Molecular and cellular biology of cancer

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Bi9910en

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1. Introduction

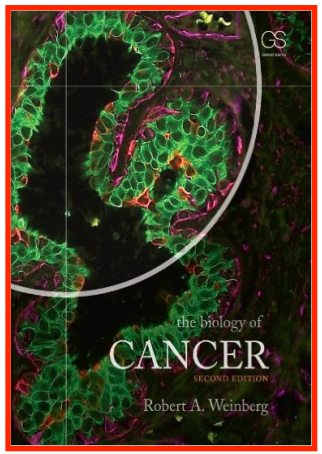
terminology, historical background, classification of tumors, oncoviruses

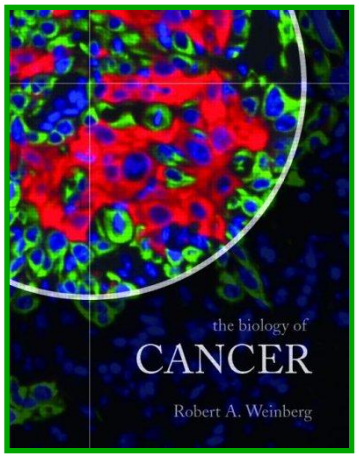
Course organization

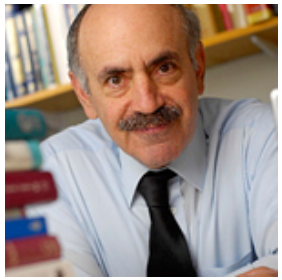
- Course ending: **EXAM**
  - **Written part**: obligatory; 25 open questions, max. 50 points, min. 30 points
  - **Oral part**

Supplemetary literature I

- Robert Allan Weinberg: **„The Biology of Cancer“** (*GS Garland Science*, **2014**; 2007)



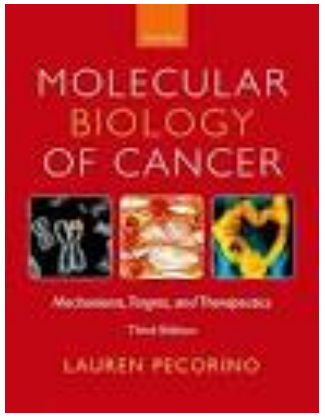


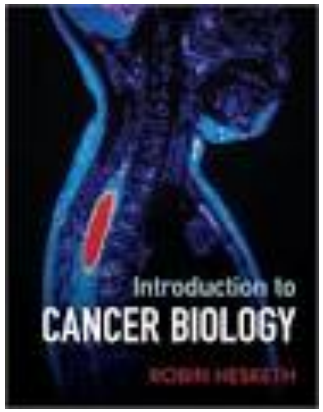


Additional textbooks of cancer biology

- Lauren Pecorino: **„Molecular Biology of Cancer. Mechanisms, Targets, and Therapeutics“** *Oxford University Press* 2012, ISBN 978-0-19-957717-0

- Robin Hesketh: **„Introduction to Cancer Biology“** *Cambridge University Press* 2013, ISBN 978-1-107-60148-2





Supplementary literature II

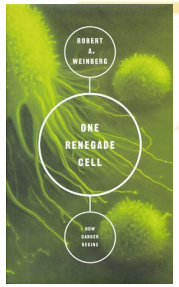
- Hanahan D. and Weinberg R.A.: **Hallmarks of Cancer**. *Cell* (**2000**) 100: 57-70.

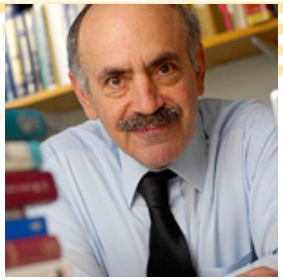
- Hanahan D. and Weinberg R.A.: **Hallmarks of Cancer: The Next Generation**. *Cell* (**2011**) 144: 646-674.

- Weinberg R.A.: **Coming full circle – from endless complexity to simplicity and back again.** *Cell* (**2014**) 157: 267-271.

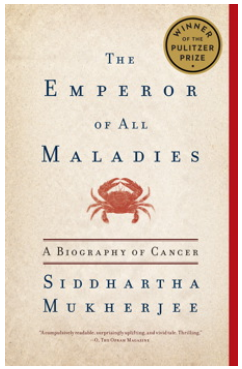
„Supplemetary literature III“

- Robert Allan Weinberg: **One renegade cell: How cancer begins**. *Science Master Series,* 1998





- Siddhartha Mukherjee: **The Emperor of all Maladies.** A Biography of Cancer. *Scribner* 2010

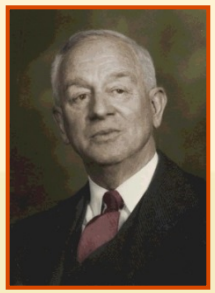


History: outline

- 400 BC **Hippocrates** desribed cancer as long finger-like extensions (resembling crab legs) protruding into healthy tissues:
  - Greek:
    karkinos (καρκίνος) = crab; onkos (ὄγκος) = mass, volume
  - Latin: cancer = crab

