

Lecture 21 Cancer Biology II

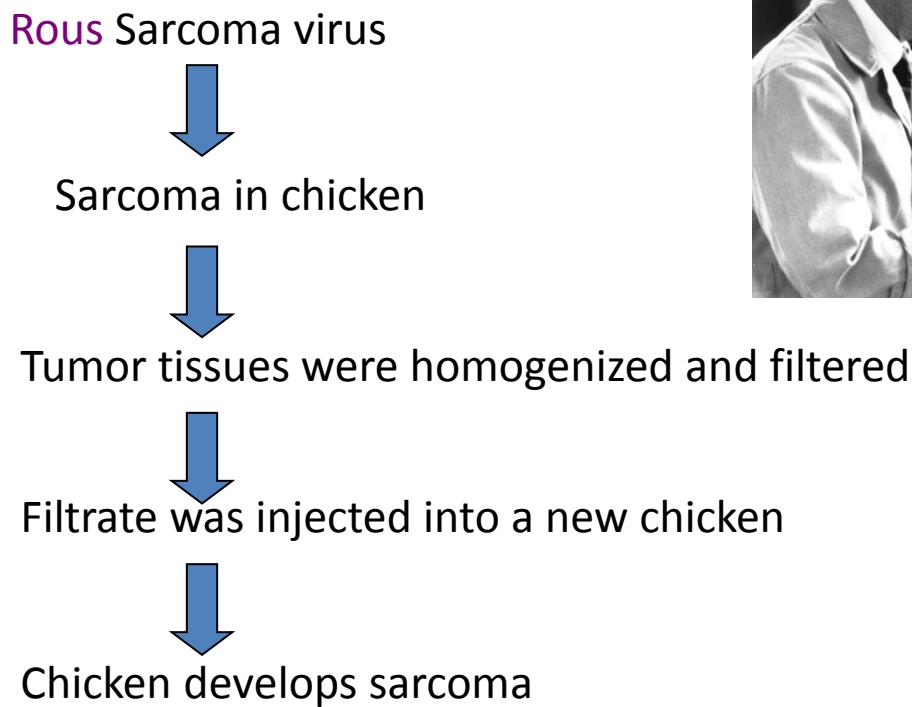
- I. Cancer critical genes
- II. Multi-step tumorigenesis
- III. Cancer stem cells
- IV. Cancer metastasis
- V. Cancer treatment

Cancer critical genes

- How were cancer critical genes discovered?
- Oncogene/proto-oncogene
- Tumor suppressor

How were cancer critical genes discovered?

- Src discovery



~ 1911

- ~ 20% of all tumors are associated with virus infection, how to explain the cause for the other 80% of human cancers?

To answer this question, let us first start from the cause of virus-induced human cancers.

RSV RNA virus structure

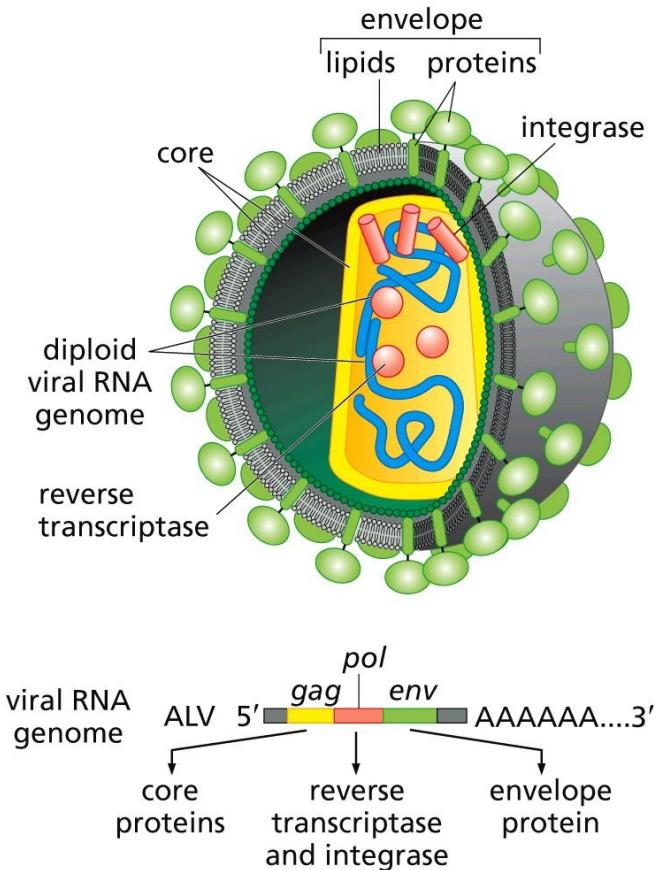


Figure 3.4a The Biology of Cancer (© Garland Science 2014)

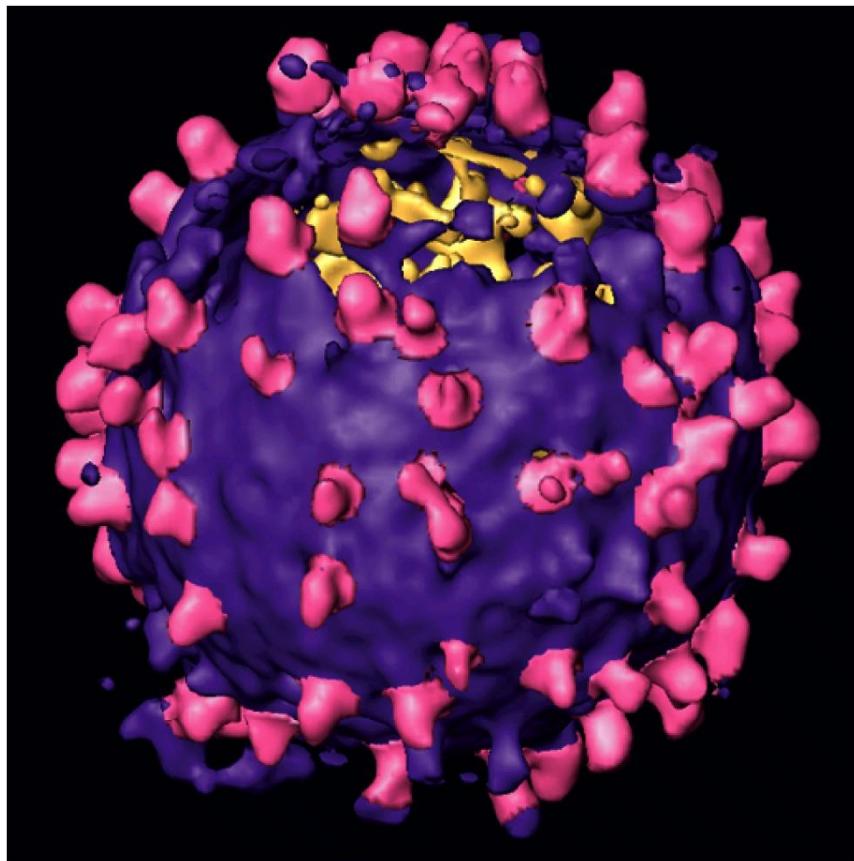


Figure 3.4b The Biology of Cancer (© Garland Science 2014)

How is RNA virus genome inserted into host genome

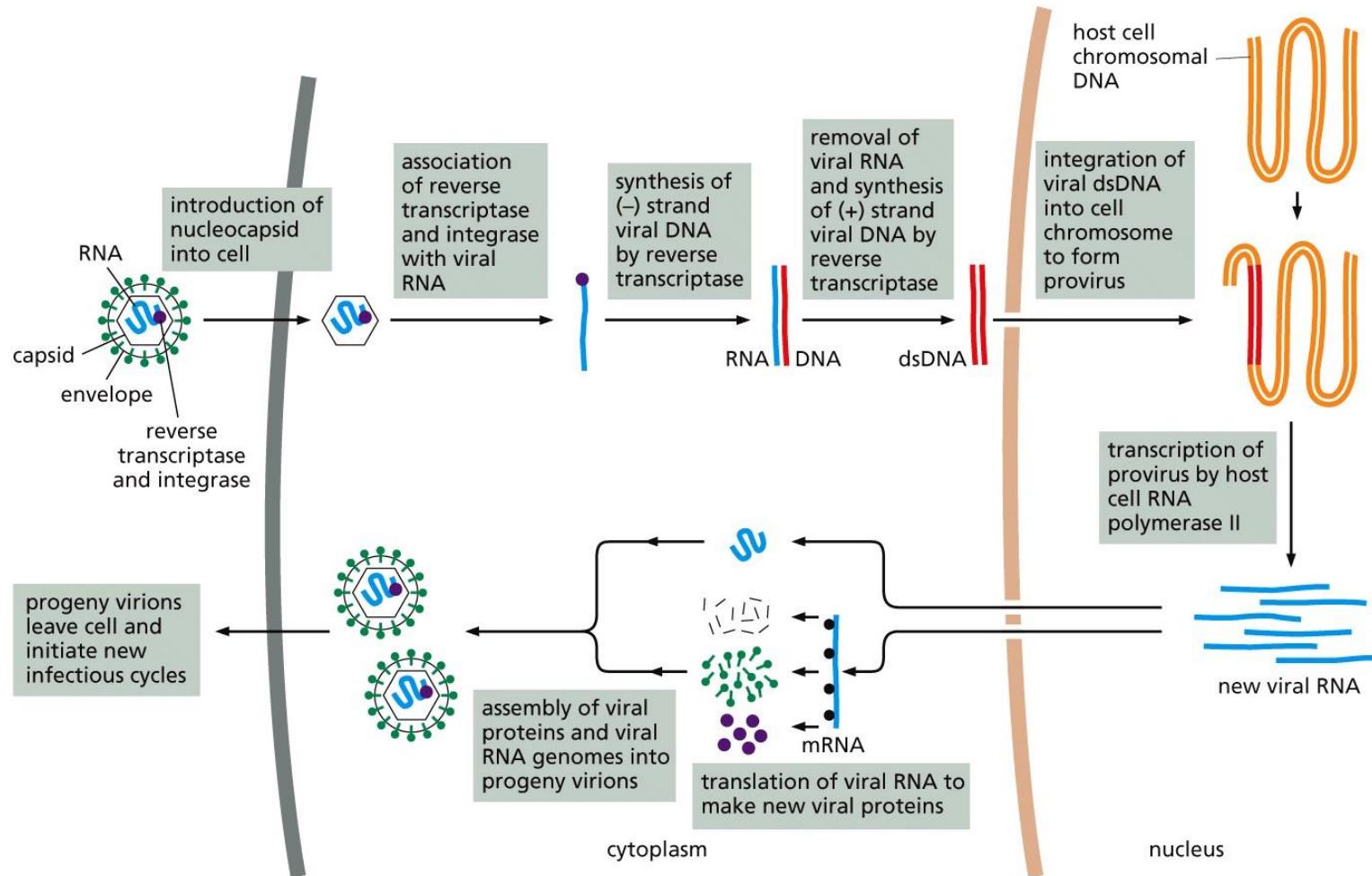


Figure 3.18 The Biology of Cancer (© Garland Science 2014)

SEM images for virus budding from cells

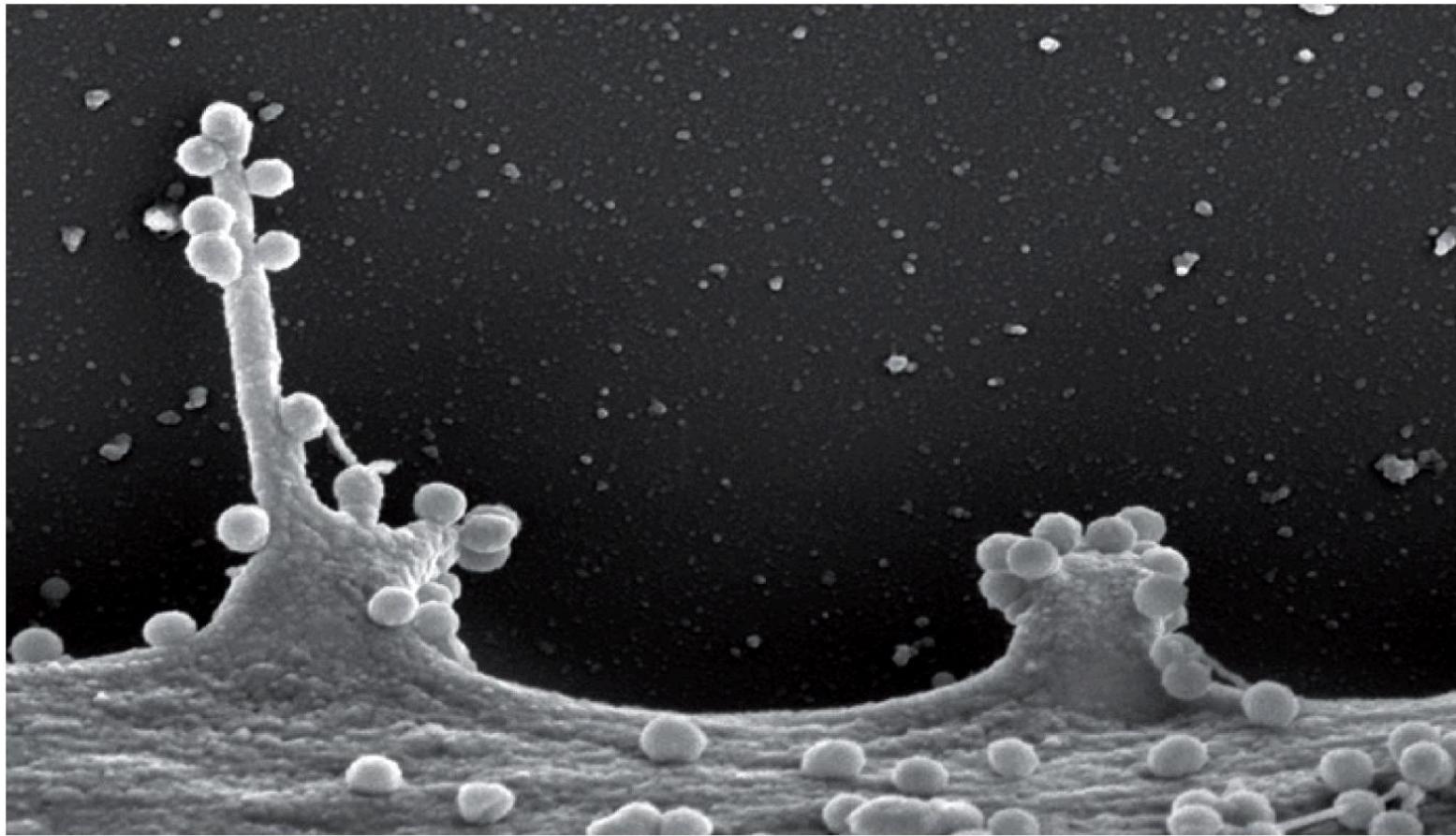


Figure 3.4c The Biology of Cancer (© Garland Science 2014)

RSV infected cells persisted indefinitely...

- California Institute of Technology, Pasadena, in the lab of Renato Dulbecco, his postdoc Harry Rubin found that RSV infected chicken embryo fibroblasts persisted indefinitely, later he and others found that the cells shared many traits that RSV-caused tumors possess.
such as morphology, growth into piles, altered metabolism, etc.

Cell transformation by RSV

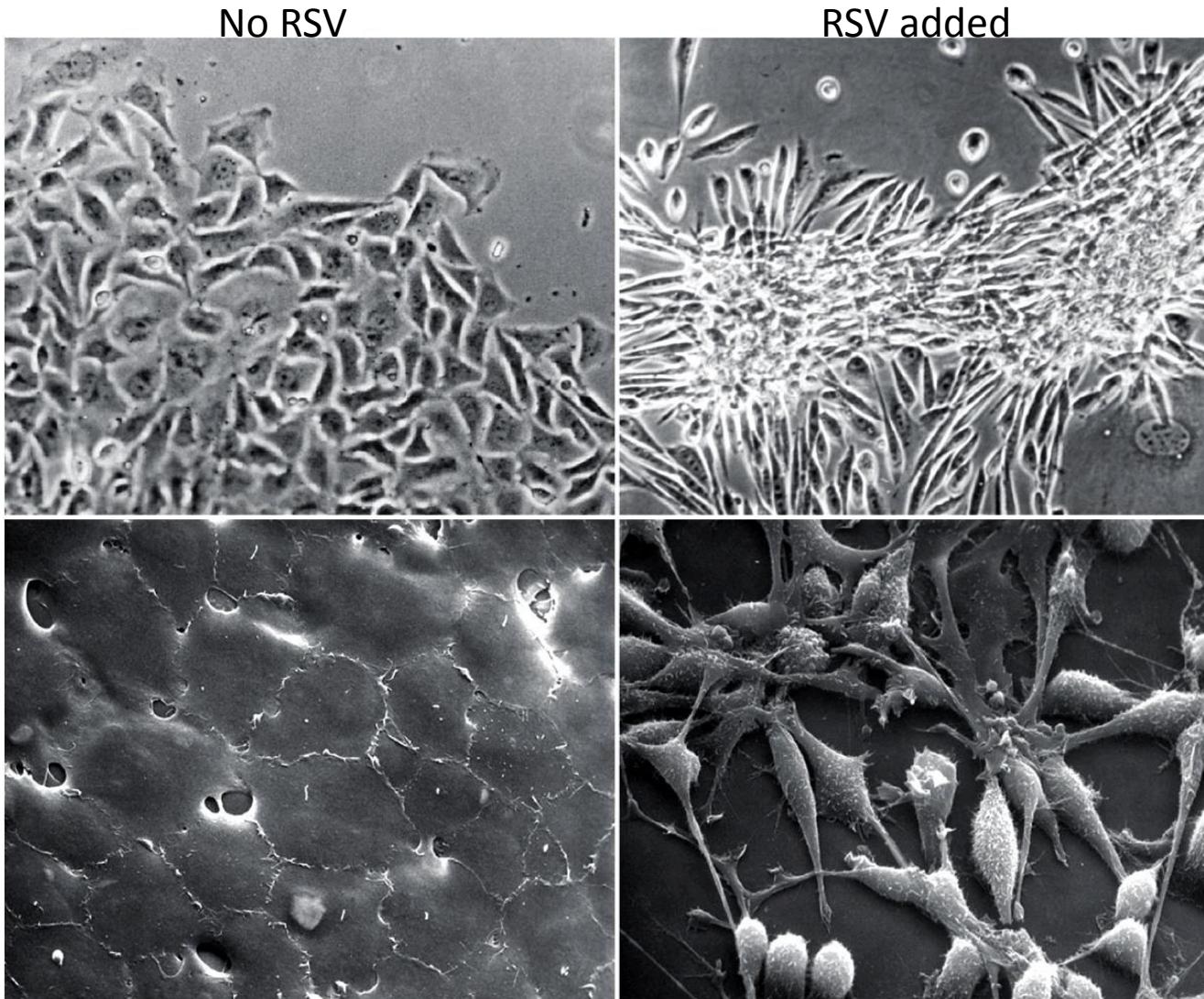


Figure 3.7b The Biology of Cancer (© Garland Science 2014)

RSV infected cells form tumor in immunocompromised mice



Figure 3.13 The Biology of Cancer (© Garland Science 2014)

- For those transformed cells, even after many generations, cells still carry the genome of the virus, the existence of the genome is necessary for maintaining transformation phenotype.

