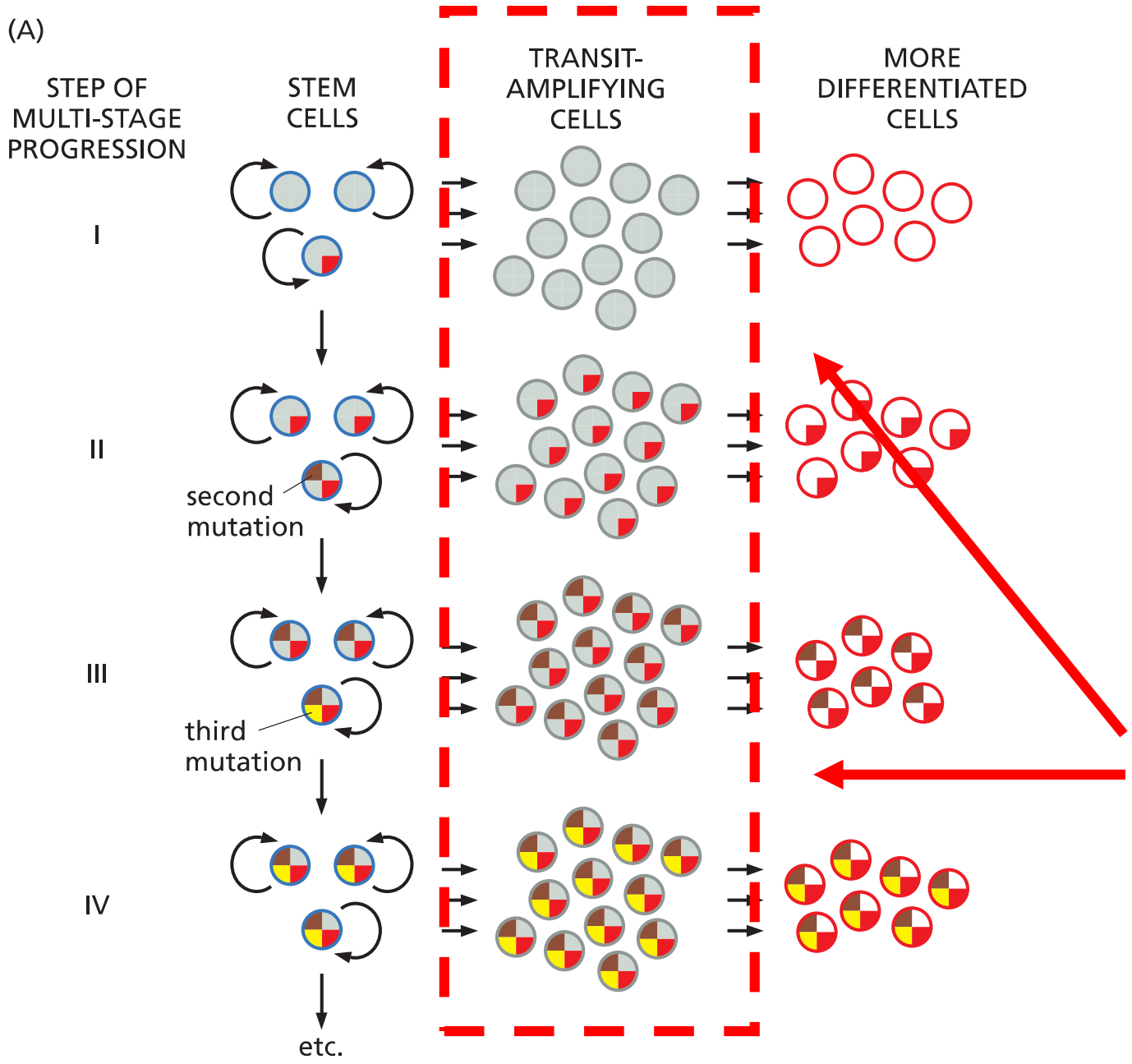


However, this scheme has its flaws:

Which cells are most likely to sustain the mutations that lead to a more advantageous phenotype?

1. The stem cells are relatively small in number. Therefore small target size.
2. The stem cells generally proliferate far less often than do the transit-amplifying/progenitor cells. (Typically the vast bulk of the mitotic activity in a tissue is presented in the transit-amplifying/progenitor compartment. Therefore, far less opportunity for somatic mutations being sustained in the stem cell compartment.