History: outline

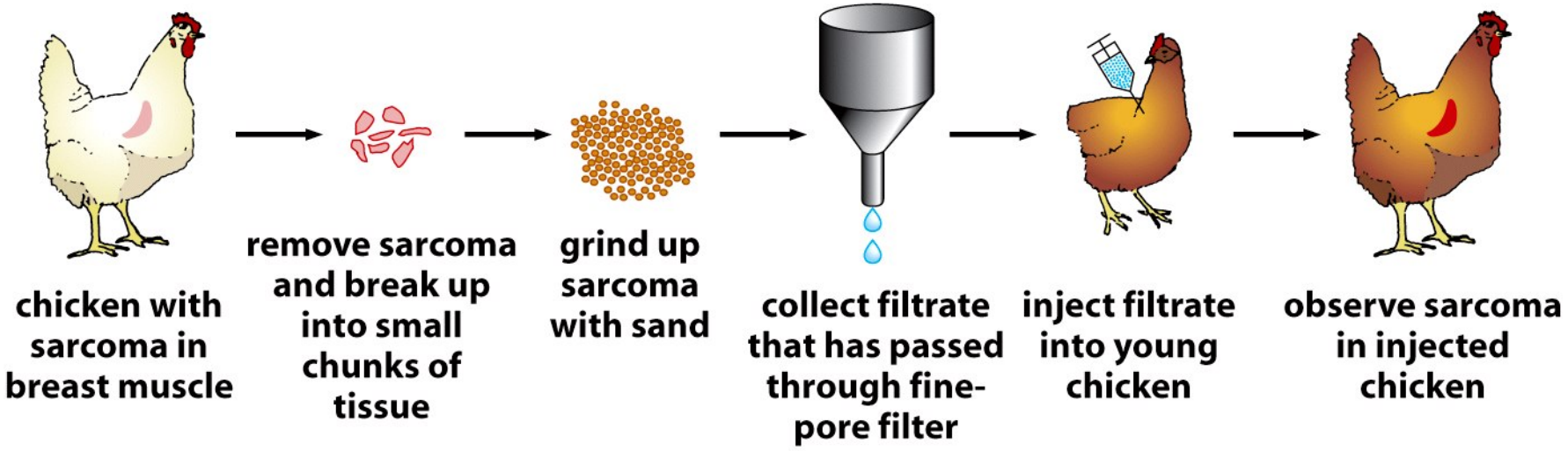
- Descriptive (epidemiologic) data:
  - 1775 −scrotal skin cancer in chimney sweeps (associated with exposure to soot and poor hygiene conditions)
  - 1842 − higher breast cancer mortality in nuns (association with nulliparity and absence breast feeding)
  - 1902 − radiation-induced skin cancer
    - carcinogen − **mutagen**
  - beginning of 20th century − familial aggregation of cancer



History: outline

- 1909 – **Francis Peyton Rous** − cancer transmitted by infectious substance in hen

  study of tumor-inducing viruses (**oncogene** - a fragment of viral genome causing a tumor) (1966 – Nobel prize; first nominated in 1926)



Weinberg RA. The Biology of Cancer. *Garland Science* 2007

History: a landmark in 1971

- 1971 – president Nixon announced „**War on Cancer**“, supported by a huge subventions by US goverment, prevailing conviction that cancers are caused by viruses (research focus on DNA-oncoviruses)
- 1976 – Bishop, Varmus – discovery of **c**-*src* (**protooncogenes**): linked with mitogenic signal pathway

  investigations of acutely transforming viruses lead to the identification of more than 30 different oncogenes
- Non-acute transforming viruses (insertion mutagenesis): 25 different proto-oncogenes discovered
- 1982 –identification of *ras* oncogene lead to the discovery that v-oncogene and protooncogene may differ in only one nucleotid!

History: outline

- Human cancers caused by retroviruses were not found
- (but later the knowledge of retroviruses enabled fast discovery of the cause of AIDS)

- 1969 - Henry Harris (cell fusion) − **tumor suppressors** – recessive genes- brakes)

- 1971 – Alfred **Knudson** – retinoblastoma – „two hits hypothesis“

- 1989 – Bert **Vogelstein** – distinct mutations in specific genes (oncogenes and tumor suppressors) are connected with discrete stages of the progression of colorectal carcinoma

- DNA tranfer (transformation, transfection)…

History: outline

- biochemistry: study of oncoproteins, their localizations, interactions
- molecular biology: isolation, characterization and targeted expression of eukaryotic genes
- cellular biology: molecular mechanisms regulating cell growth and division
- genetics of somatic cells and viruses: functional tests of specific genes
- …

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p21: **WAF1** - „*wild type p53-activated fragment*“
cip1 - „*Cdk-interacting protein 1*“
sdi1 - „*senescent cell-derived inhibitor 1*“

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- **epigenetics**

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gen: *CDKN1A* (6p21.2)

History: outline

- „**Omics**“ era: genomics, transcriptomics, proteomics, epigenomics, kinomics, metylomics, glycomics, metabolomics…

➢ …Robert Allan Weinberg: **Coming full circle – from endless complexity to simplicity and back again**. *Cell* (2014) 157: 267-271.

History: outline

- **1971**: president Nixon declared „War on Cancer"…
- **2012**: World Oncology Forum, Lugano, Switzerland

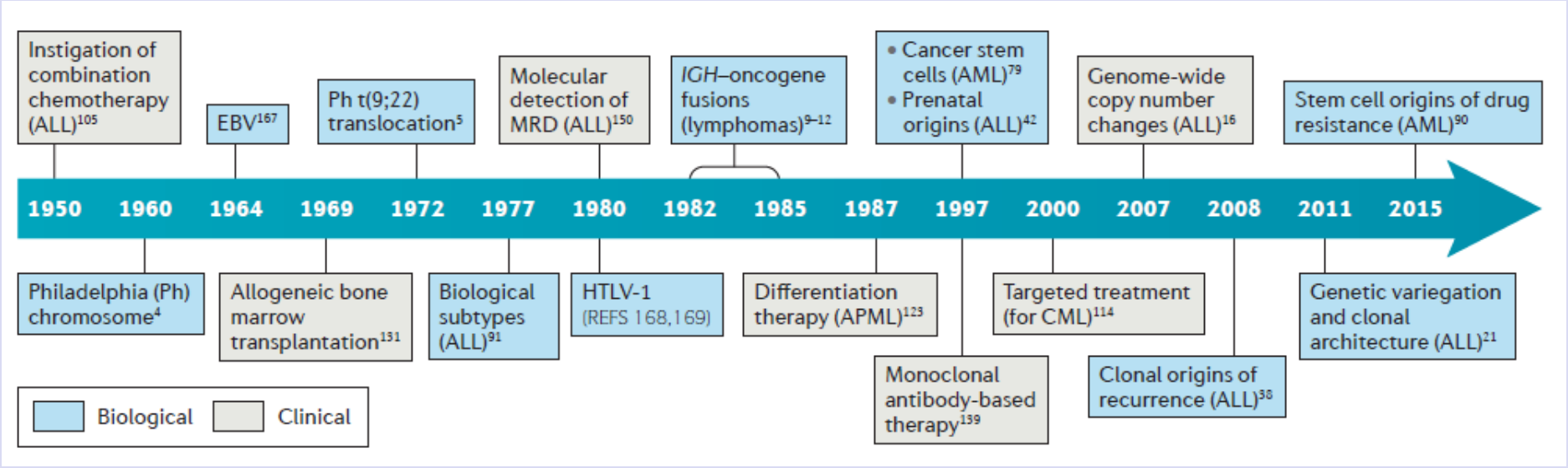
- Hanahan D. **The cancer wars 2. Rethinking the war on cancer**. *Lancet* 2014, 383: 558-563