Hunt down the gene that is responsible for cellular transformation

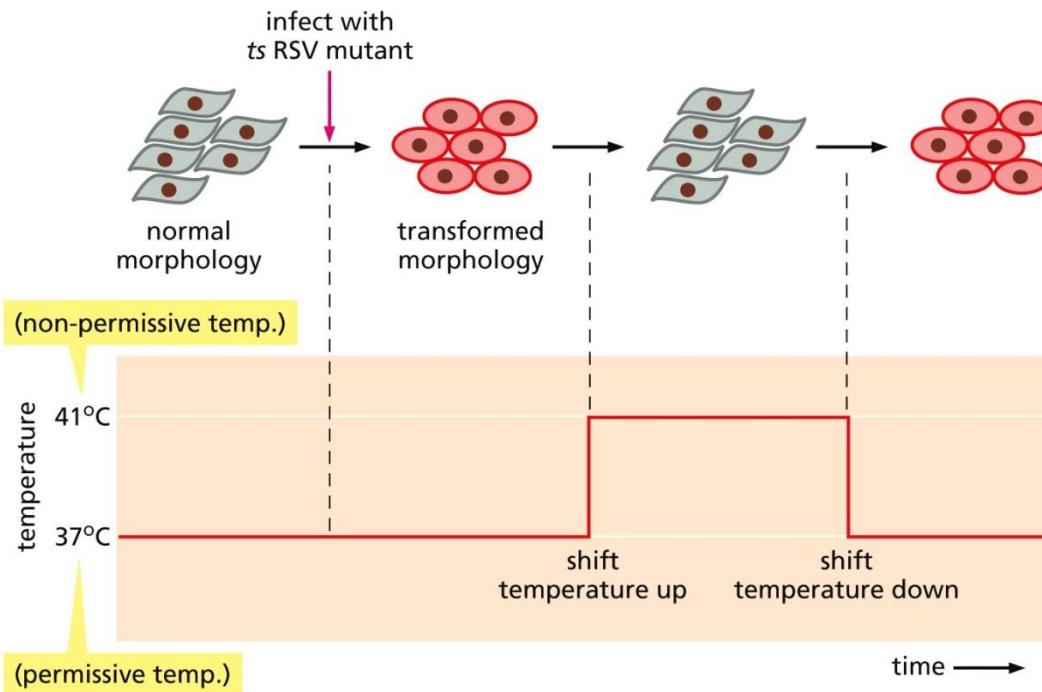


Figure 3.8 The Biology of Cancer (© Garland Science 2014)

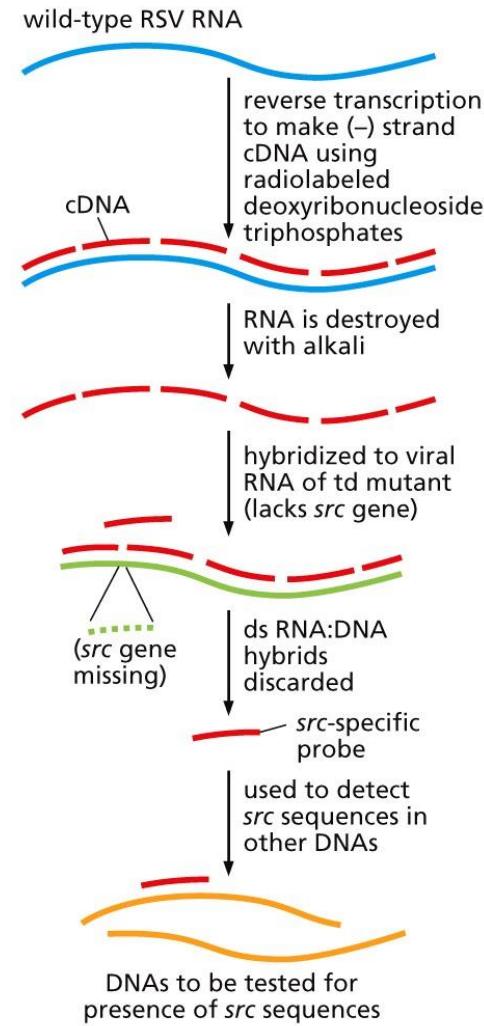


Figure 3.20 The Biology of Cancer (© Garland Science 2014)

How might these oncogenes derive from?

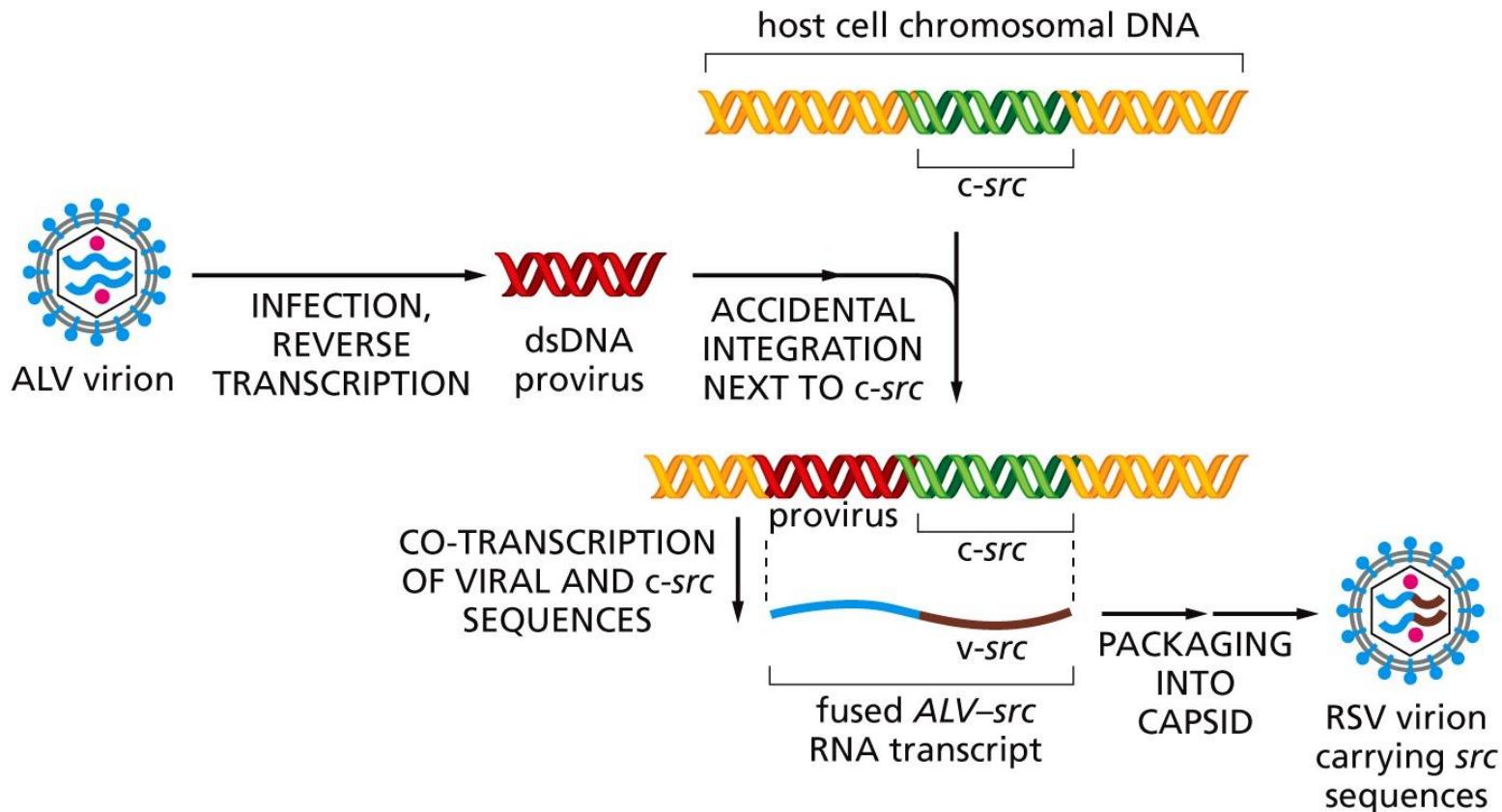


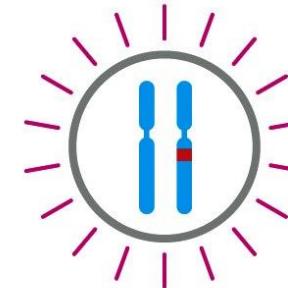
Figure 3.22 The Biology of Cancer (© Garland Science 2014)

Oncogenes and tumor suppressors

(A) overactivity mutation (gain of function)



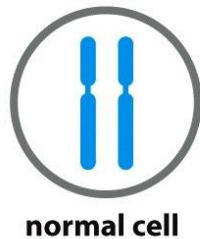
single mutation event
creates oncogene



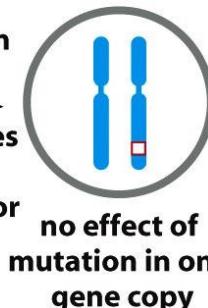
activating mutation
enables oncogene to
promote cell transformation

cells
en route to
cancer

(B) underactivity mutation (loss of function)

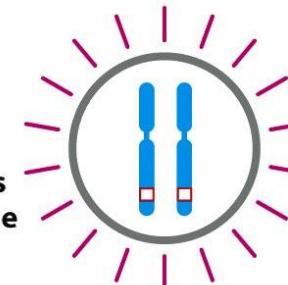


mutation
event
inactivates
tumor
suppressor
gene



no effect of
mutation in one
gene copy

second
mutation
event
inactivates
second gene
copy



two inactivating mutations
functionally eliminate the
tumor suppressor gene,
promoting cell transformation

Oncogenes/proto-oncogenes

- Gain of function of oncogenes leads to tumor formation
- Proto-oncogene normally does not trigger tumorigenesis, but its overactivation or overexpression leads to tumor formation

Tumor suppressors

Loss of function of tumor suppressors leads to tumor formation

Examples:

- Retinoblastoma
- P53
- ARF
- Tsc1
- Neurofibromin 1, etc.

Through family tree certain cancers were found to be inherited in a recessive manner

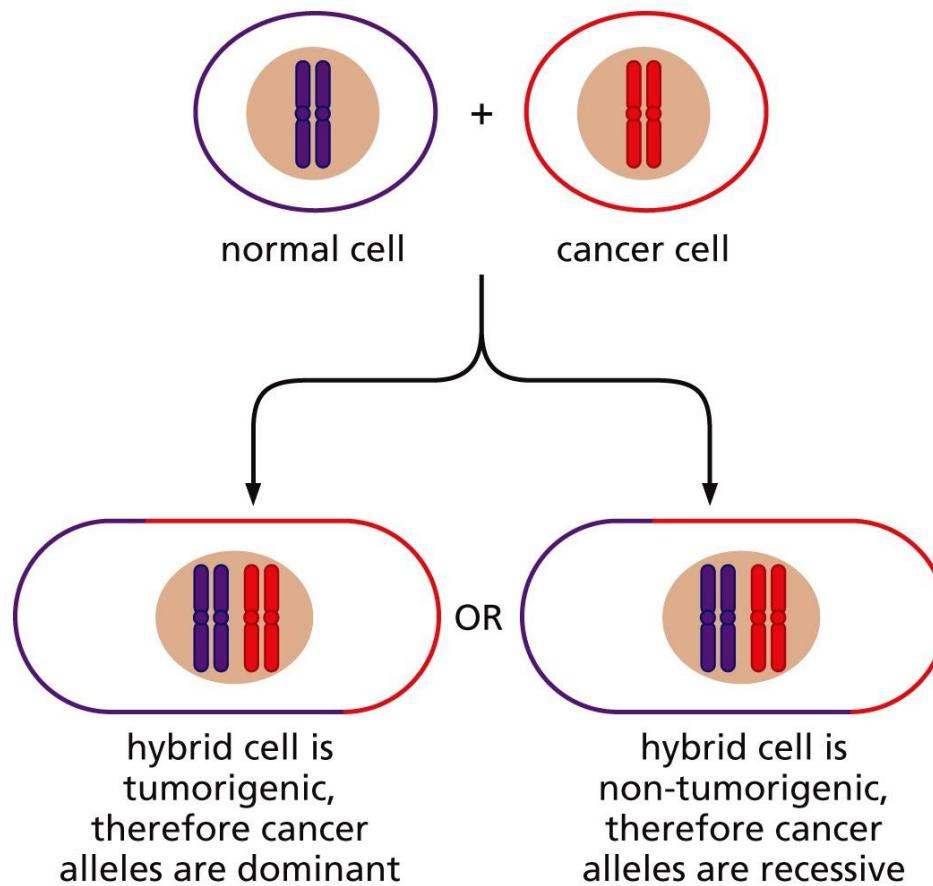


Figure 7.2 The Biology of Cancer (© Garland Science 2014)

Rb gene identification

- infected individual has a deletion of a certain band in his chromosome 13
- Both copy of Rb genes need to be defected to confer cancer phenotype

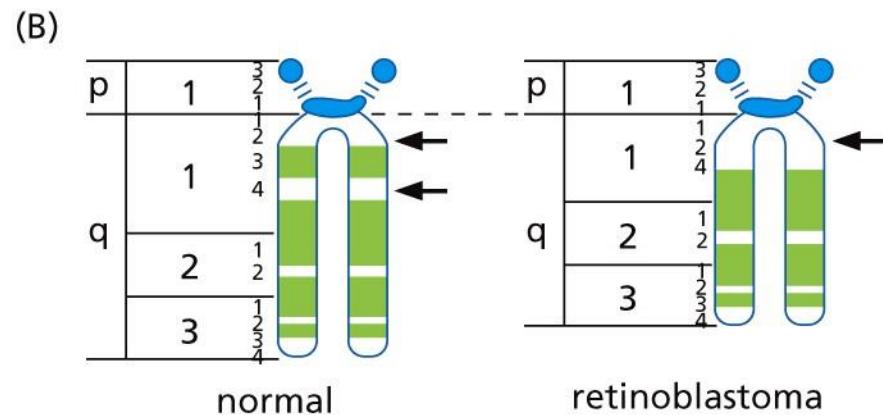
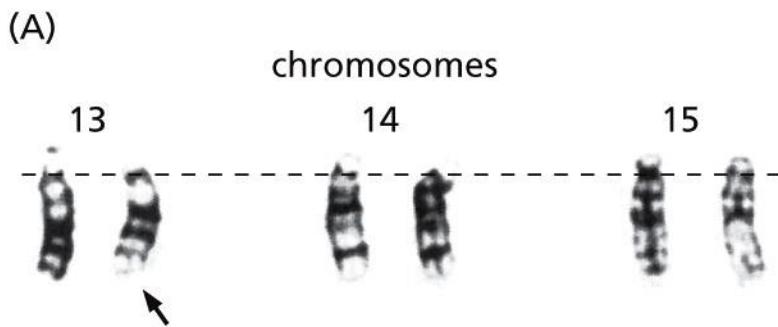


Figure 7.10 The Biology of Cancer (© Garland Science 2014)

- Molecular mechanisms for cancer critical genes with examples

- Ras mutation in human cancers
 - Rb loss of heterozygosity
 - p53 mutation or loss
 - HPV
 - APC mutation in colon cancers

(1) Ras mutation

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 (N)
Bladder	10 (H, 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, *Nature Rev. Cancer* 3:11–22, 2003.