Hence, it is far more likely that the transit-amplifying compartment rather than the same cell compartment is the source of the mutations that generate novel variants.



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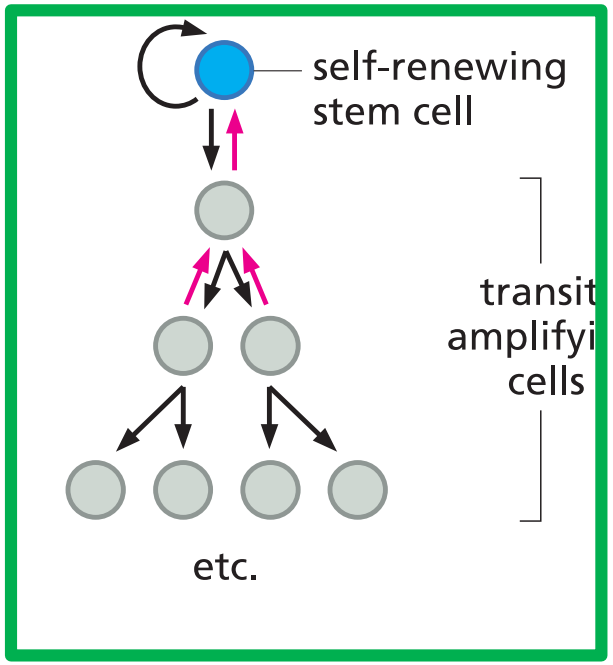