- ➢ We won battles NOT war
- ➢ New strategy needed: „overarching and holistic battlespace war plan against cancer"

1. Attack all (more) hallmarks of cancer cells
2. Attack not only cancer cells, but also their „supporters"
3. Consider „geographic" aspect of the conflict (primary tumor vs metastases)

History: outline II

- Landmarks in leukemia research, that pave the way for biological and clinical discoveries in oncology



Greaves M. *Nat Rev Cancer*. 16: 163-172, **2016**

Terminology

Tumor, neoplasm

- new and abnormal tissue in multicellular organism that does not have a physiological function in this organism and its growth not controlled

- genetically conferred abnormal gain of tissue mass that has clonal character and its growth is not coordinated with the growth of the surounding tissues and with homeostasis

Classification of tumors I:

according to the capacity to invade surrounging tissues

- **benign**: stay in their primary location without invading other sites of the body. They do not spread to local structures or to distant parts of the body.
- **malignant (cancerous)**: invade surrounding tissues, spread to distant sites via the bloodstream or the lymphatic system, give rise to the secondary tumors (metastases) at other parts of the body

- According to the spatiotemporal occurence we distigush primary and secondary tumors

  (! *secondary* – (i) therapy-related; (ii) metastases

  lethal may be also non-metastatic tumor)

Terminology

Cancer

- a disease caused by an uncontrolled division of cells in a part of the body that spread to other parts of the body

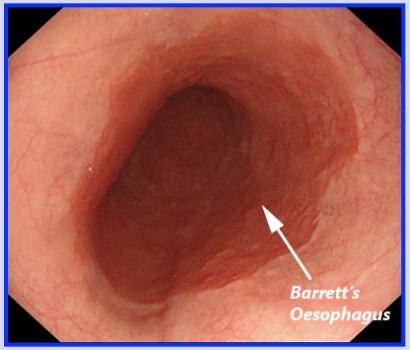
Normal → malignant tissue precancerous changes

- **hyperplasia** - increased cell production, but cells are phenotypically normal

- **dysplasia** – the presence of abnormal cells within a tissue or organ, cytological changes including cell size, shape, nucleo/ cytoplasmic ratio, altered mitotic activity; and abnormal frequency of cell types

- **metaplasia** – transient changes of cell phenotype caused by external signals; the replacement of a mature, differentiated cell type by another cell type that does not typically occur in the tissue in which it is found, often at boundary of two types of epithelial tissues: esophagus – stomach − e.g Barrett esophagus

Barrett esophagus



- The condition of the intestinal metaplasia in esophagus as a result of gastroesophageal reflux disease (GERD, stomach acid flowing backwards into the esophagus). The nonkeratinized squamous epithelium that typically covers the esophagus is not resistant to the acidic stomach fluids. It becomes inflammed and eventually if the irritation persists, squamous epithelium transforms into nonciliated columnar epithelial cells that is more acid-proof.
- Barrett esophagus can be reversed by treating the underlying GERD; however, if the condition persists, esophageal cells can become dysplastic, which can eventually lead to cancer.
- It belongs among „precancerous conditions“, it increases the risk of esophageal cancer 30x
- It could be manifested only by heartburn

Normal → malignant tissue

- **polyps**, **papilomas**, **warts** – structures visible by eye, contain same cell types as normal tissue, but exhibit expansive growth and create macroscopic mass, dysplastic, do not penetrate basement membrane, **benign**

- **invading tumors** – penetrate basement lamina of epithelial tissues, **malignant**

- **metastatic tumors** – also **malignant,** form secondary foci, not only invading but able to survive in circulation and adapt to the new conditions at the distant site

Classification of tumors II:

according to the cells/tissues they arise from

- **carcinomas** – tumors of epithelial tissues (around 80-90% human tumors)
- **sarcomas** – solid tumors of connective tissues − muscles, bones, cartilage
- **leukemias** a **lymphomas** − derived from haematopoietic and immune cells
- **neuroectodermal** tumors – derived from neural tissue
- **germinal** tumors – derived from totipotent germ cell
- **mixed** tumors