Most oncogenic mutations on Ras occur on amino acid 12

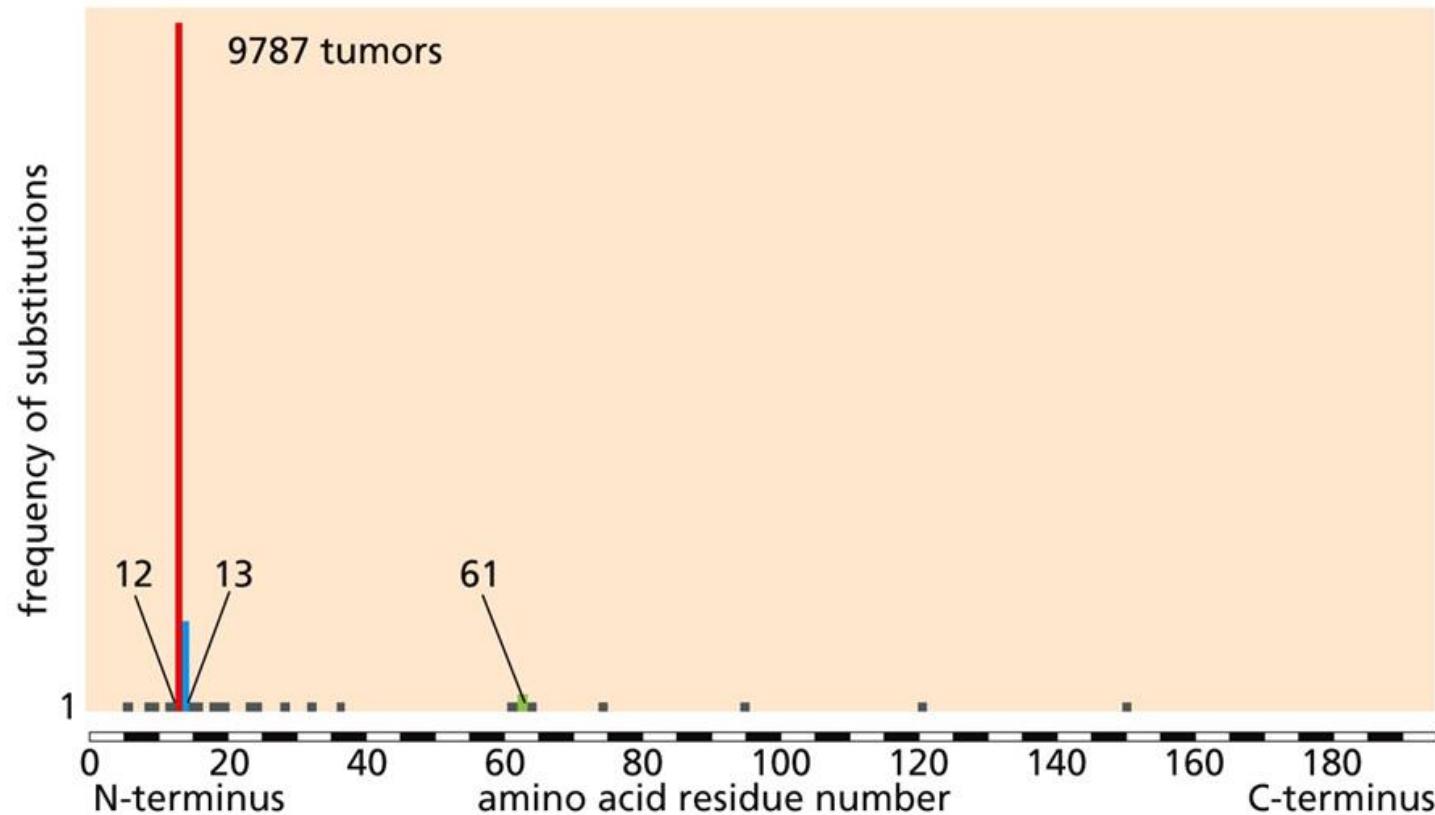


Figure 4.10 The Biology of Cancer (© Garland Science 2014)

(2) Rb loss of heterozygosity- (LOH) induces retinoblastoma

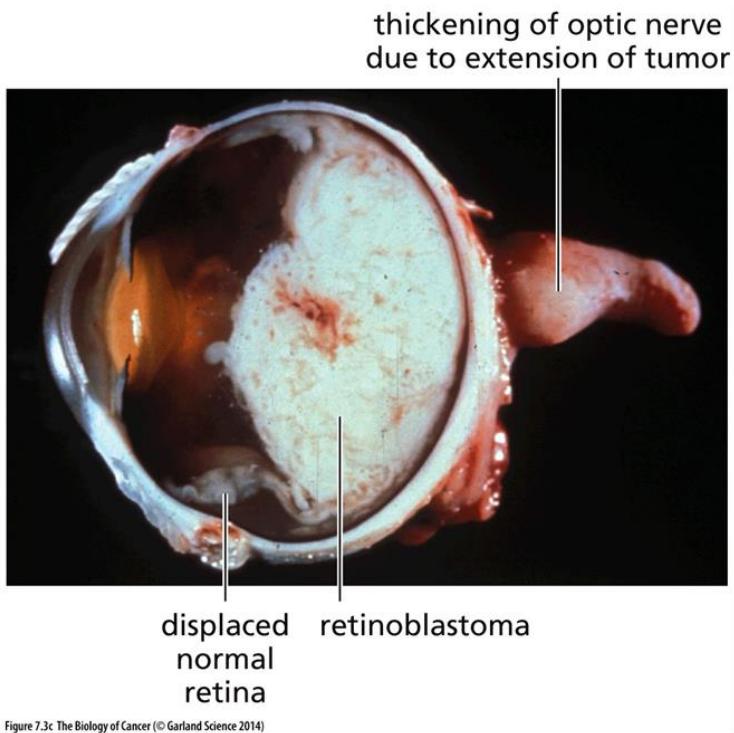


Figure 7.3d The Biology of Cancer (© Garland Science 2014)

Figure 7.3c The Biology of Cancer (© Garland Science 2014)

Rb and cell cycle control

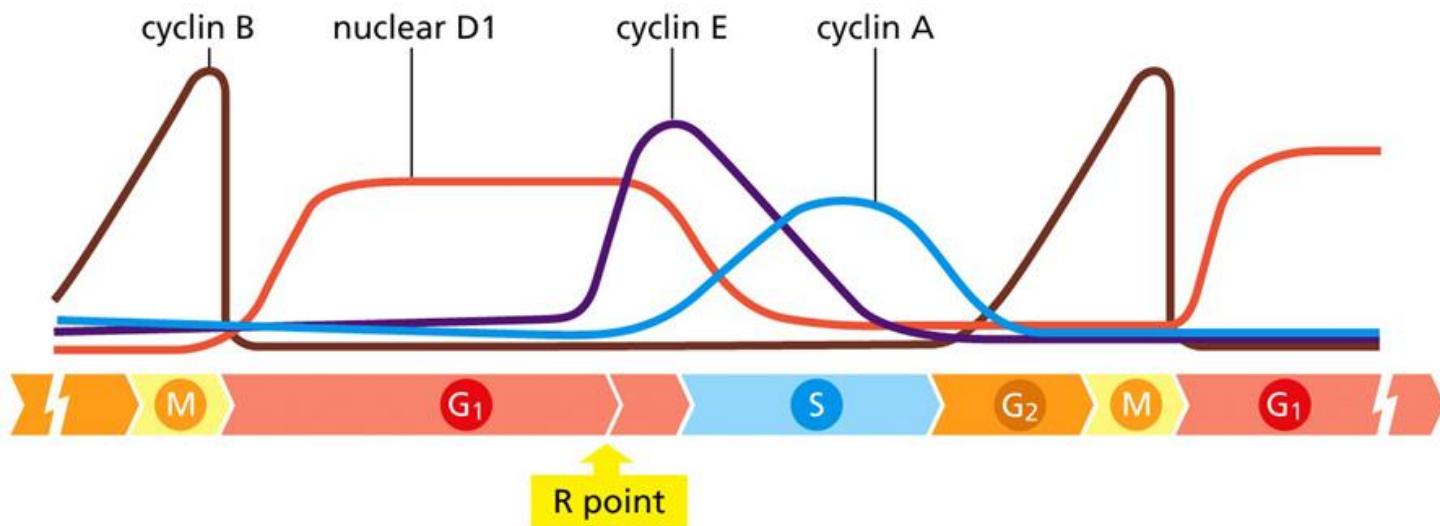


Figure 8.10 The Biology of Cancer (© Garland Science 2014)

Phosphorylation of Rb promotes cyclin E/cdk activity

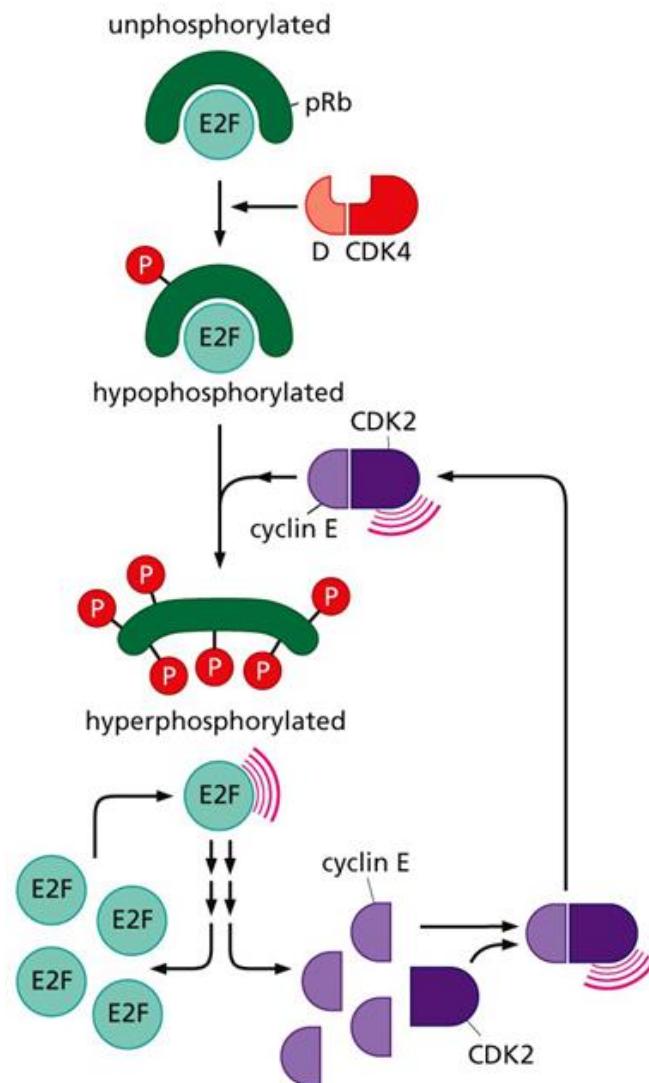


Figure 8.25a The Biology of Cancer (© Garland Science 2014)

Mutation of p53 in human cancers

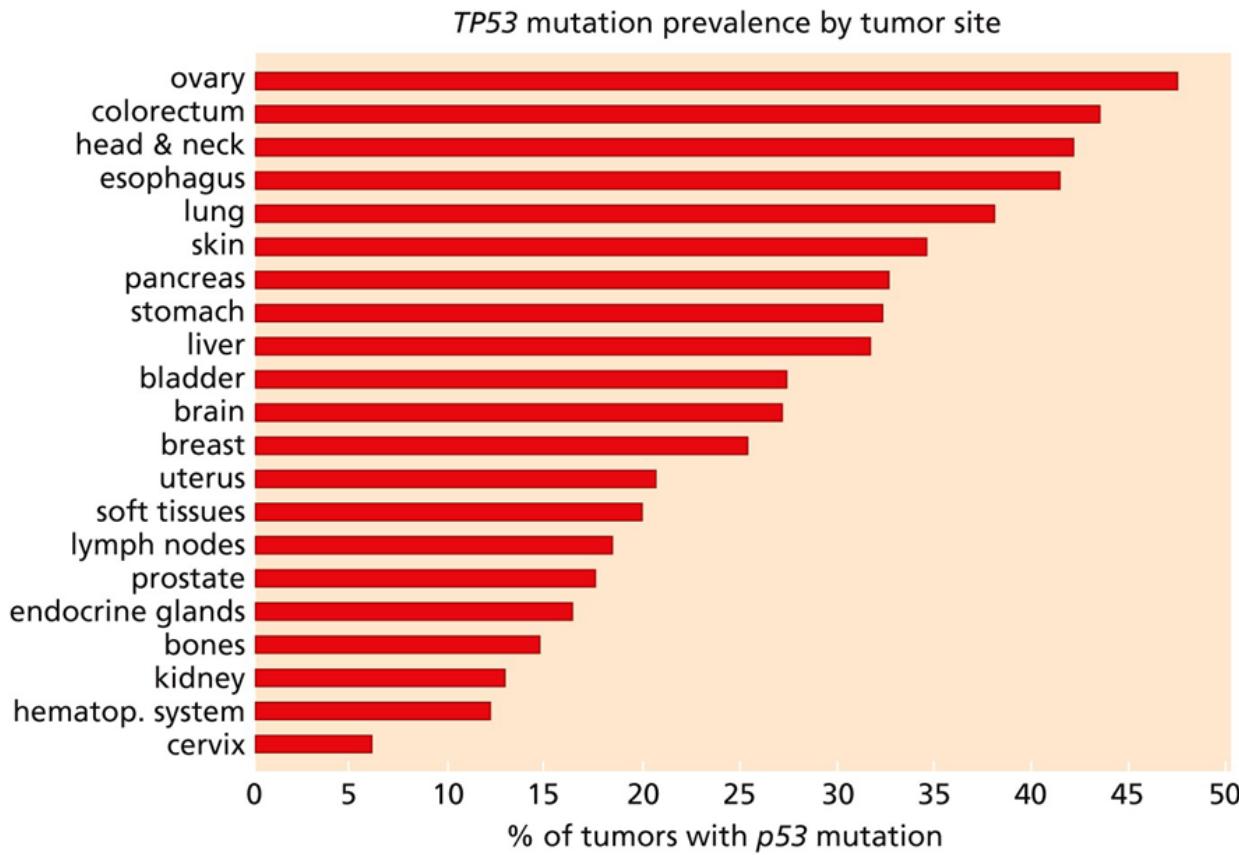


Figure 9.4 The Biology of Cancer (© Garland Science 2014)

Models of action of p53 tumor suppressor

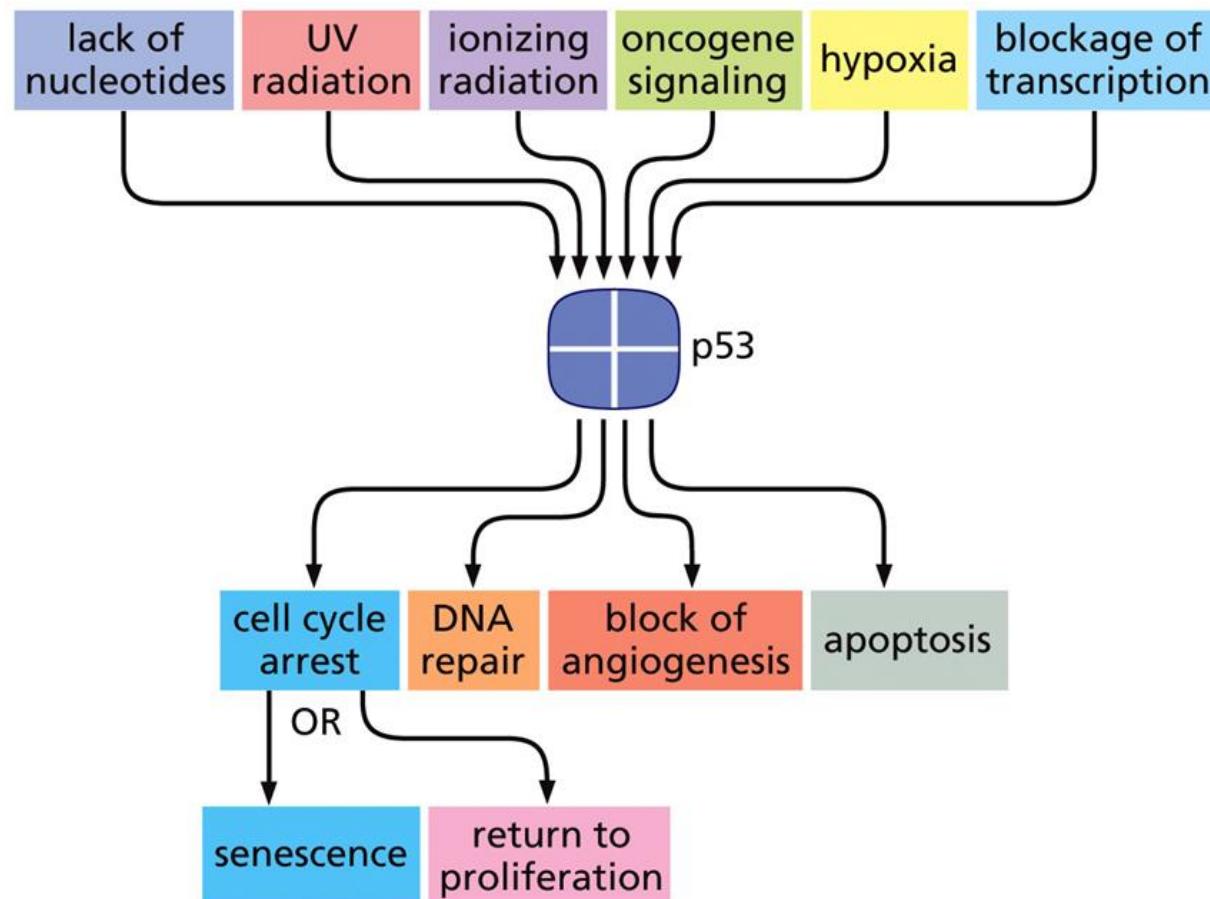
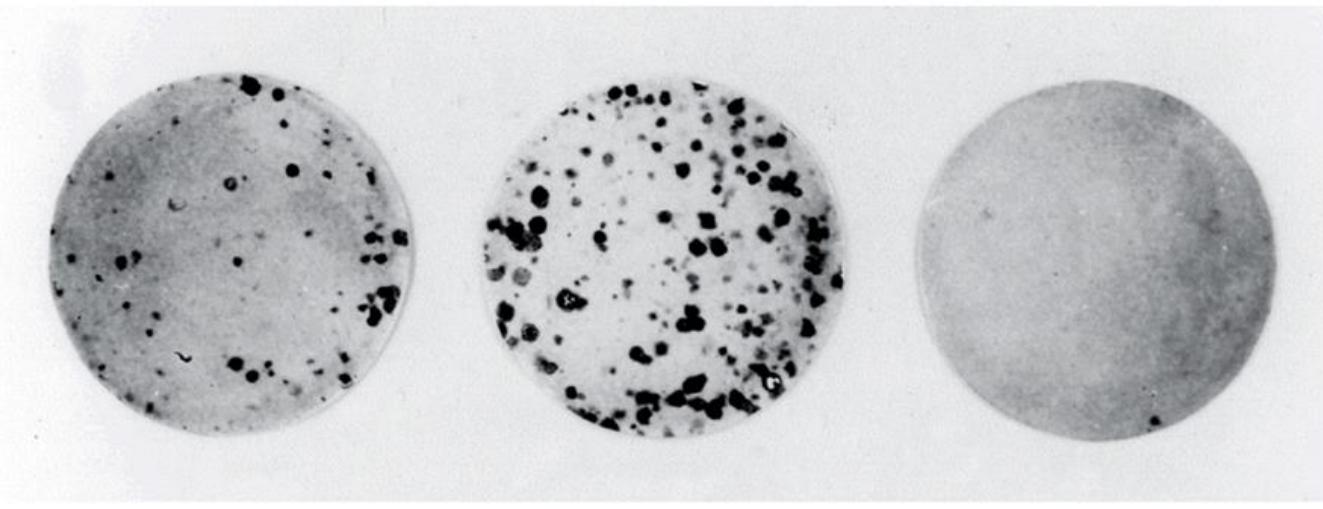