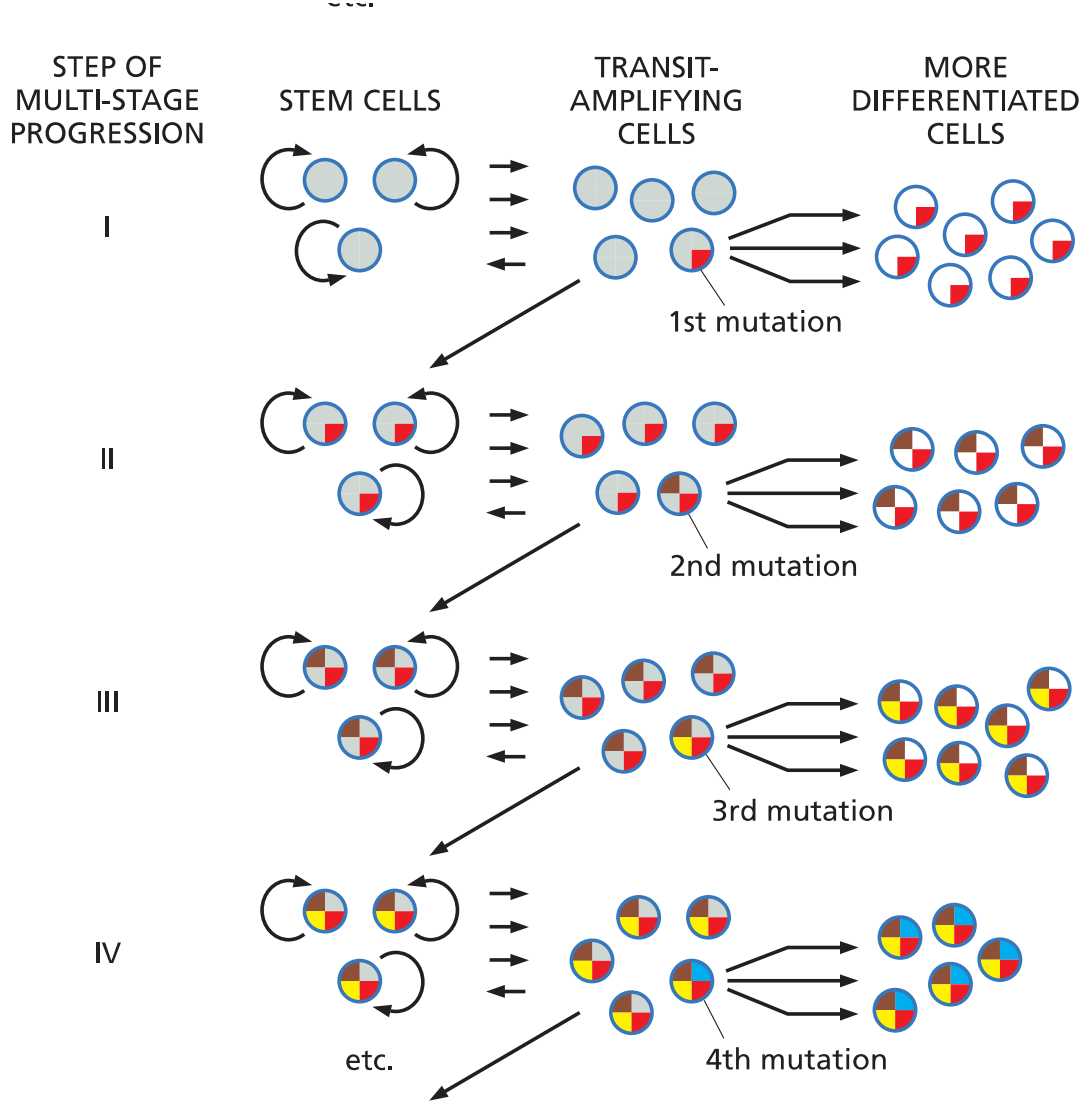
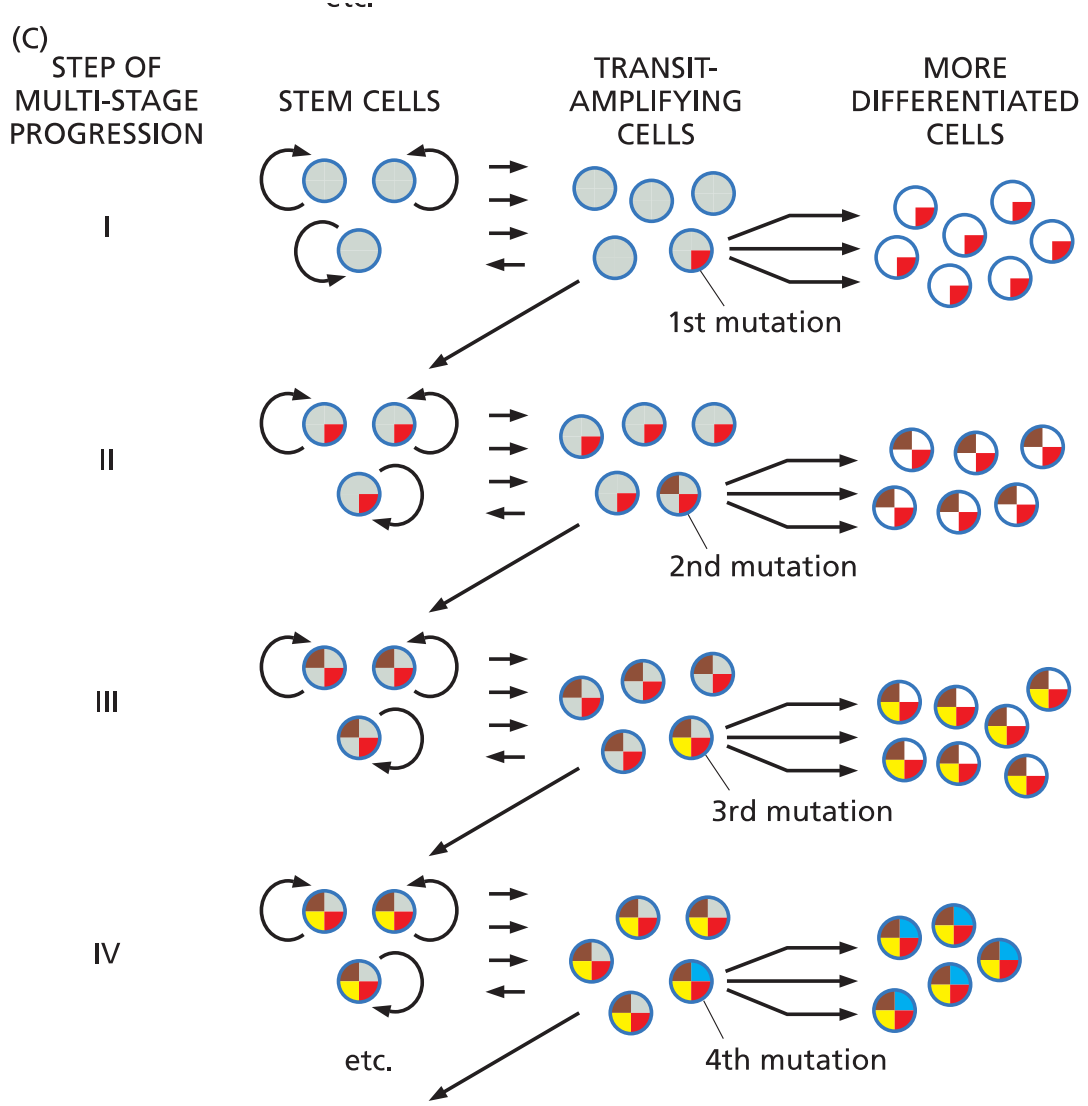
Hence, it is far more likely that the transit-amplifying compartment rather than the same cell compartment is the **source of the mutations** that generate novel variants.