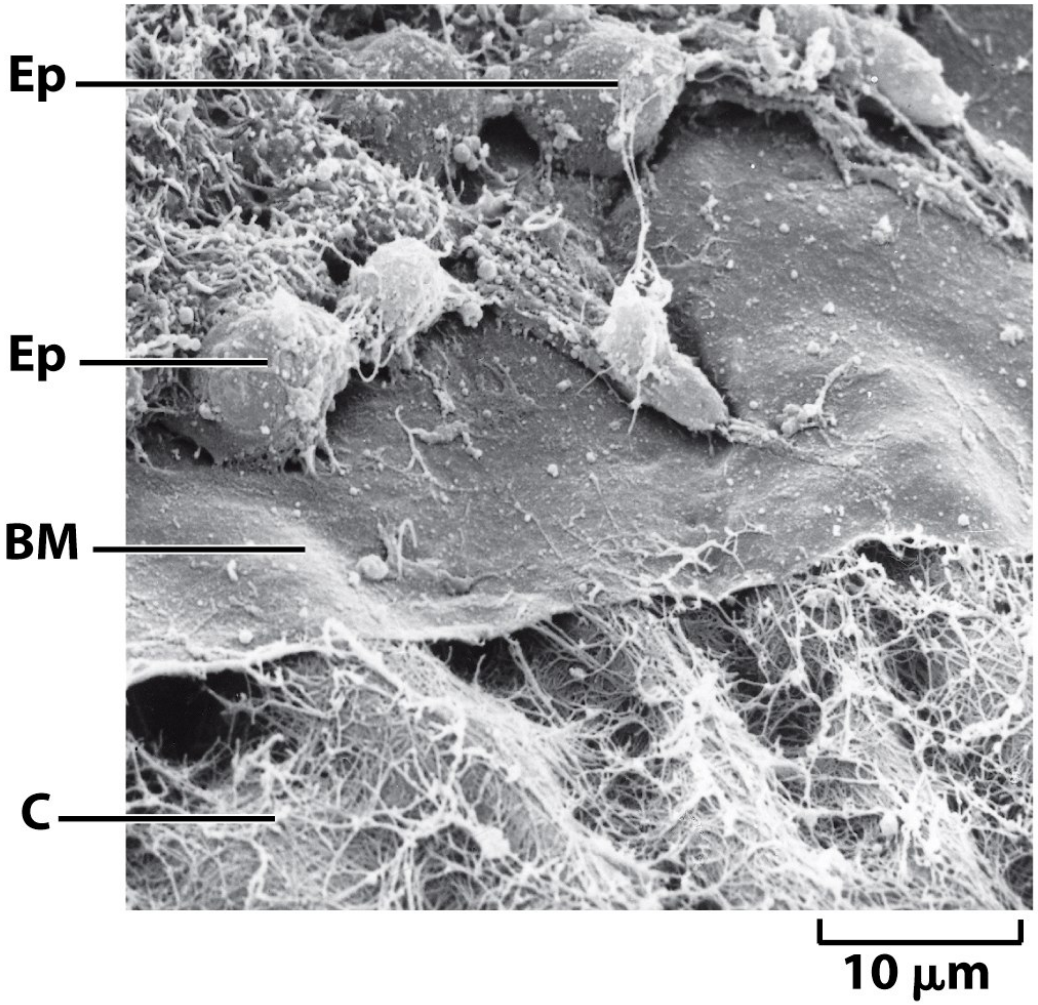
Classification of tumors II:

according to the cells/tissues they arise from

Solid tumors - create a single mass or many masses, that grow in organ systems and can occur anywhere in the body (carcinomas, sarcomas, neuroectoderm tumors, etc)

Liquid tumors (blood cancer) - circulate throughout the body via the bloodstream, develop in the blood, bone marrow, or lymph nodes (leukemias, lymphomas, myelomas)

Carcinomas



Derived from z **epithelial cells**:

Epithelial (**Ep**) layer form inner lining of body cavities and outer surface of body. The basement membrane (BM) sits between epithelium and the underlying connective tissue that containes stromal cells and collagen fibres (C).

Weinberg RA: The Biology of Cancer. *GS*, 2007

Carcinomas

- **80-90 %** of tumors

- **Spinocellular / squamous cell carcinomas** – originate in cells that form protective layers (the surface of the skin, the lining of the hollow organs of the body, and the lining of the respiratory and digestive tracts)

- **adenocarcinomas** – derived from glandular epithelium with exocrine function which lines certain internal organs and makes and releases substances in the body, such as mucus and other fluids

- **mixed** – coexistence of both types

Sarcomas

- **1%** of tumors

- Tumors of connective tissues arising from mesenchymal or ectodermal tissues:

- fibrosarcomas − derived from fibroblasts, cells producing collagen and extracellular matrix
- liposarcomas − from adipocytes, cells that store lipids in the cytoplasm
- osteosarcomas − from osteoblasts, cells that produce bone matrix
- leiomyosarcomas a rhabdomyosarcomas - from myocytes (muscle cells)
- angiosarcomas – from precursors of endothelial cells

Leukemias a lymphomas

- Tumors derived from haematopoetic and immune cells

- leukemias – from circulating or bone marrow cells

- lymphomas – from B and T lymphocytes, form clusters (solid tumors), often in lymph nodes

Neuroectodermal tumors

- Tumors derived from various cells of central and periferal nervous system

- gliomas
- glioblastomas
- neuroblastomas
- schwannomas
- meduloblastomas

- **Around 1.3%** of diagnosed tumors, but represent cca 2.5% of cancer-associated deaths

Other tumors

- some tumors do not belong to any of the classes:

- melanomas − derived from melanocytes, cells producing melanin (pigment), neural crest-derived cells located in the bottom layer of the skin's epidermis
- small cell lung carcinomas– lung cells that display neuroendocrine markers

- **transdifferentiation** – switch/transformation of the cell type, acqusition of new differentiation markers
- **dedifferentiation** – not possible to track the type of the tissue that gave rise to the tumor: anaplastic tumors (**1 to 2 %** of tumors)

Classification of tumors III:

according to the location in the body where the cancer first developed.

- **lung carcinoma**
- **colorectal carcinomas**
- **breast carcinoma**

- **acute myeloid leukemia**
- **acute lymphocytic leukemia**
- **etc.**

Intermezzo: Classification of solid tumors in clinical praxis

For every oncological patient pathologist asses:

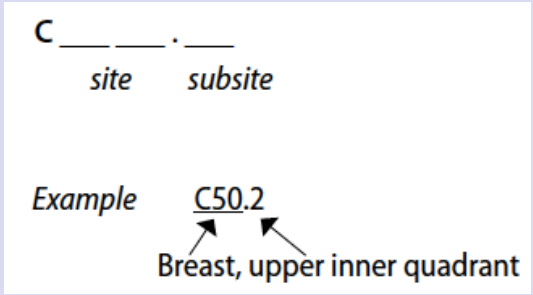
- typing/grading
- staging
- rating

„Typing“

The International Classification of Diseases for Oncology (ICD-O-3) is designed to categorize tumors:

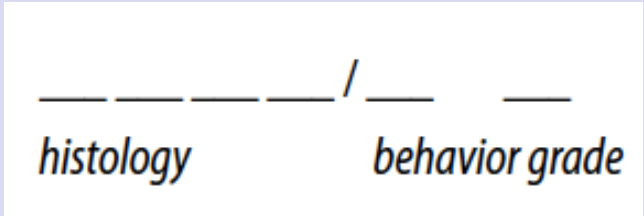
Unique numerical code used for coding the site (topography) and the histology (morphology) of the neoplasm