Figure 9.8 The Biology of Cancer (© Garland Science 2014)

Example



ras + p53
deletion mutant

ras + p53 val-135
point mutant

ras + p53
wild type

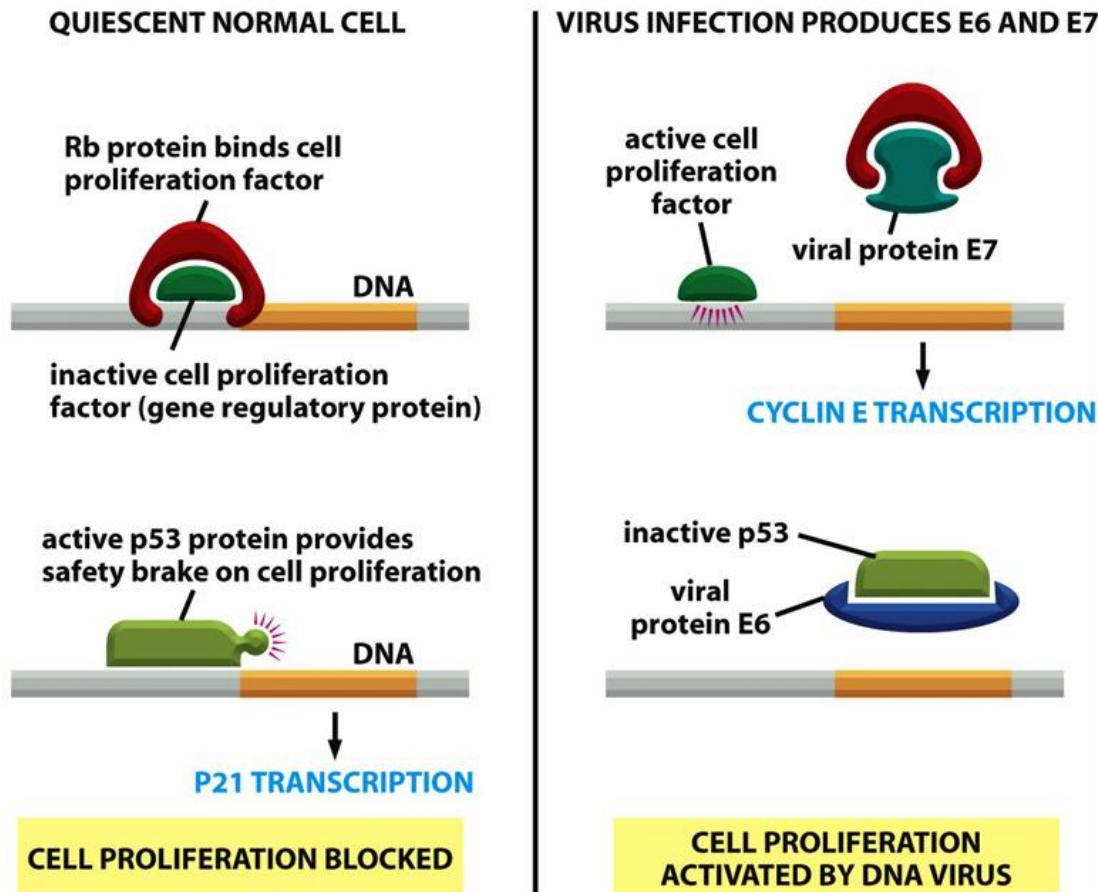
Figure 9.3 The Biology of Cancer (© Garland Science 2014)

p53 V135: gain of function



02.3_Visualization_of_Cancer_I_Lymphoma.wmv

Mode of action for HPV



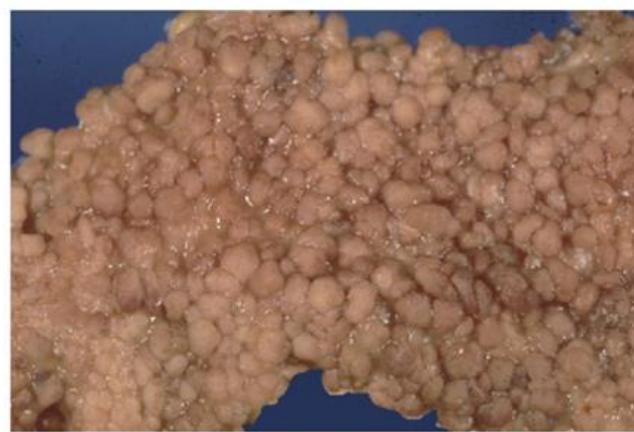
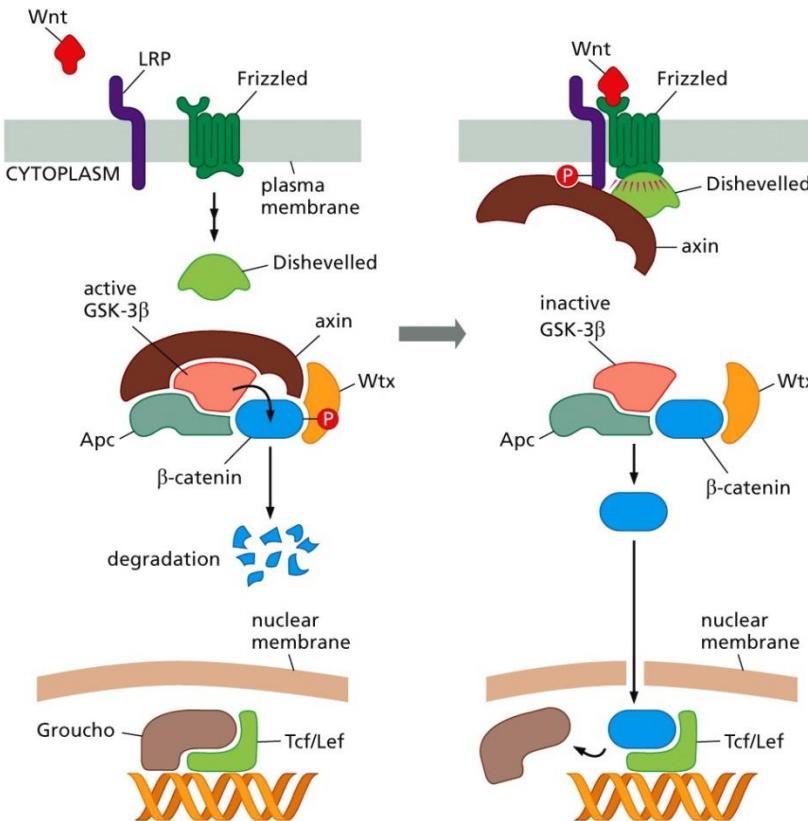
APC mutation and human colon cancers

Table 20–2 Some Genetic Abnormalities Detected in Colorectal Cancer Cells

GENE	CLASS	PATHWAY AFFECTED	HUMAN COLON CANCERS (%)
<i>K-Ras</i>	oncogene	receptor tyrosine-kinase signaling	40
β -catenin ¹	oncogene	Wnt signaling	5–10
<i>Apc</i> ¹	tumor suppressor	Wnt signaling	> 80
<i>p53</i>	tumor suppressor	response to stress and DNA damage	60
<i>TGFβ receptor II</i> ²	tumor suppressor	TGF β signaling	10
<i>Smad4</i> ²	tumor suppressor	TGF β signaling	30
<i>MLH1</i> and other DNA mismatch repair genes	tumor suppressor (genetic stability)	DNA mismatch repair	15 (often silenced by methylation)

The genes with the same superscript act in the same pathway, and therefore only one of the components is mutated in an individual cancer.

- APC LOH induces polyposis, which usually happens before colon cancer



Normal colon

Adenomatous polyposis

II. Multi-step tumorigenesis

Cancer takes many years to develop

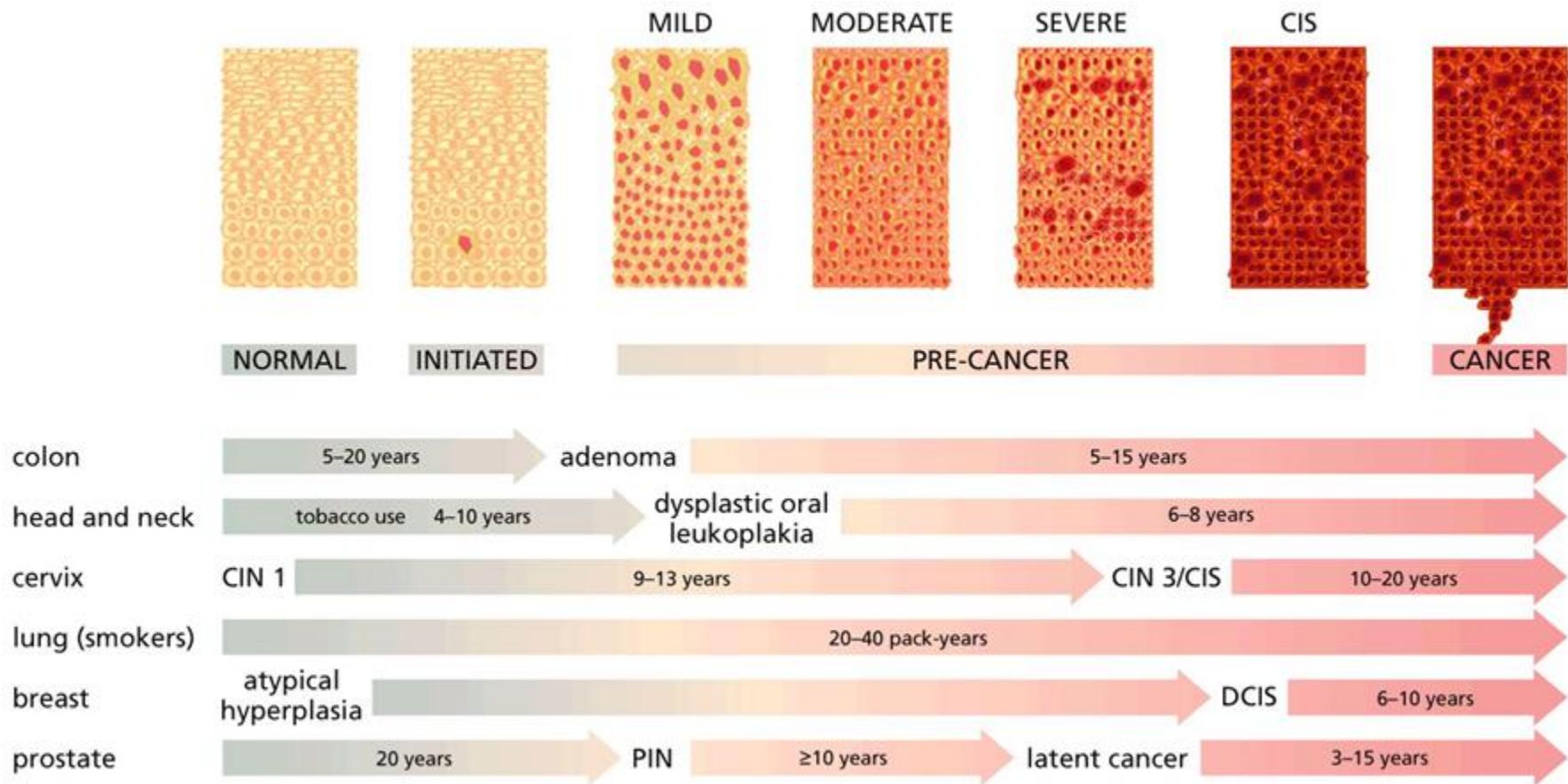


Figure 11.8a The Biology of Cancer (© Garland Science 2014)

Darwinian evolution and clonal succession

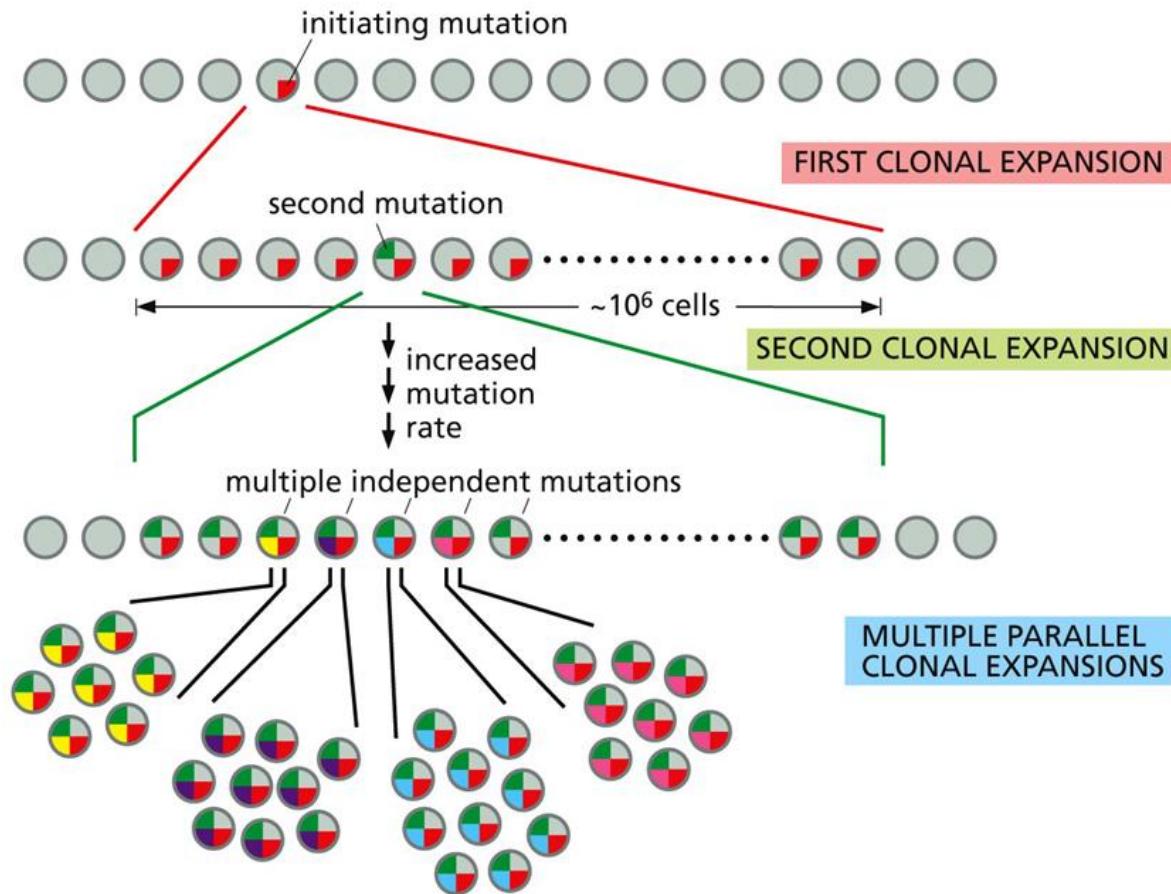


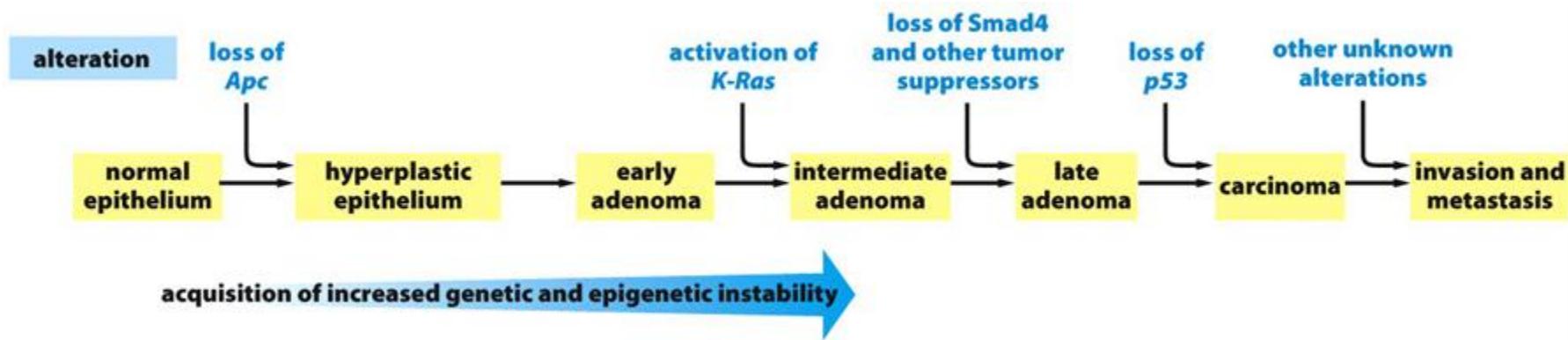
Figure 11.20a The Biology of Cancer (© Garland Science 2014)

Driver versus passenger mutations

- **Driver mutation:** mutation of critical genes confer advantageous phenotypes leading to the clonal expansions that driven multi-step tumorigenesis.
- **Passenger mutation:** mutations of which have no influence on cancer cell phenotype and consequently are irrelevant to tumor progression

Genetic analysis from 21 Gliomas, only 8% of the mutations belong to driver mutation
In other types of cancers, the percentage is even lower.