If the mutations are sustained in the transit-amplifying compartment, how are they <u>sustained and perpetuated</u> in the descendant population?

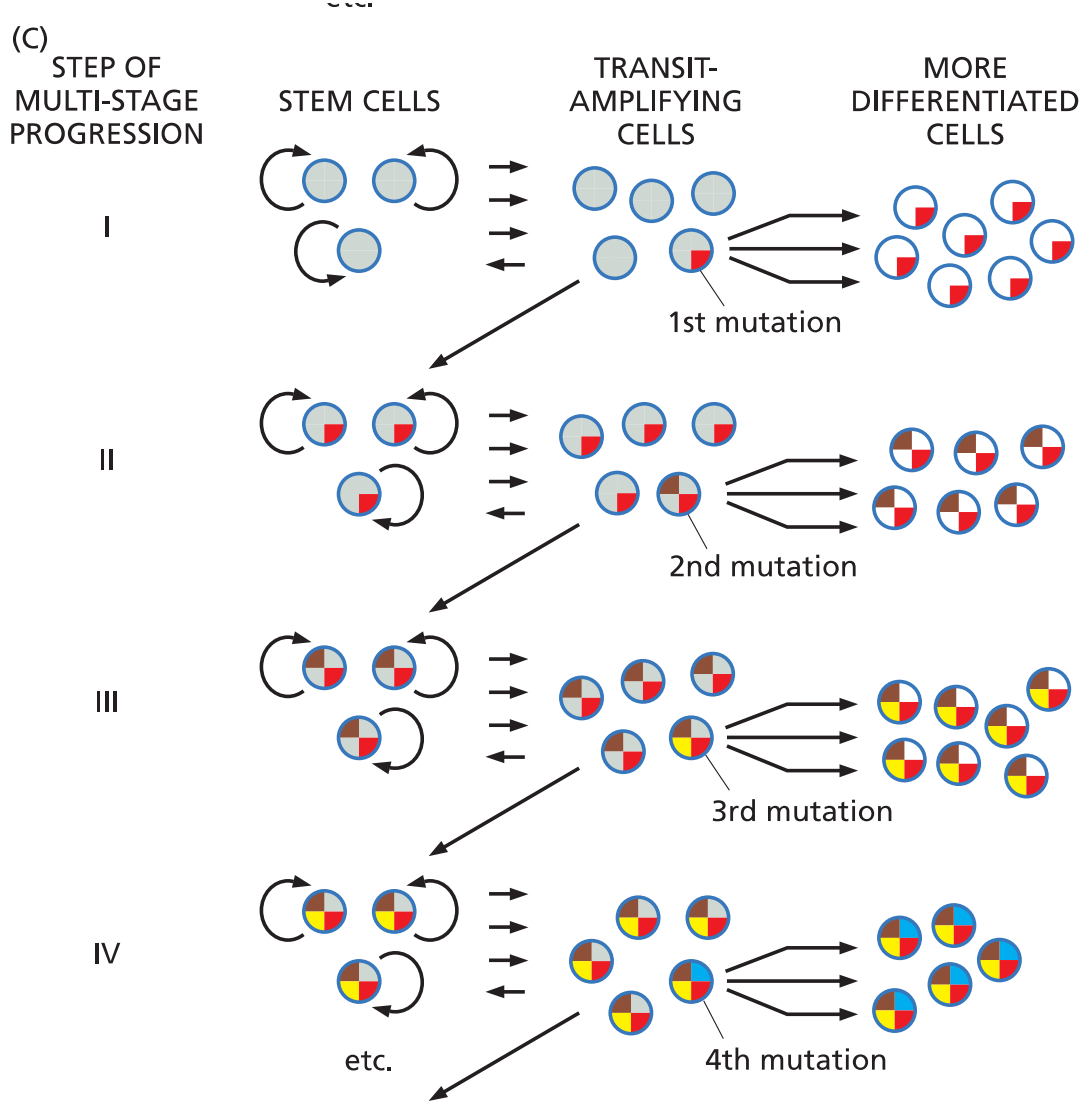






If the mutations are sustained in the transit-amplifying compartment, how are they sustained and perpetuated in the descendant population?