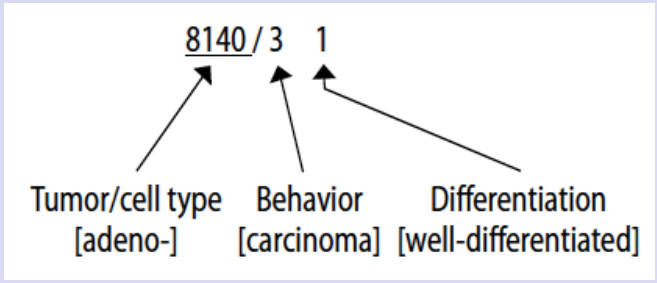
Structure of topography (site) code:



Structure of morphology code:

- 4 digits cell type (histology)
- 1 digit behavior
- 1 digit grade, differentiation or phenotype





„Typing“

The International Classification of Diseases for Oncology (ICD-O-3) is designed to categorize tumors:

Morphology code:

First number after slash denotes biological properties (**behaviour**):

- **0**: benign
- **1**: uncertain behaviour
- **2**: carcinoma *in situ*
- **3**: malignant, primary site
- **6**: malignant, metastasis
- **9**: malignant, uncertain whether primary or metastasis

Example: code 8850/0 stands for lipoma

code 8850/3 stands for liposarcoma

„Typing“

Morphology code:

Second number after slash denotes differentiation (**grading**) − only for malignant tumors!

Differentiation describes how much a tumor resembles the normal tissue from which it arose.

1	Grade I	Well differentiated
		Differentiated, NOS
2	Grade II	Moderately differentiated
		Moderately well differentiated
		Intermediate differentiation
3	Grade III	Poorly differentiated
4	Grade IV	Undifferentiated
		Anaplastic
9	Grade or differentiation not determined, not stated or not applicable	

Example of complete code:

Diagnostic term:	
Poorly differentiated squamous cell carcinoma, upper lobe of lung	
C34.1	**8070/33**

„Staging“

Refers to the anatomical extent of the cancer:

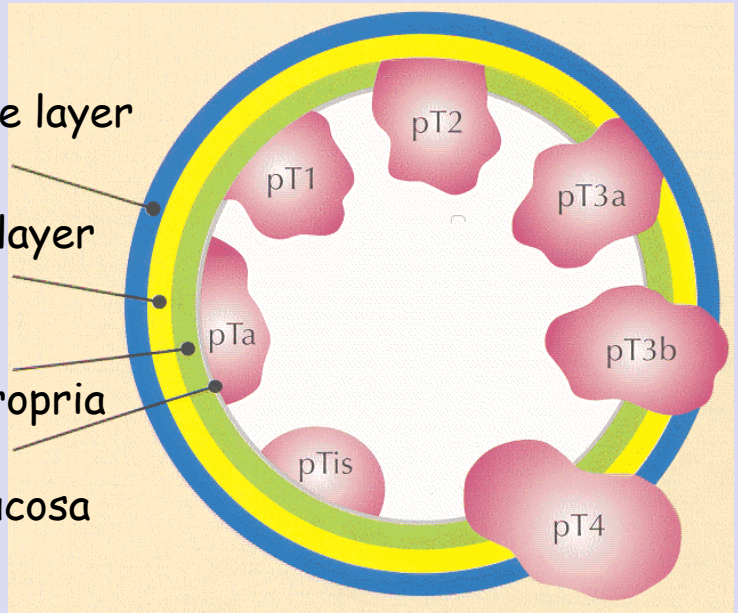
- the size of the tumor
- whether the tumor crossed specific anatomical barriers
- whether the cancer has spread to nearby lymph nodes
- whether the cancer has spread to a different part of the body

TNM staging system:

“T” category describes the primary tumour site and size:

Tx: tumor cannot be assessed
Tis: carcinoma in situ
T0: no evidence of tumor
T1, T2, T3, T4: size and/or extension of the primary tumor

example: bladder carcinoma



„Staging“

TNM staging system:

…

“N” category describes the regional lymph node involvement
Nx: lymph nodes cannot be assessed
N0: no regional lymph nodes metastasis
N1, N2, N3: regional lymph node metastasis present

“M” category describes the presence or otherwise of distant metastatic spread
M0: no distant metastasis
M1: metastasis to distant organs (beyond regional lymph nodes)

Example: T3N1M0

„Rating“

Molecular classification − presence/absence of specific molecules

- Some of them are **diagnostic** (e.g. specific chromosomal translocation in lymphomas and some sarcomas)
- Some are **prognostic** and determine the choice of therapy (e.g. −N-*MYC* amplification in neuroblastoma, hormone receptors *(ER, PrR* in breast carcinoma).
- Others are targeted by specific anti-cancer drugs thereby **predict** response to the therapy (např. *HER2/Neu* for Herceptin, Rituximab, Glivec,..).