- Genetic changes underlying the development of colorectal carcinoma



Successive mutation of cancer genome during the development of colon cancers.

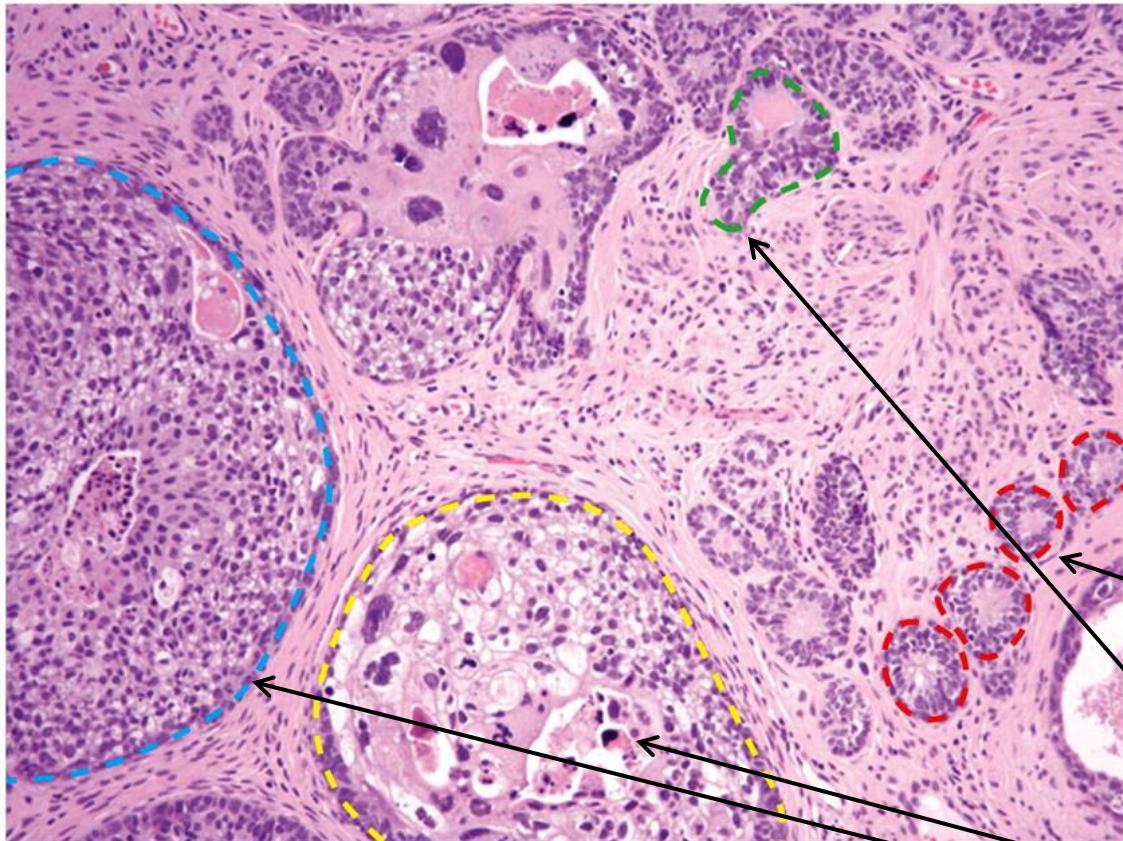
APC: important tumor suppressor in suppressing Wnt signaling pathway

K-Ras: small GTPase when mutated will activate multiple pathways to promote proliferation.

Smad4: important mediator in TGF-beta signaling pathway.

p53: master regulator in control cell cycle, death, genome integrity, etc.

Diversification within a tumor



Four populations of cervical cancer cells:
Due to distinct differentiation programs activated by the various carcinoma cells rather than genetic diversification.

Small basaloid islands

Columnar cell carcinoma

Squamoid cell carcinoma

Figure 11.21a The Biology of Cancer (© Garland Science 2014)

III. Cancer stem cells

Take a tumor mass, make single cell suspension



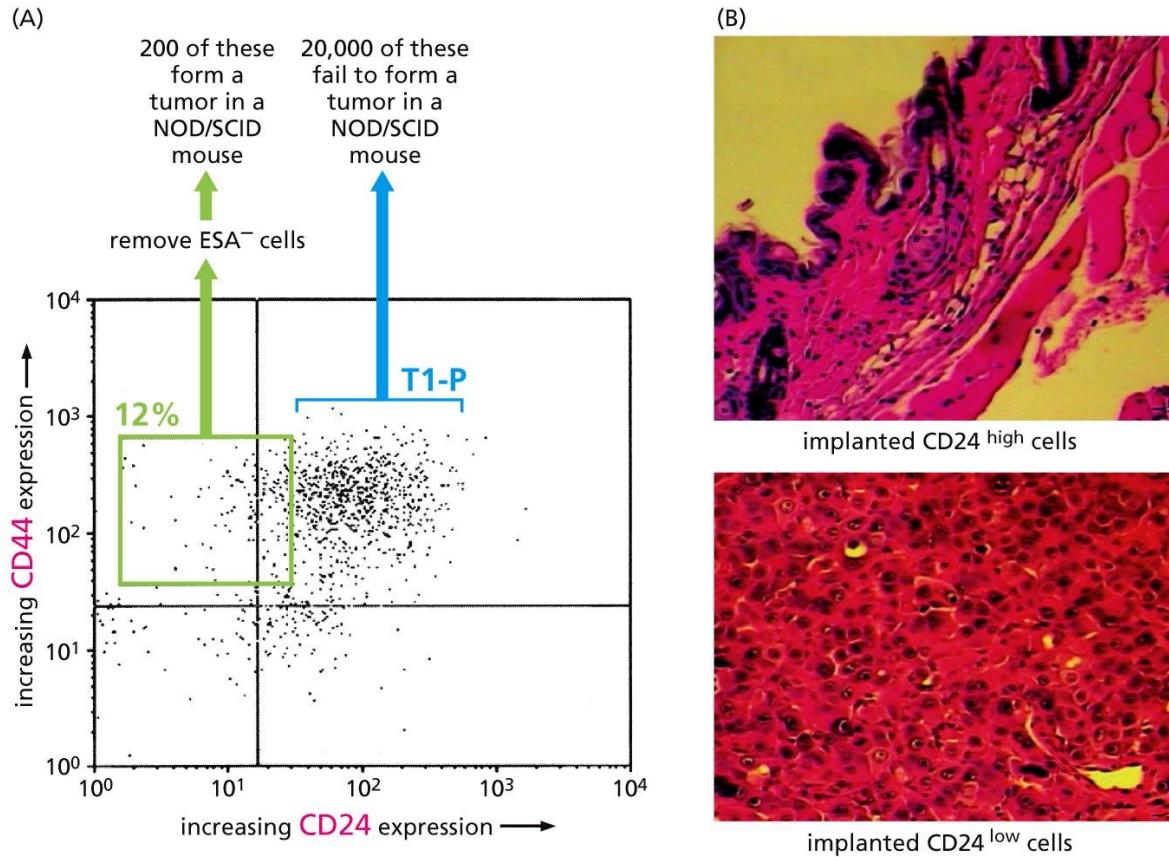
Inject single cell into immunodeficiency mice to induce tumor formation



<<1% of the isolated cancer cells can induce a new tumor formation

Those that can induce tumor formation has the characteristics of **cancer stem cells**: They are capable of indefinite self-renewal, but they also give rise to rapidly cells that have limited capacity to self-renewal. They are responsible for maintaining the Population of cells in a tumor.

Cancer stem cells



Based on surface marker
To sort two populations of Cancer cells. Only one Population can initiate new Cancer formation.

Enrichment of brain tumor stem cells

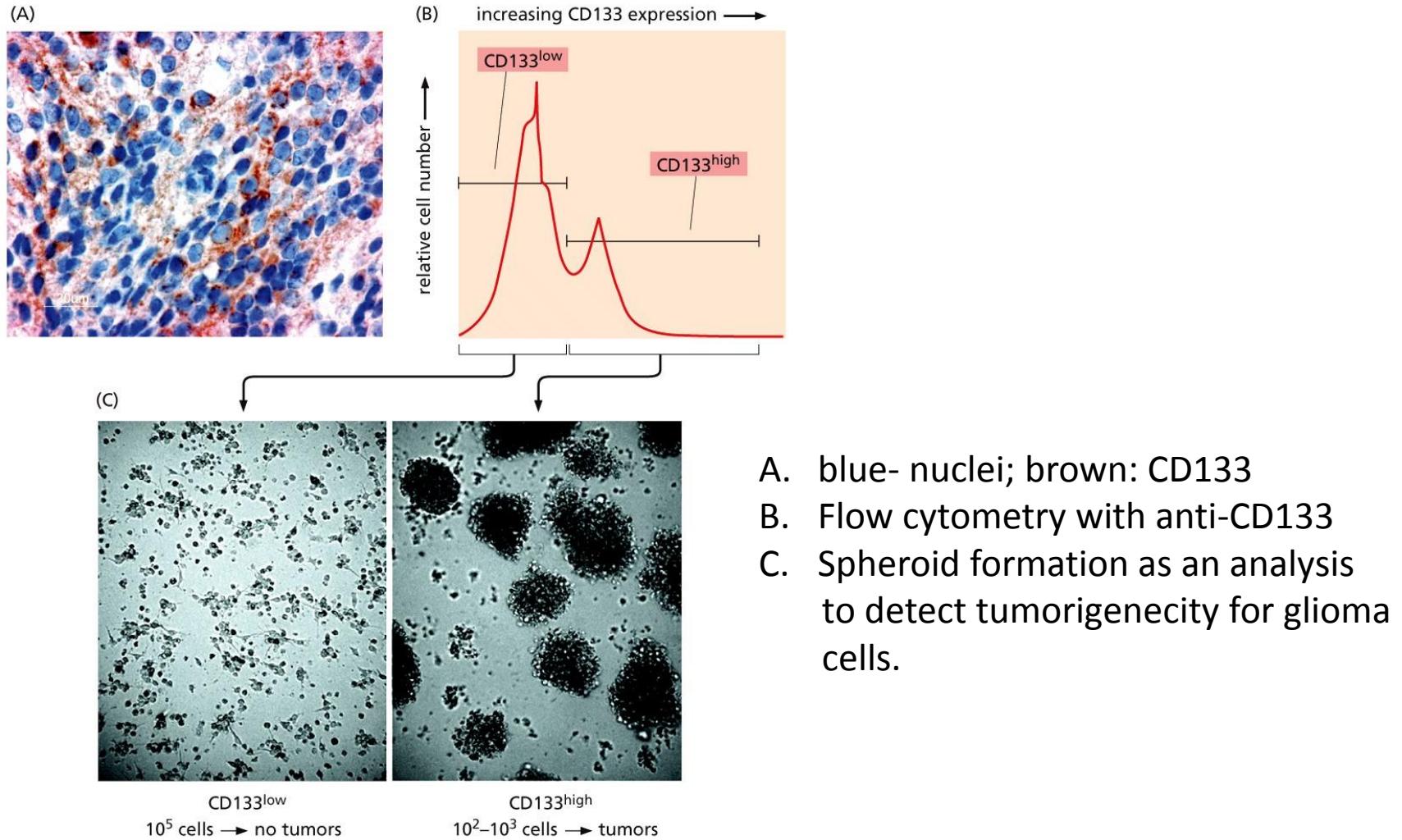
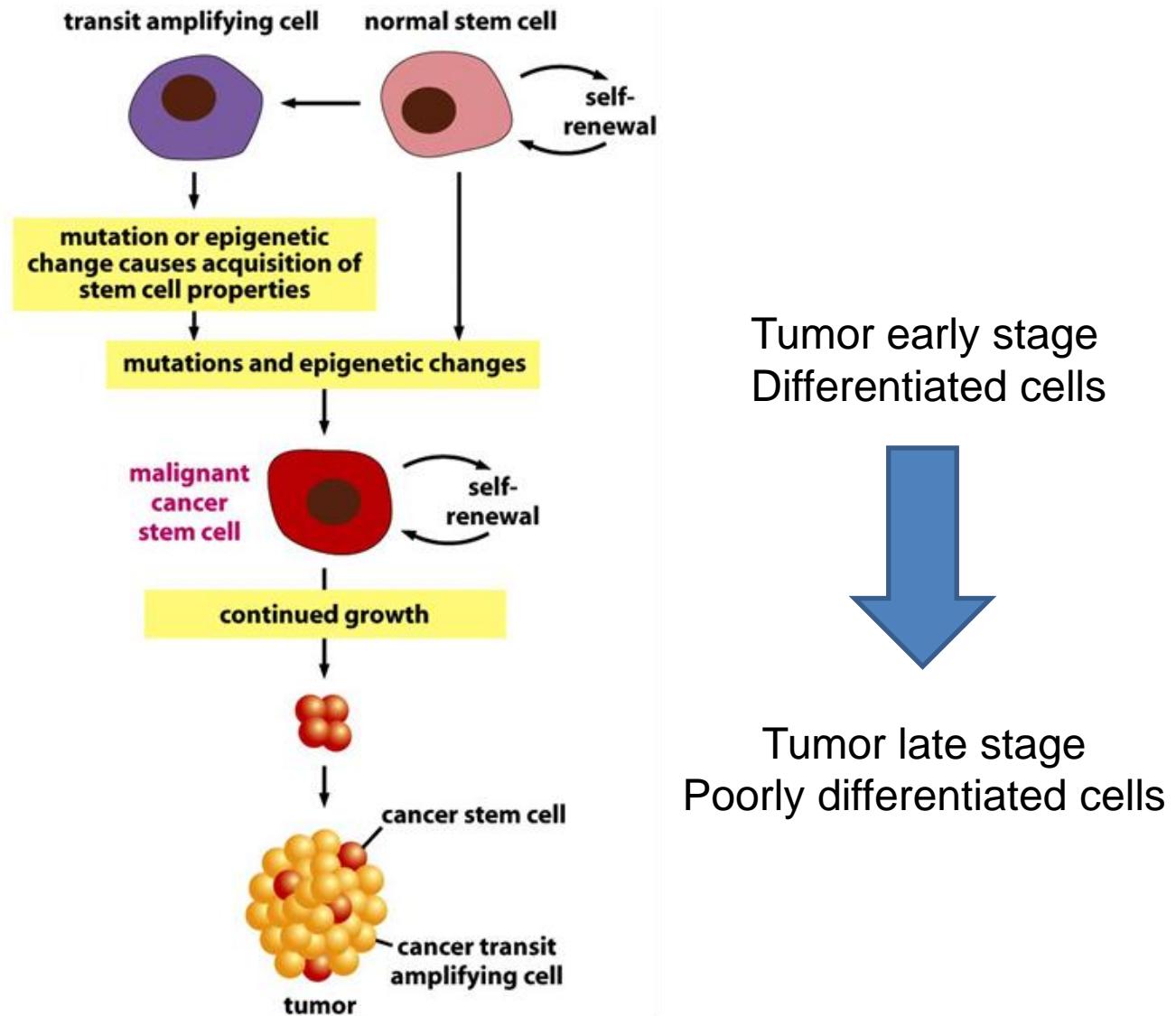


Figure 11.17 The Biology of Cancer (© Garland Science 2014)

Cancer Stem Cells

- Cancer stem cell have the similar property as stem cells in normal tissues, they are self-renewing and has unlimited proliferation potential.
- Cancer stem cells must have the property of initiating new tumor formation.
- Cancer stem cell localization has no common patterns.
- Autocrine signaling involving TGF- β and Wnt factors enables both types of SCs to maintain their residence in this state.