And even if this is true, which cells are the objects of selection?



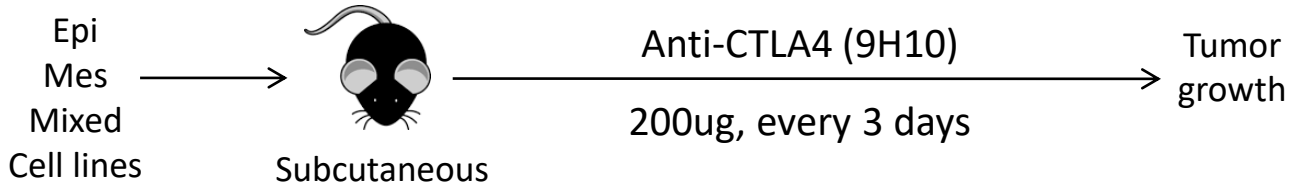
If the mutations are sustained in the transit-amplifying compartment, how are they sustained and perpetuated in the descendant population?

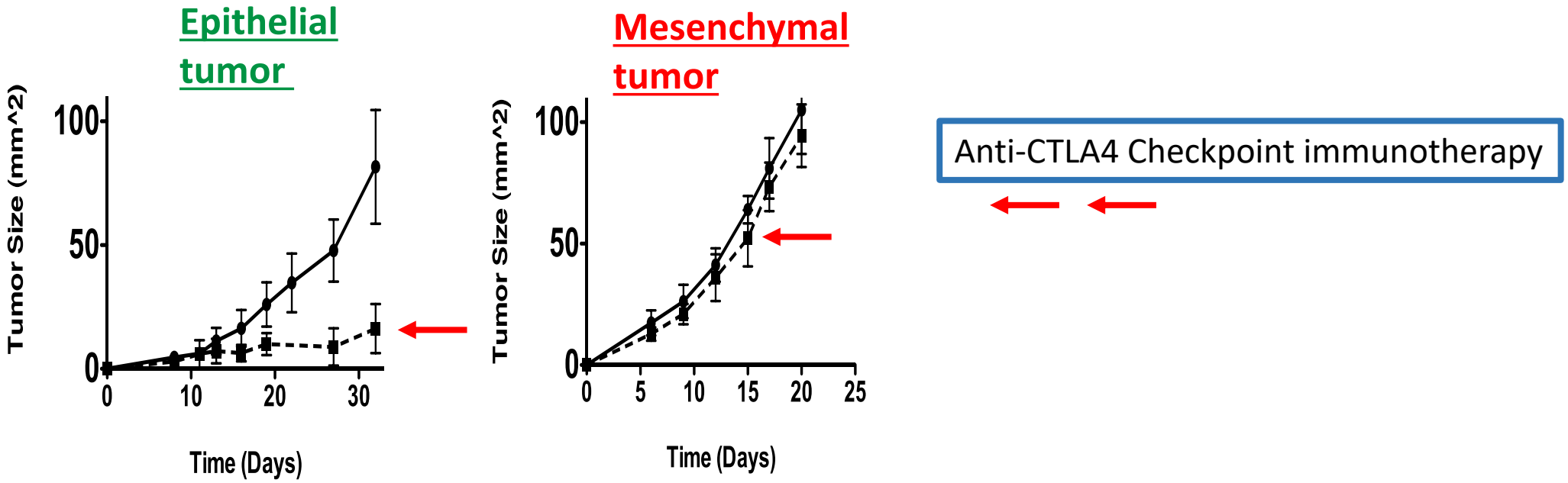
And even if this is true, which cells are the objects of selection?