Cancerogenesis

- Process of the development and progression of tumor

- multistep

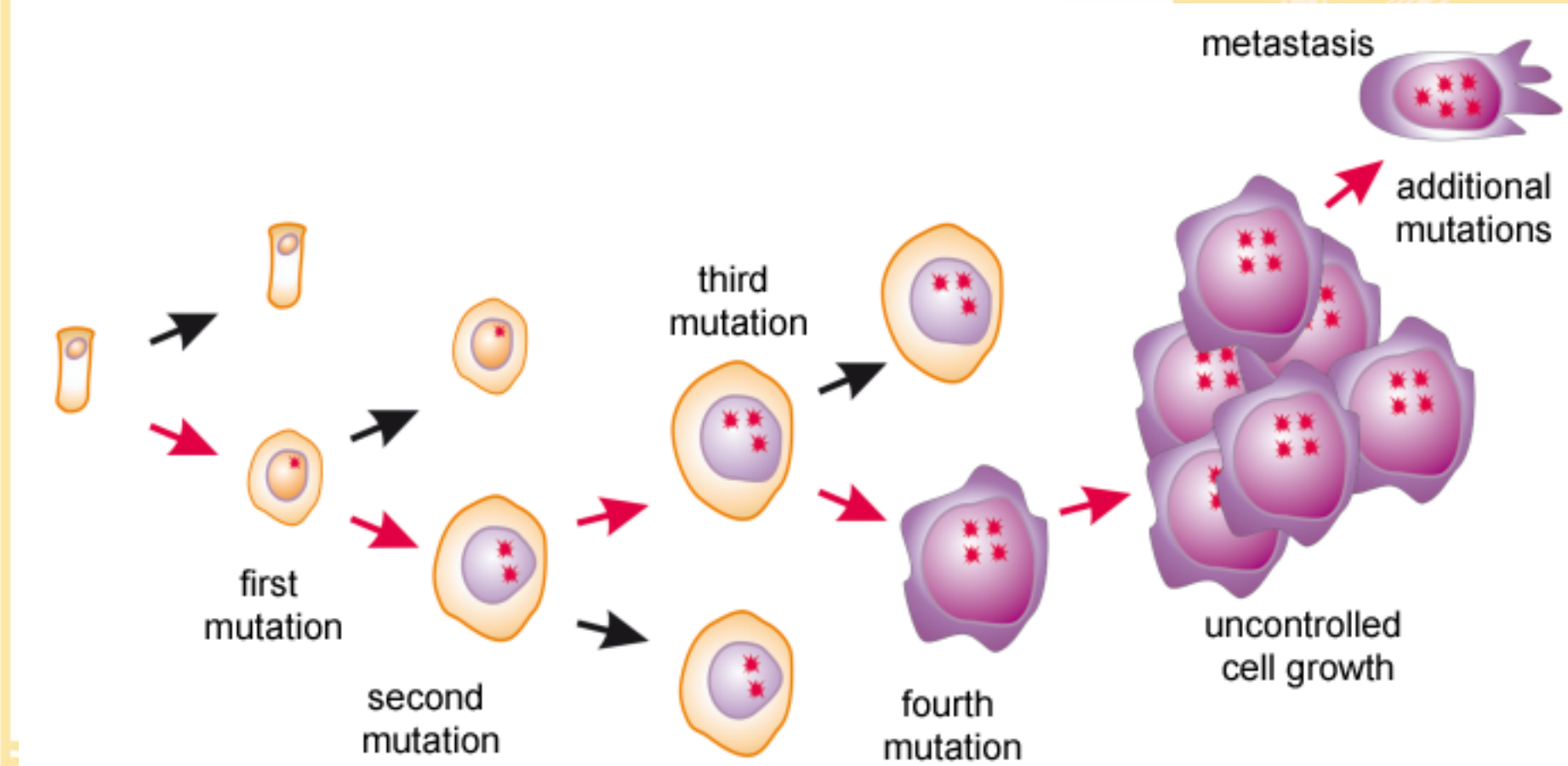
- Based on gradual accumulation of genetic and epigenetic changes

alias: tumorigenesis, carcinogenesis

(Neoplastic) transformation

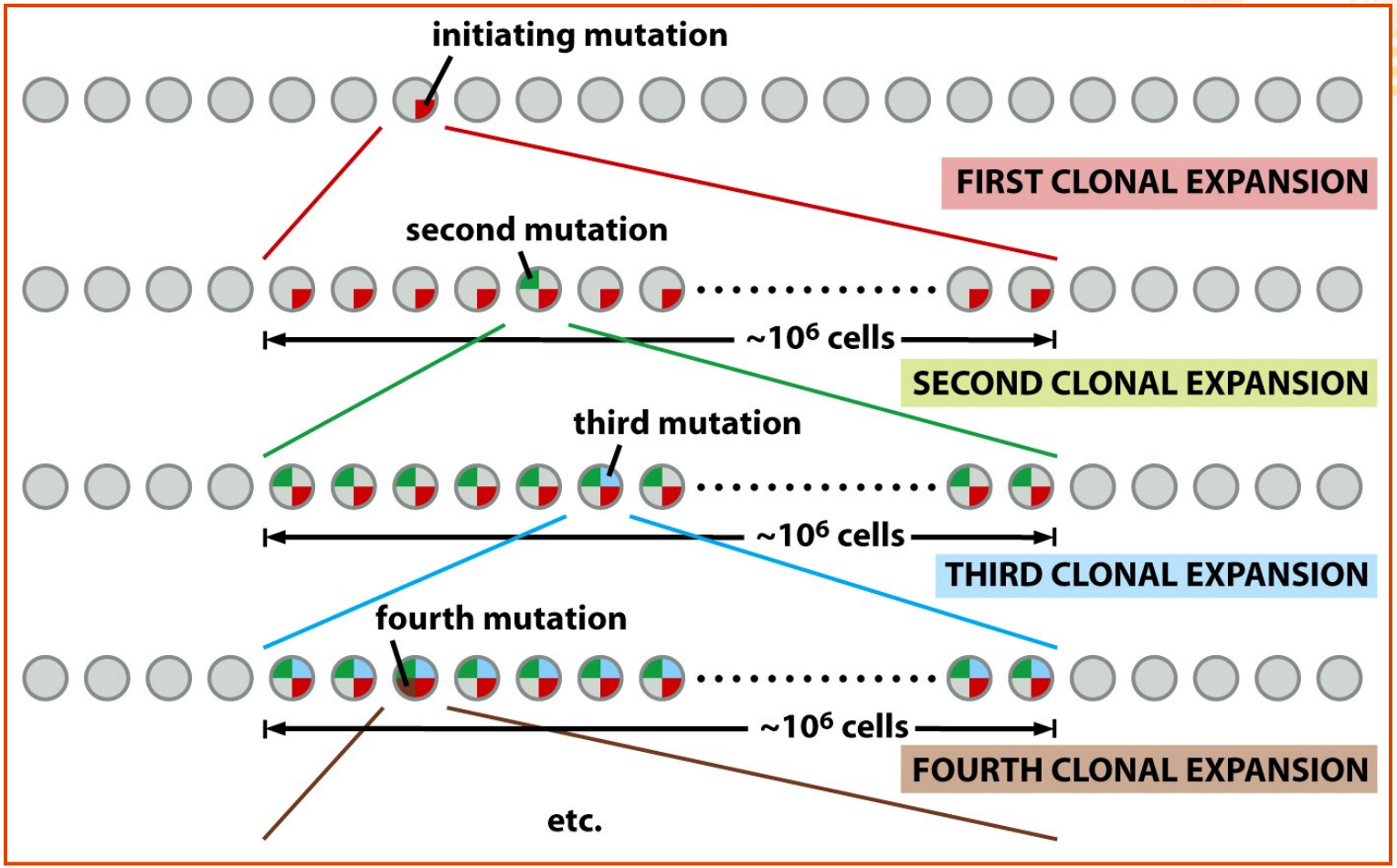
- Transformation of somatic cell into a cancer cell