Cancers **may** arise from cancer stem cells



IV. Tumor metastasis

Overview of metastasis---causing 90% of all cancer death

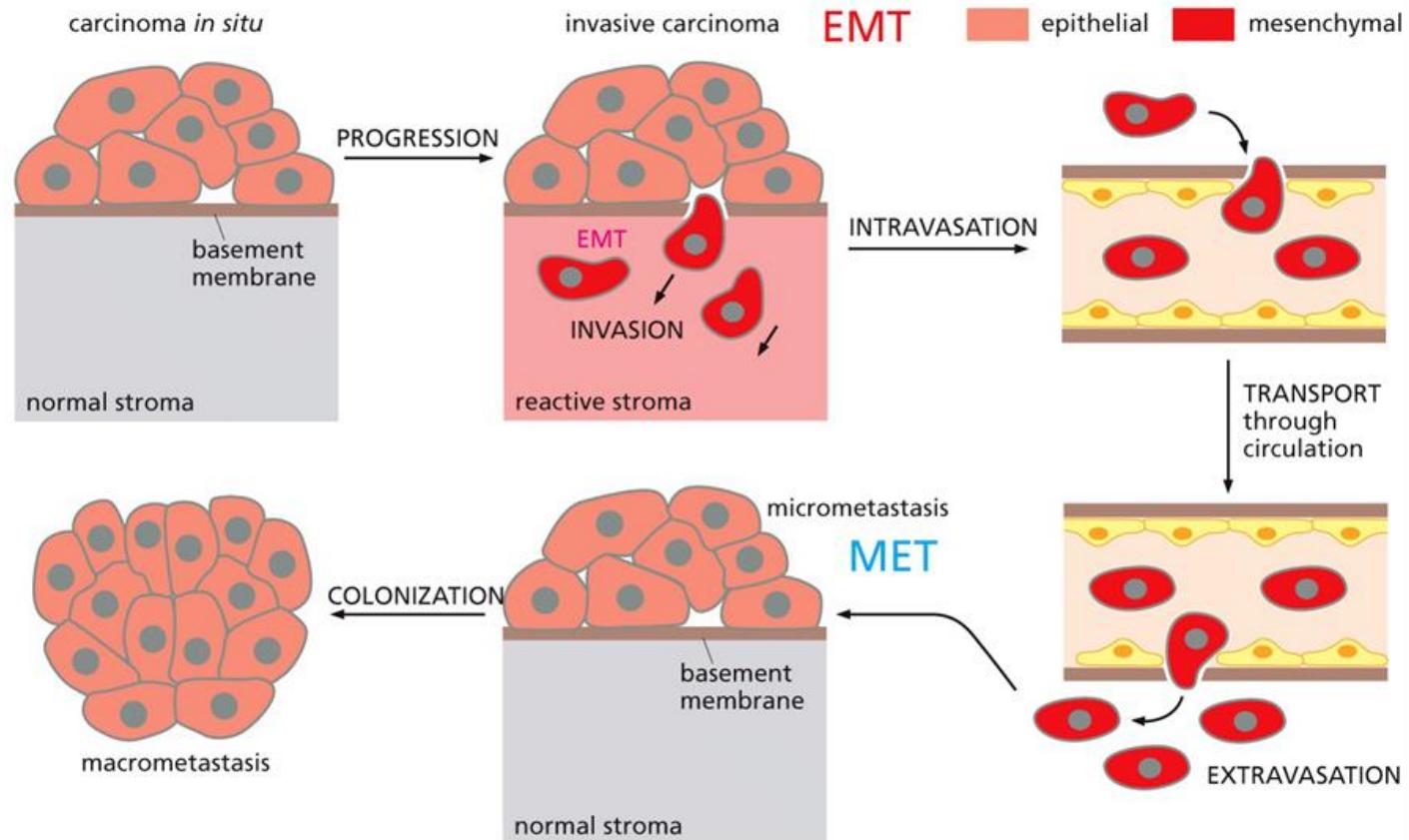


Figure 14.18b The Biology of Cancer (© Garland Science 2014)

- Epithelial-mesenchymal transition is important in metastasis

EMT promoting transcription factors promotes metastasis

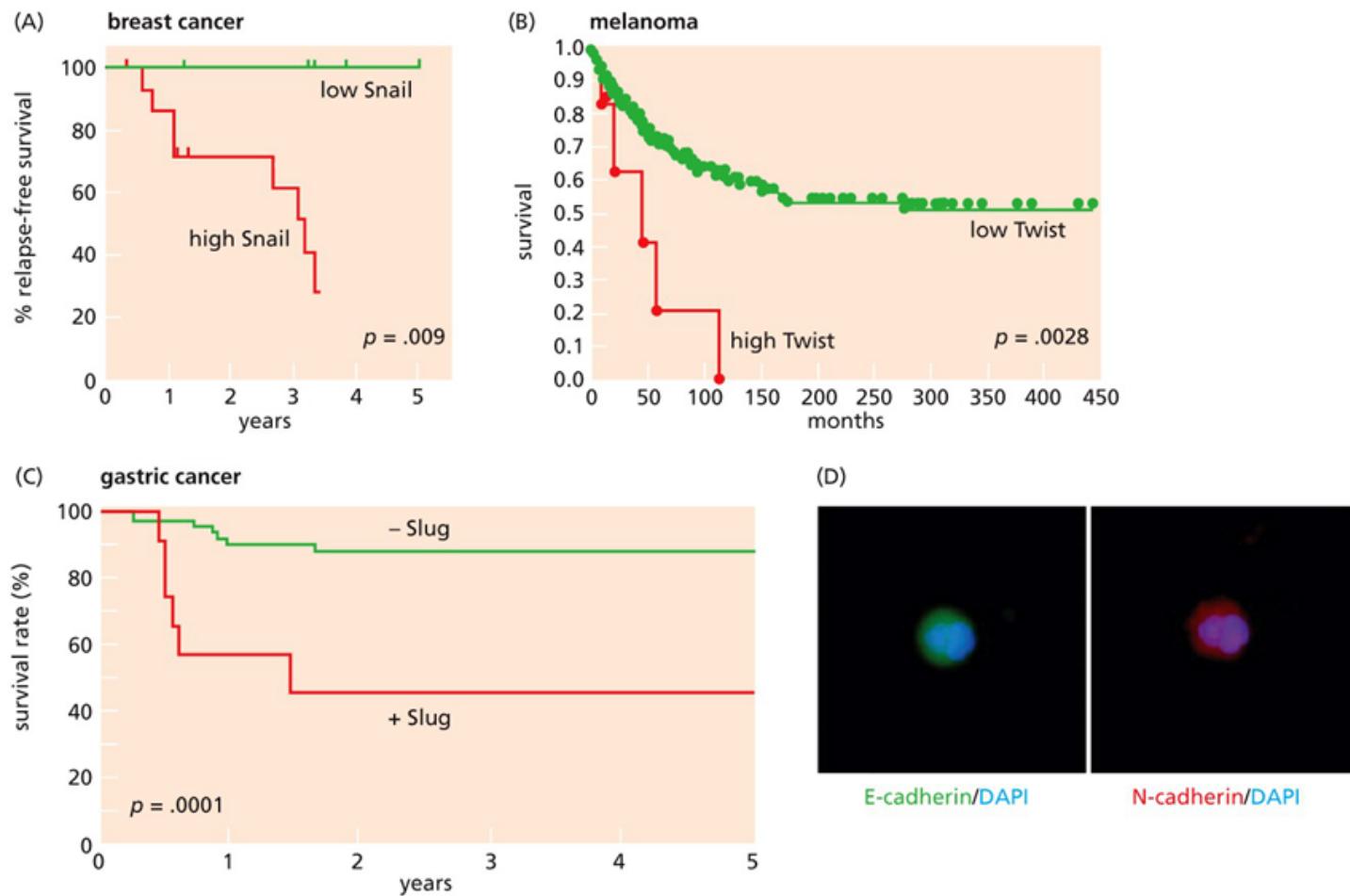


Figure 14.31 The Biology of Cancer (© Garland Science 2014)

EMT is associating to increased migration of epithelial cells

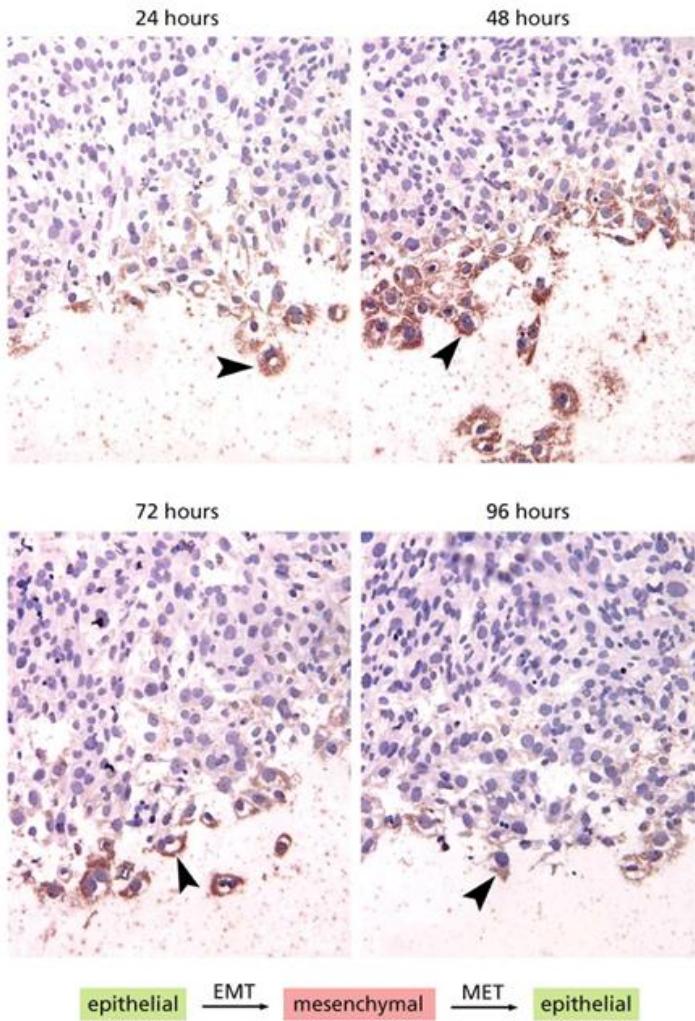
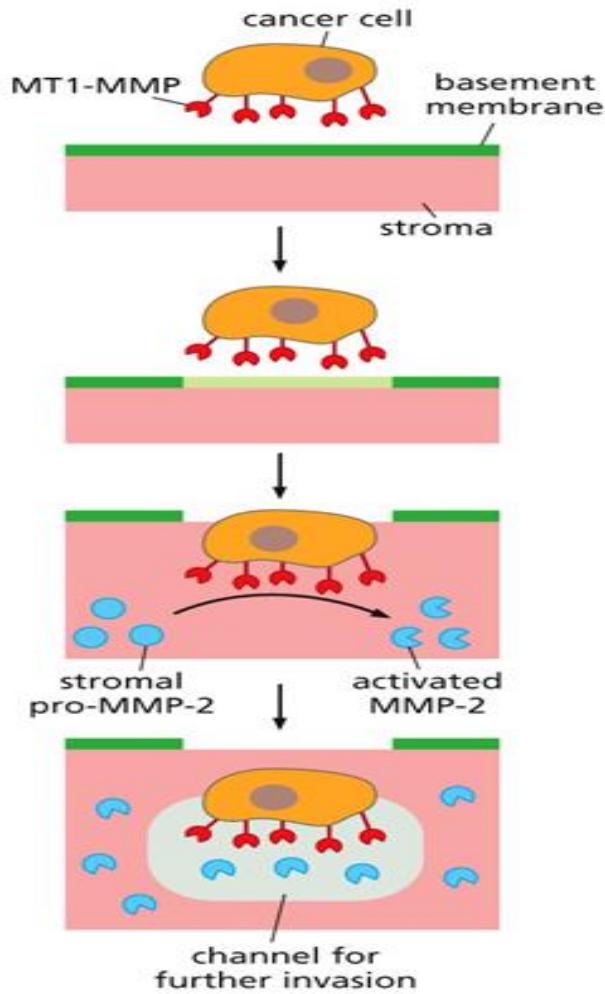


Figure 14.26 The Biology of Cancer (© Garland Science 2014)



14.2_Mechanisms_of_Brain_MetastasisFormation.mov

Increased activity of MT-MMP, and many factors promote cancer invasion



MT-MMP: membrane type matrix metalloproteinase
It is an important protease family, many members are frequently found to be overexpressed in cancers.
MMPs can cut or digest ECM proteins and free cancer cells from the boundary.

Figure 14.34 The Biology of Cancer (© Garland Science 2014)

V. Cancer treatment

- Traditional cancer treatment (before 1975)
 - Surgery
 - Chemotherapy
 - Radiotherapy

still take a major part
in cancer therapy
- Present and future in cancer treatment

Mortality rates for cancers in the past decades

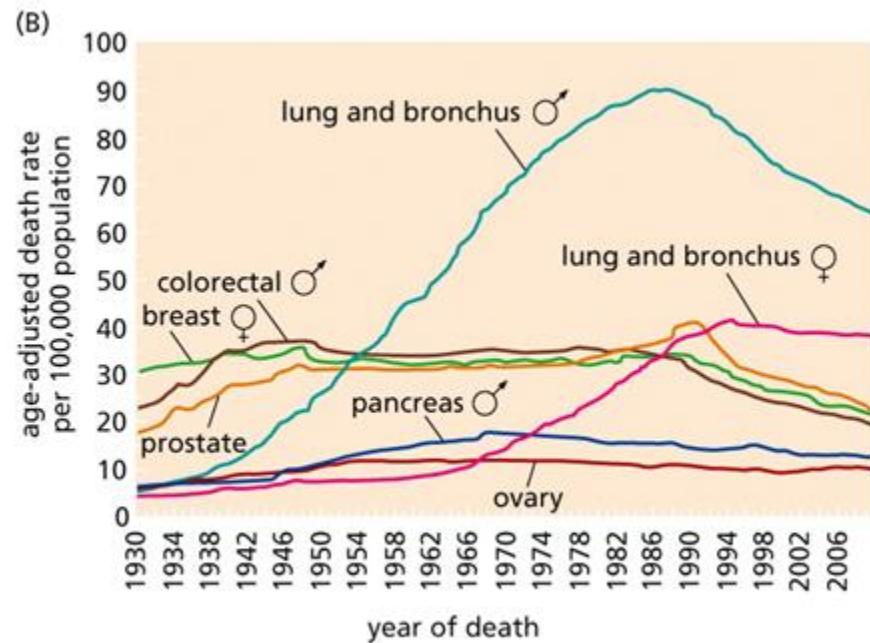
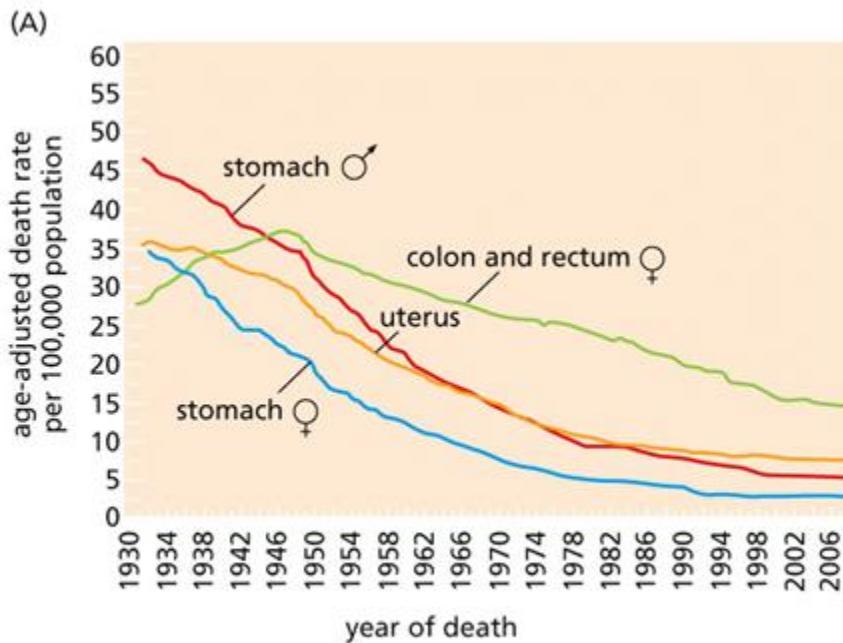


Figure 16.1 The Biology of Cancer (© Garland Science 2014)

improved food storage, awareness in Helicobacter pylori
early detection and surgery increase survival rates for
Certain types of cancer

Chemotherapy drugs

Examples for some of them, all are generally cytotoxic:

Table 16.2 Examples of antimetabolites used to treat cancer

Name	Chemical structure	Targeted reaction	Examples of clinical use
methotrexate	folate analog	formation of tetrahydrofolate	breast cancer, lymphomas
6-mercaptopurine	purine analog	purine biosynthesis	leukemia, NHL
doxorubicin	natural product ^a	intercalating agent, inhibits topoisomerase	wide range
thioguanine	guanine analog	purine biosynthesis	acute granulocytic leukemia
fludarabine	purine analog	ribonucleotide reductase, DNA replication	chronic lymphocytic leukemia, NHL
cladribine	adenosine analog	adenosine deaminase	hairy-cell leukemia
bortezomib	peptide analog	proteasomal degradation	multiple myeloma
paclitaxel	natural product ^a	microtubule destabilization	lung, ovarian, breast cancer
etoposide	natural product ^a	DNA unwinding	lung cancer, sarcomas, glioblastoma
mitoxantrone	topoisomerase inhibitor	DNA unwinding	AML, breast cancer, NHL
irinotecan	topoisomerase inhibitor	DNA unwinding	colorectal carcinoma
vinblastine	natural product ^a	microtubule assembly	Hodgkin's lymphoma
vorinostat	hydroxamic acid	histone deacetylation	cutaneous T-cell lymphoma
azacitidine	pyrimidine analog	DNA methylation	myelodysplastic syndrome

Abbreviations: NHL, non-Hodgkin's lymphoma; AML, acute myelogenous leukemia.