(Unlikely to be the stem cells, which lack the display of certain advantageous phenotypes.)
(Unlikely to be the more differentiated cells, unless they can generate less-differentiated cells)>

And then there is the question of **intratumoral inter-clonal collaboration**

Differential response of Epi. and Mes. tumors to checkpoint immunotherapy





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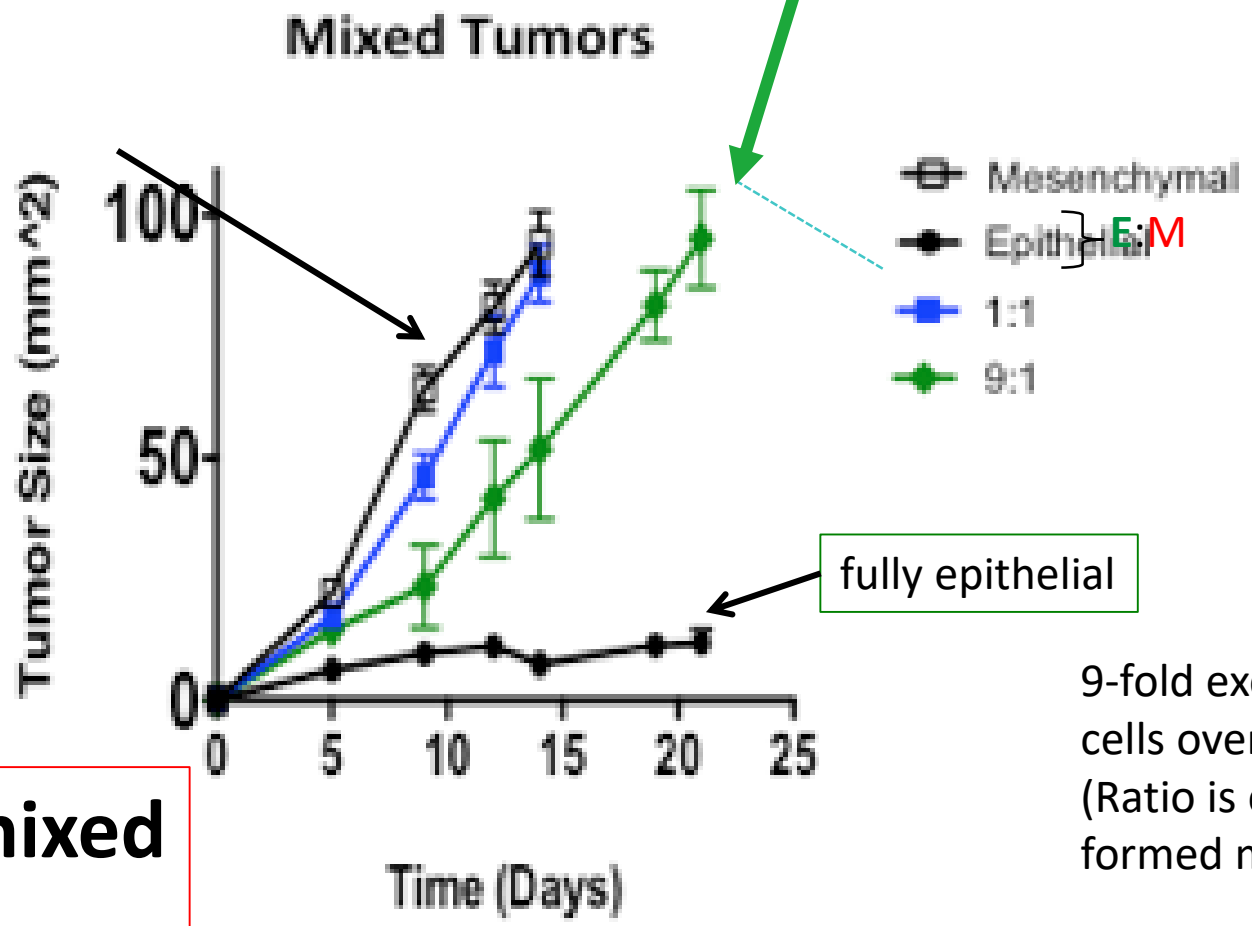
And then there is the question of **intratumoral inter-clonal collaboration**

Minority (10%) **mesenchymal** subpopulations can protect majority **epithelial** populations from elimination by anti-CTLA4 therapy

fully mesenchymal



fully epithelial

9-fold excess of **epithelial** cells over **mesenchymal** cells. (Ratio is conserved in fully formed mixed tumors)

Make mixed tumors

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