Cancerogenesis is driven by gradual accumulation of (epi)genetic changes



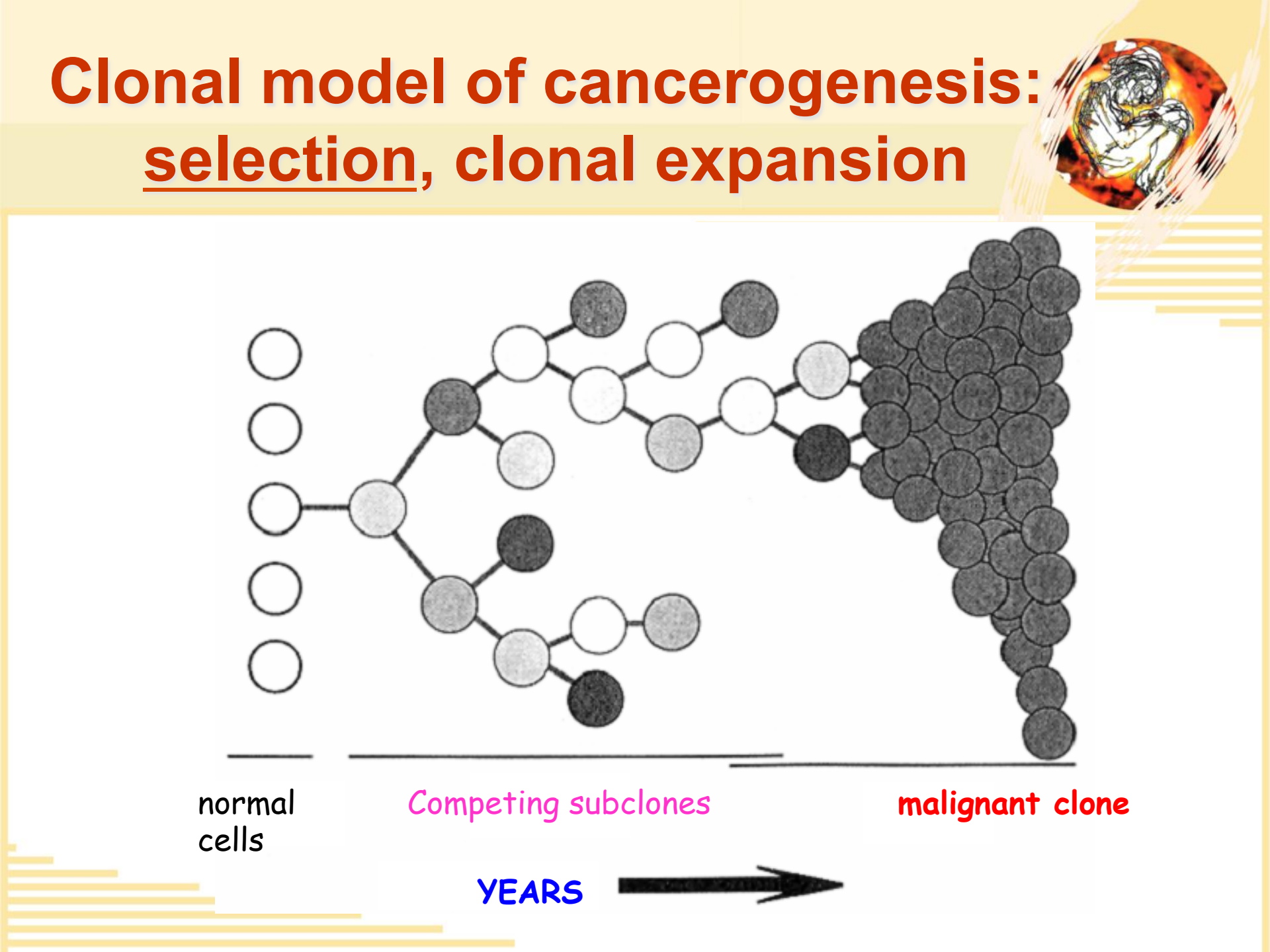
Koptíková Jana, 2016

Multistep cancerogenesis accompanied by sequence of clonal expansions

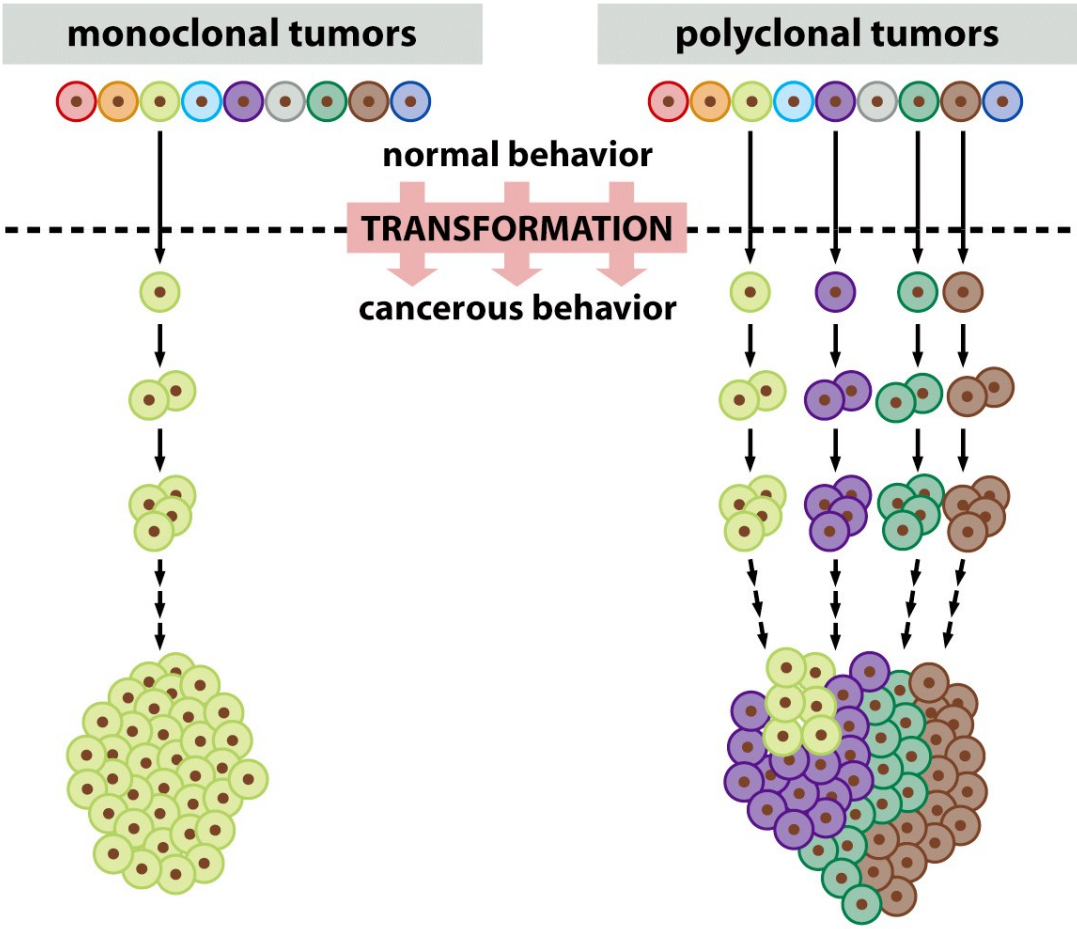


Weinberg RA. The Biology of Cancer. *Garland Science* 2007

Clonal model of cancerogenesis: selection, clonal expansion

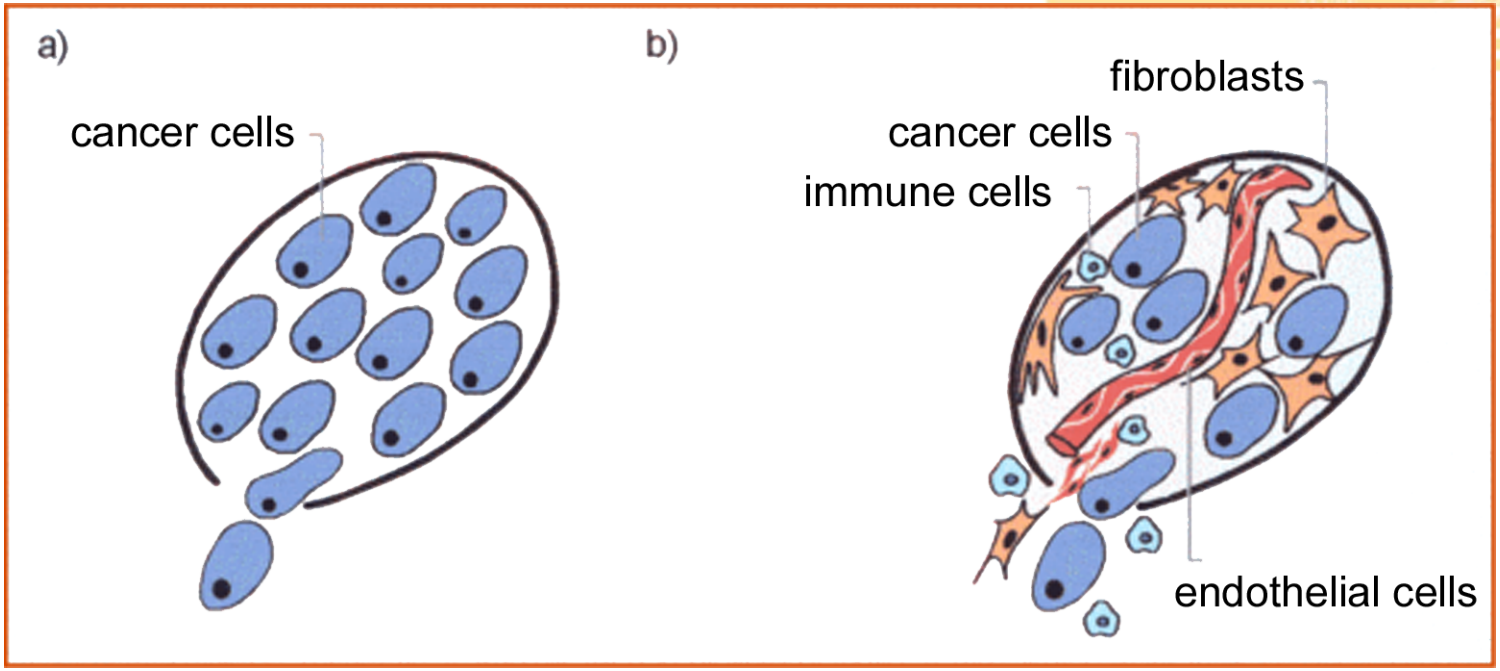