^aComplex structure.

Chemotherapeutic drug in combination

Table 16.4 Examples of multi-drug treatment protocols

Acronym	Components	Mechanisms of action	Application
ABVD	doxorubicin, bleomycin, vinblastine, dacarbazine	intercalation, DNA strand breaks, microtubule inhibition	Hodgkin's lymphoma
CHOP	cyclophosphamide, hydroxydaunorubicin, vincristine, prednisone	alkylating, DNA intercalation, microtubule inhibition, steroid antagonist	non-Hodgkin's lymphoma
FOLFOX	fluorouracil, leucovorin, oxaliplatin	pyrimidine analog, folic acid antagonist, DNA cross-linking	colorectal cancer
TIP	paclitaxel, ifosfamide, platinum agent cisplatin	microtubule antagonist, alkylating, DNA cross-linking	testicular cancer

More specific cancer drug development

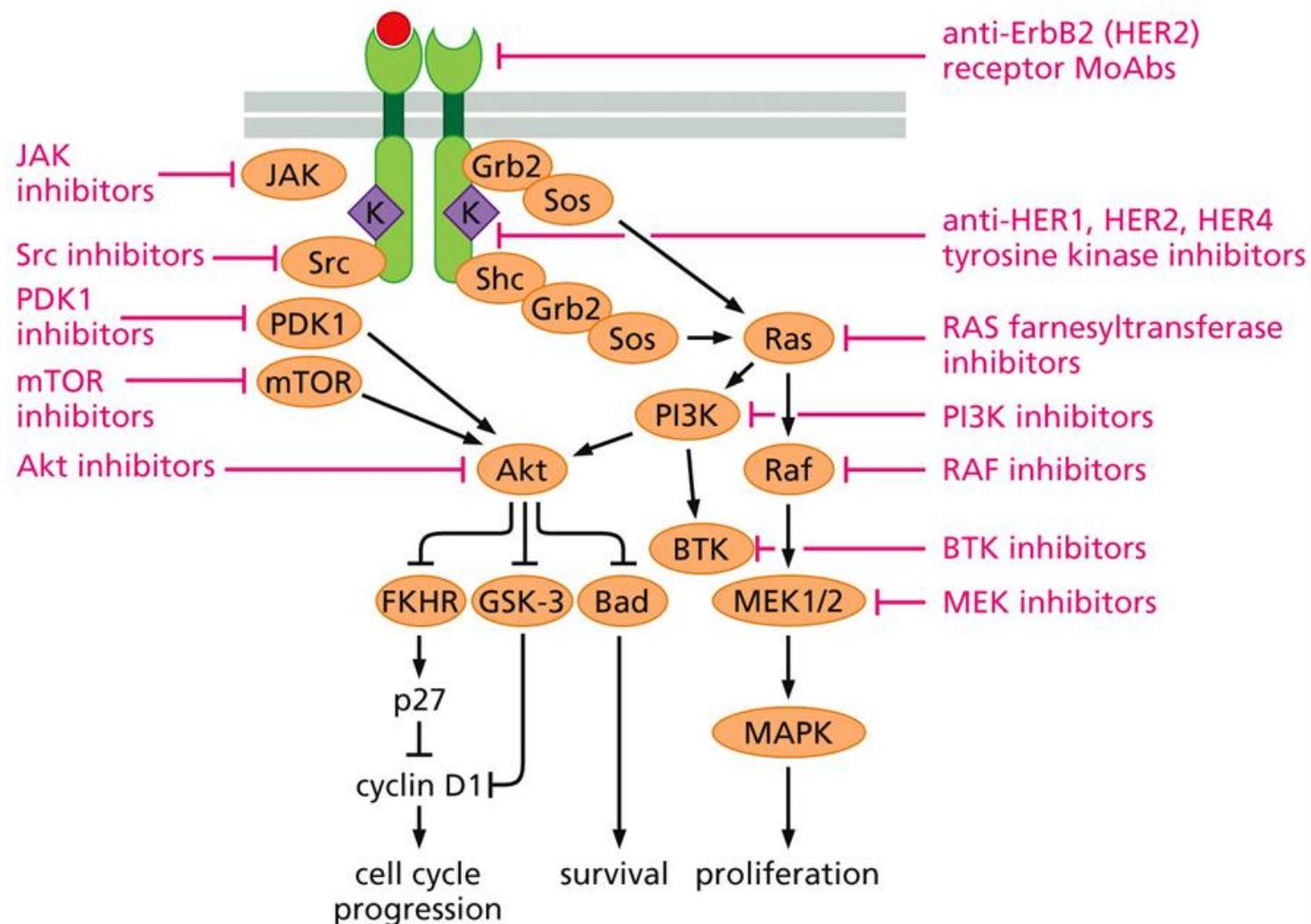


Figure 16.9 The Biology of Cancer (© Garland Science 2014)

The successful case of Gleevec

- Target BCR-Abl, which is the initiating oncogene to induce chronic myelogenous leukemia (CML), greatly increase the lifespan of this type of leukemia patients.

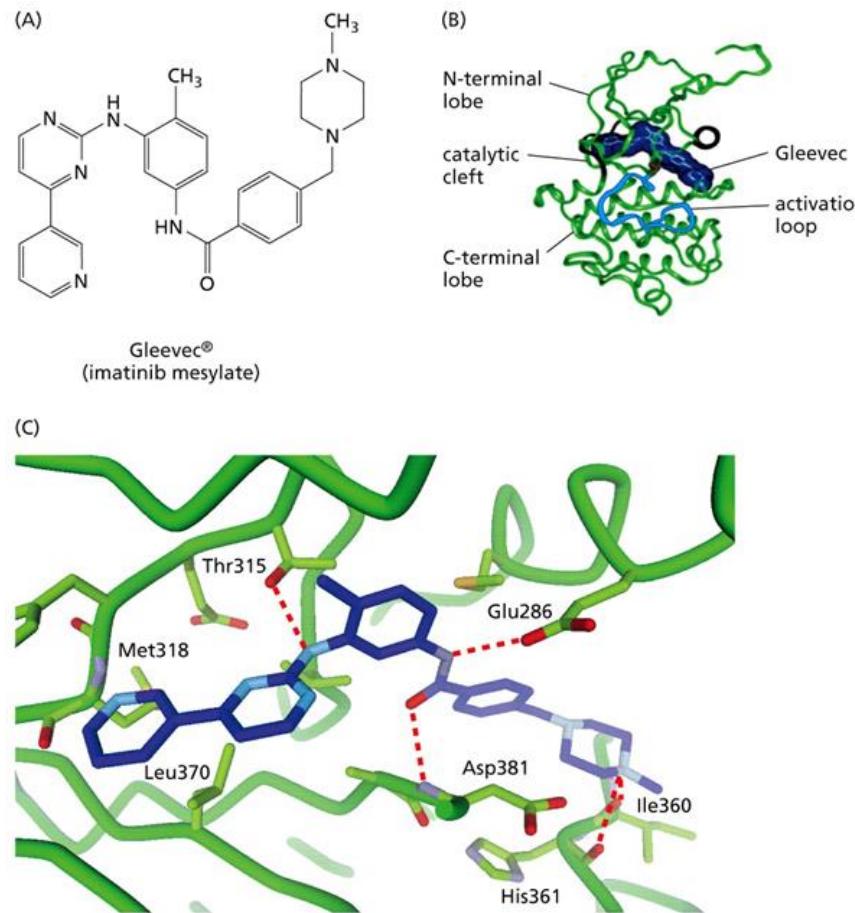


Figure 16.10 The Biology of Cancer (© Garland Science 2014)

- Gleevec more specifically targets BCR-Abl versus other kinases

All kinase folds are similar

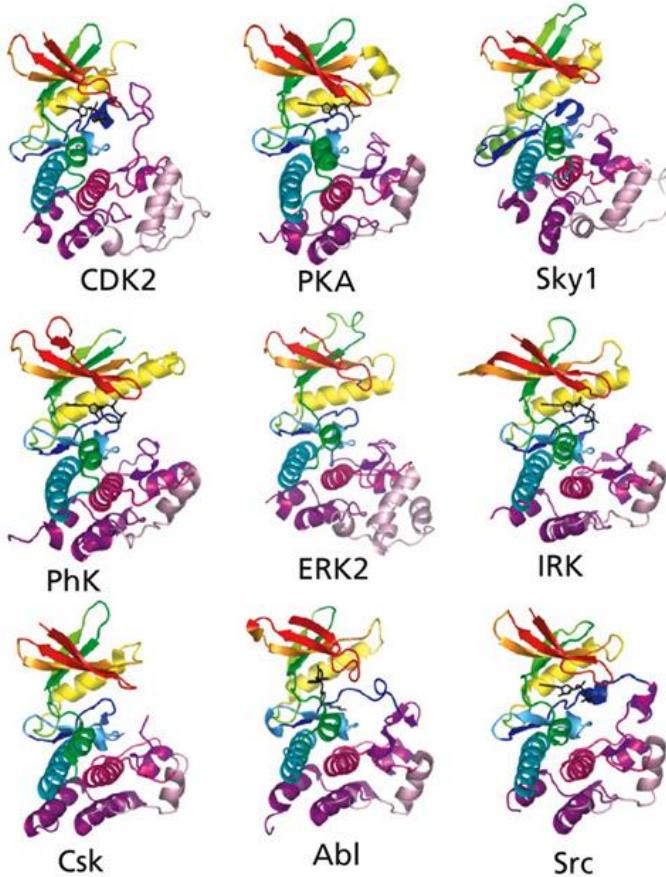


Figure 16.12a The Biology of Cancer (© Garland Science 2014)

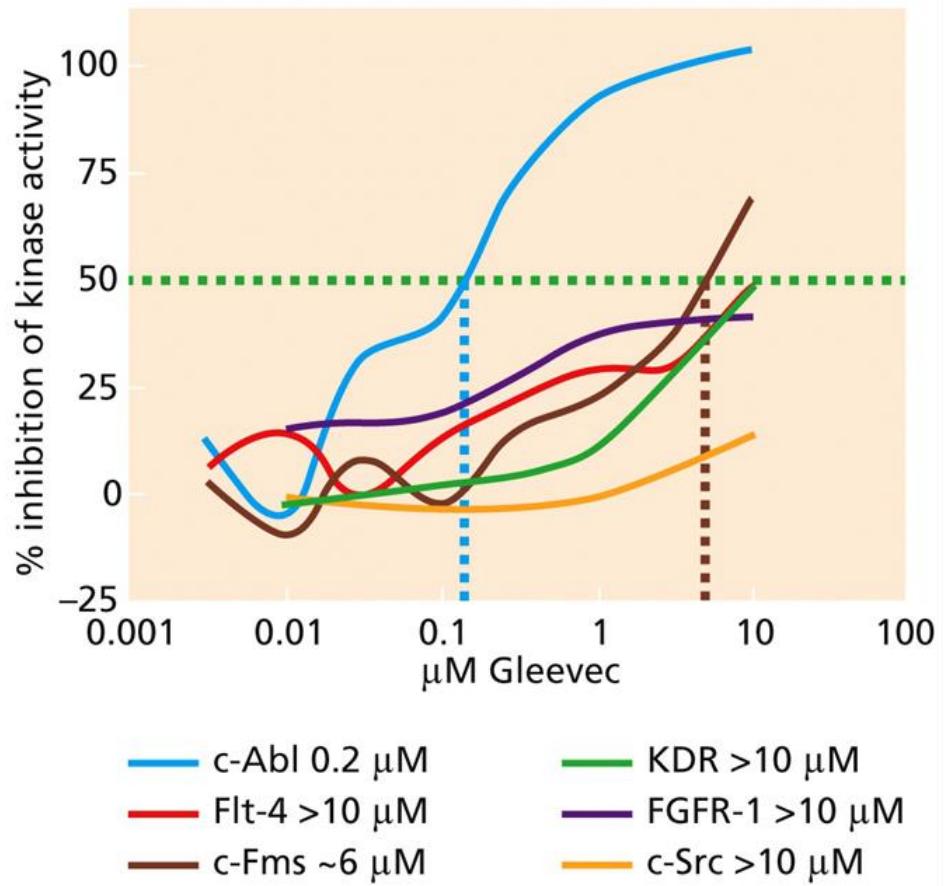


Figure 16.14 The Biology of Cancer (© Garland Science 2014)

Over the time, cancer develop resistance to cancer drugs...

Table 16.5 Mechanisms of acquired resistance to anti-cancer therapies^a

Nature of resistance	Mechanism of resistance
Multi-drug resistance ^b	increased expression of drug export pumps
Pan-drug resistance ^c	unknown
Drug detoxification ^d	enzymatic detoxification of drug molecule
Acquired drug resistance	<p>refuge of cancer cells in drug-protected anatomical sites^e</p> <p>failure of tissue to convert pro-drug into active form</p> <p>refuge of cancer cells in an anatomical site that provides protective trophic signals^f</p> <p>massive stromalization^g</p> <p>emergence of mutant, structurally altered cellular target^h</p> <p>amplification of gene encoding targeted protein</p> <p>emergence of cells bearing alterations in genes whose products are functionally redundant with drug targetⁱ</p> <p>loss of drug importer^j</p> <p>passage through an EMT^k</p> <p>activation of anti-apoptotic regulators</p>
Physiologic activation of compensatory adaptive mechanisms	
Resistance to EGF-R inhibition	<p>up-regulation of IGF-1R signaling</p> <p>amplification of <i>Met</i> gene</p> <p>mutational activation of a <i>ras</i> gene</p>
Resistance to Smoothened inhibition	amplification of <i>Gli2</i> gene
Resistance to Bcr-Abl inhibition	amplification of <i>Bcr-Abl</i> gene

^aThe entries in this table refer to tumors that are initially responsive to an applied therapy and then exhibit resistance that is manifested as regrowth of a tumor and thus indicates clinical relapse. Resistance may emerge because of the outgrowth of a therapy-resistant subpopulation of variant cells; such variant cells may preexist in the population prior to the onset of treatment or may arise as genetic or epigenetic variants that are formed *de novo* during the course of treatment. Alternatively, resistance may arise as a normal compensatory physiologic response to an initially applied therapy-imposed inhibition; this second form of resistance presumably occurs widely throughout a tumor rather than resulting from the selective outgrowth of a therapy-resistance subpopulation.

^bAs an example, concomitant resistance to paclitaxel, doxorubicin, etoposide, and vinblastine is exhibited by cells overexpressing P-glycoprotein, a drug export transporter operating in the plasma membrane.

^cPan-drug resistance refers to resistance against all agents that are applied to a tumor and cannot be attributed to increased drug export.

^dAs an example, lack of responsiveness of glioblastomas to the temozolomide alkylating drug is often due to expression of the MGMT enzyme, which detoxifies it (see Section 12.8).

^eAs an example, a variety of metastatic growths in the brain may be protected from chemotherapy by the blood-brain barrier, which blocks agents in the circulation from entering the brain parenchyma.

^fAs an example, lymphoma cells may survive in the thymus because thymic stromal cells release survival factors in response to the genotoxic stress provoked by chemotherapy.

^gAs an example, part of the difficulty of treating pancreatic carcinomas derives from the development in these tumors of a highly desmoplastic stroma that impedes transport of drugs from the circulation to the neoplastic cells.

^hAs an example, patients treated successfully with imatinib/Gleevec will develop drug resistance because of the emergence of cells expressing a mutant, structurally altered Bcr-Abl protein that no longer permits high-affinity binding of the drug.

ⁱAs examples, individuals whose tumors exhibit responsiveness to EGF-R inhibitors may develop resistance because of the mutational activation of a *ras* oncogene or because of *crl1* amplification. Resistance of CML cells to imatinib/Gleevec may develop because of the emergence of cells expressing altered p19^{ARF}, Myc, p53, or Ras, which function to bypass the dependence of the tumor cells on the targeted Bcr-Abl oncoprotein. Resistance to B-Raf inhibition can develop through up-regulation of PDGF-R β expression or N-ras mutation.

^jHigh-grade serous ovarian carcinomas are often treated with doxorubicin that is encapsulated in a synthetic liposome. Drug-resistant cells often emerge that have lost the LDL receptor-related protein (LRP1B), a cell surface protein that appears to be responsible for internalizing the liposomes.

^kPassage through an epithelial-mesenchymal transition (EMT) results in, among other changes, the expression of drug efflux pumps in the plasma membrane and increases in expression of anti-apoptotic proteins.

Cancer immunotherapy

Reactivation of immune system in cancer patients
to inhibit cancer development

Two examples:

1. CAR-T (chimeric antigen receptor- T cell)

2. Anti-PD1 antibody

Emily Whitehead

CD19-CAR-T therapy



03/2013, NEJM



Cancer Immunotherapy breakthrough

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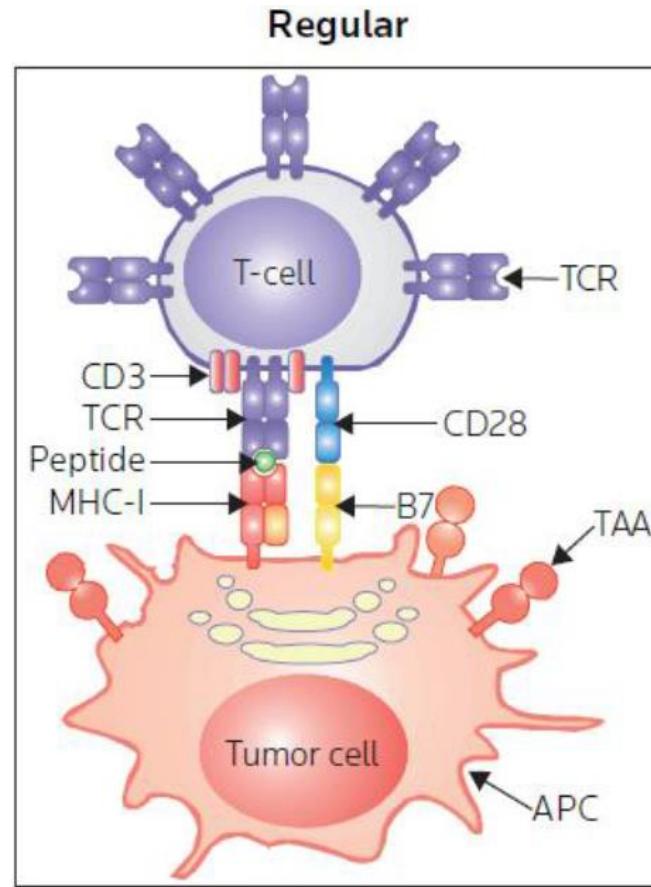
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Breakthrough of the Year 2013

CANCER IMMUNOTHERAPY

How T cells recognize antigen presenting cells (APC)



Elements required for specific T-cell response

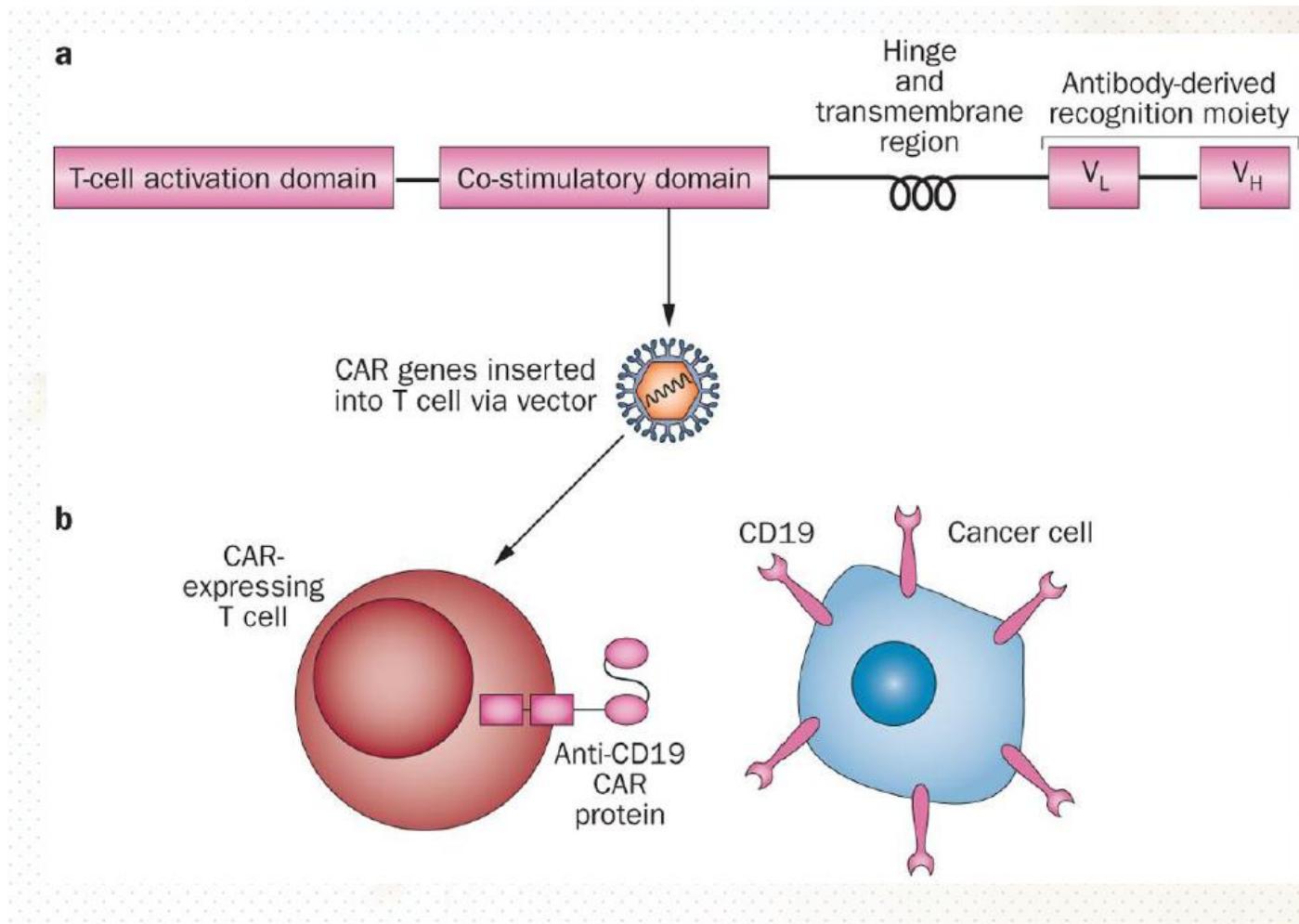
Specific T-cell receptor

Peptide antigen presentation and processing

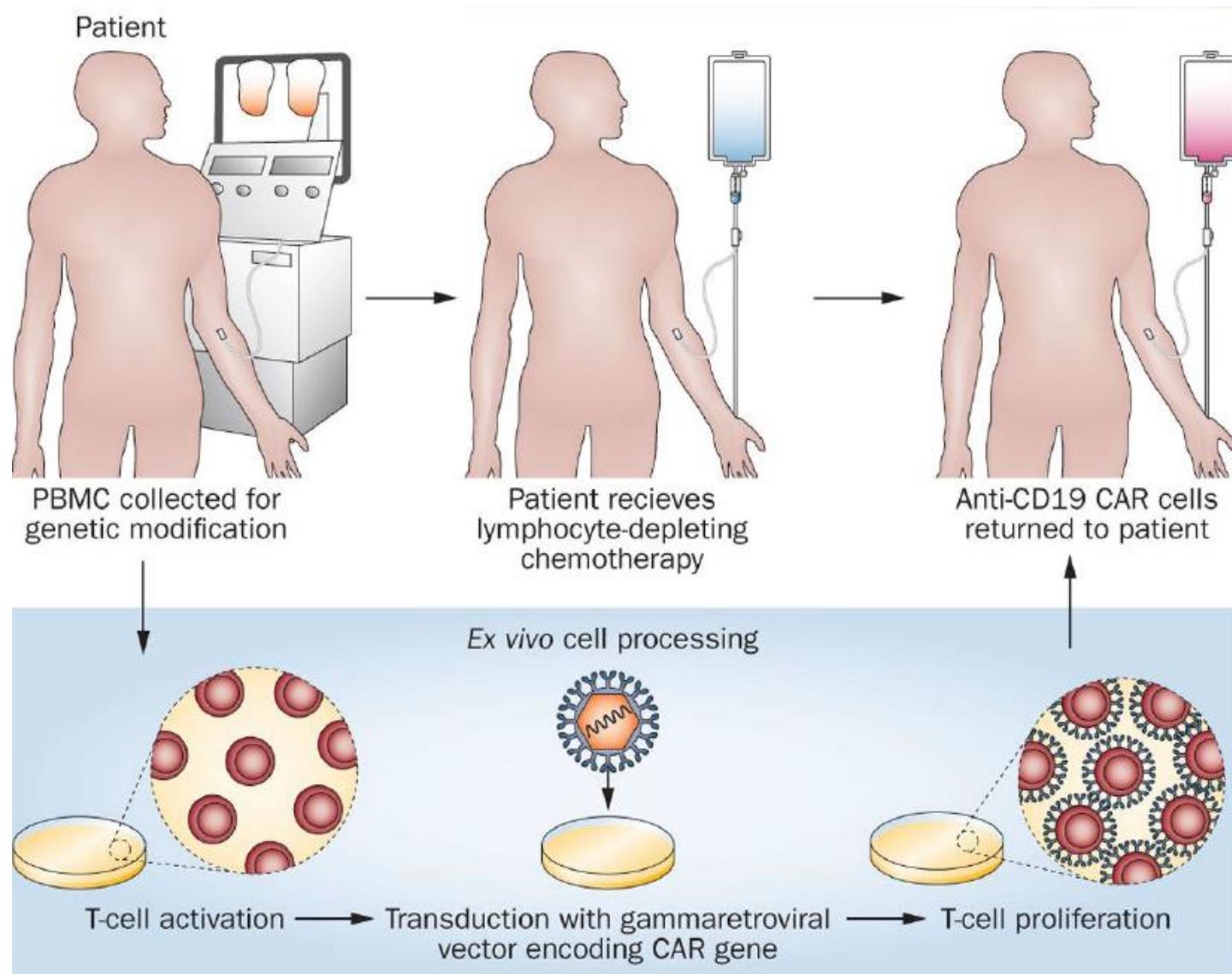
MHC-I/ β_2 -microglobulin

Costimulatory molecules

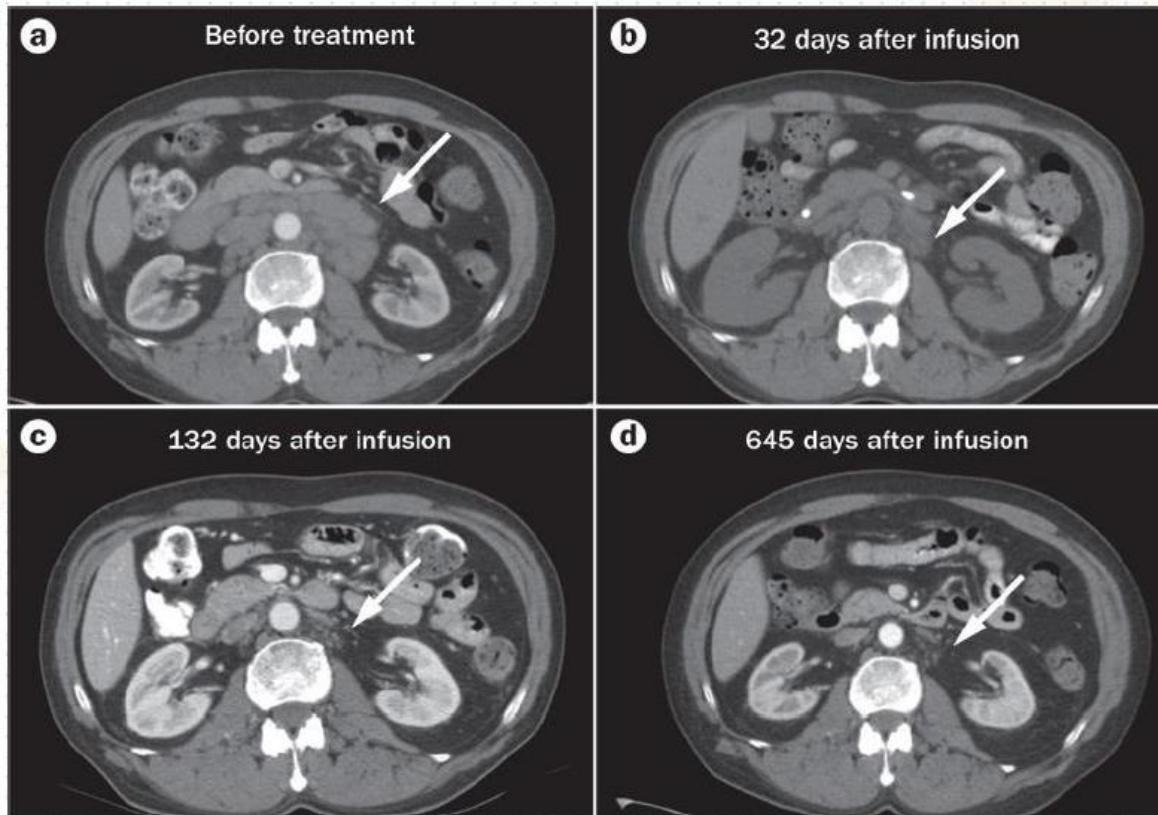
The structure of Chimeric antigen receptor expressed on T cells



The immunotherapy with CAR-T therapy

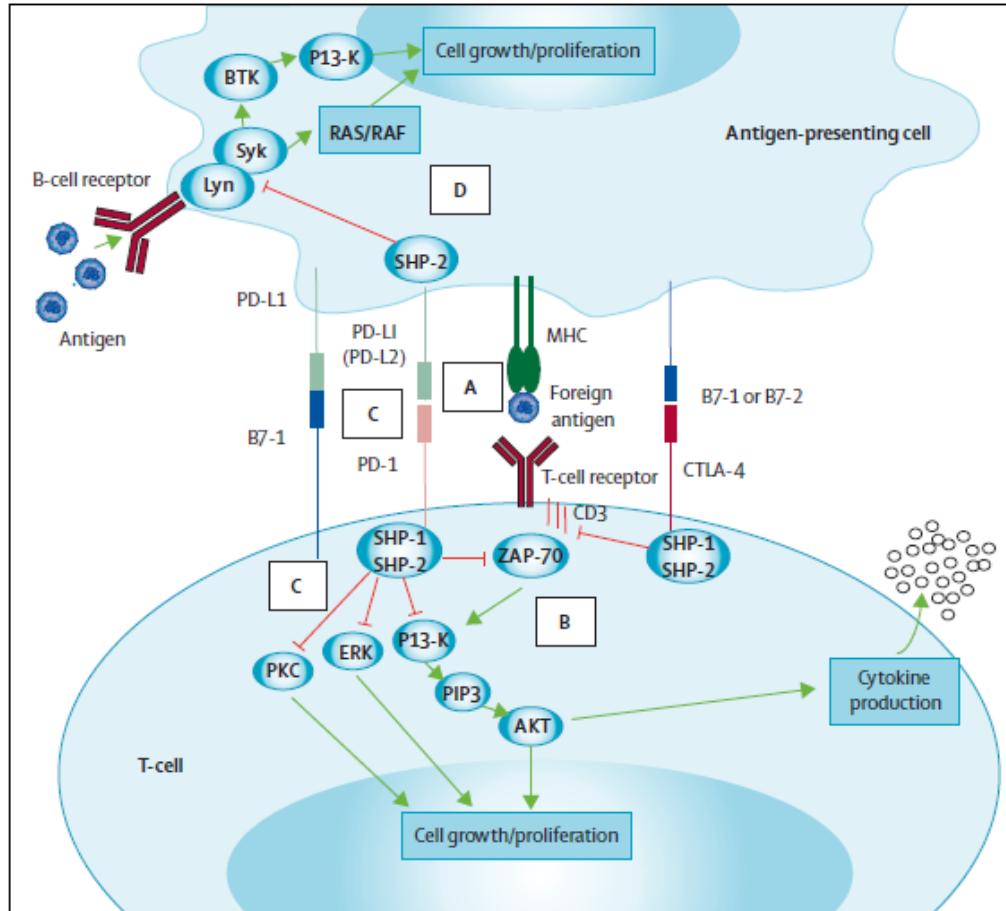


CAR-T immunotherapy is effective to reduce cancer growth



A dramatic decrease of tumor cells was observed in patients with lymphoma by CAR-T therapy

Anti-PD-1 as a immunotherapy for tumors



PD-1 (from programmed death 1) was found to be expressed in T cells. PD-1 binds to PD-L1 which is expressed in antigen presenting cells. The interaction between these two proteins serves as immune checkpoint to inhibit immune response. Due to PD-L1 and PD-1 recognition, tumor cancers failed to be detected by immunosurveillance program. Anti-PD-1 or anti-PD-L1 antibodies can block this recognition, reactivate the immune response and therefore cause the deletion of cancer cells. PD-1 immunotherapy has been widely used and is effective for ~30% of human cancers...