Are tumor monoclonal or polyclonal?



Weinberg RA. The Biology of Cancer. *Garland Science* 2007

Tumor is complex tissue



Tumor contains not only transformed cancer cells (a), but also other host cells that together with extracellular components form tumor microenvironment (b).

cancerogenesis vs. **neoplastic transformation**

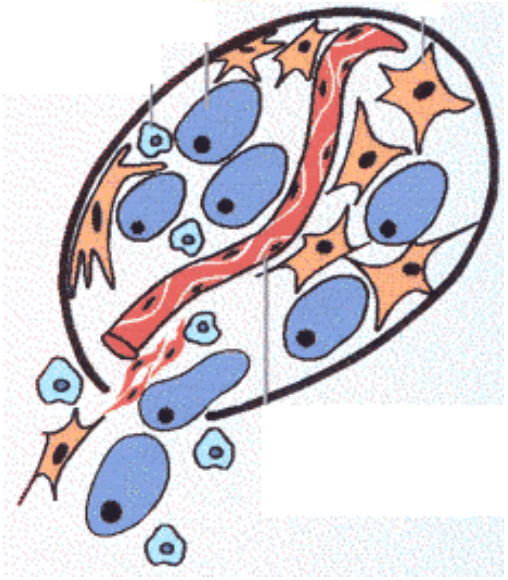
Cellular composition of solid tumor

1. Tumor parenchyma

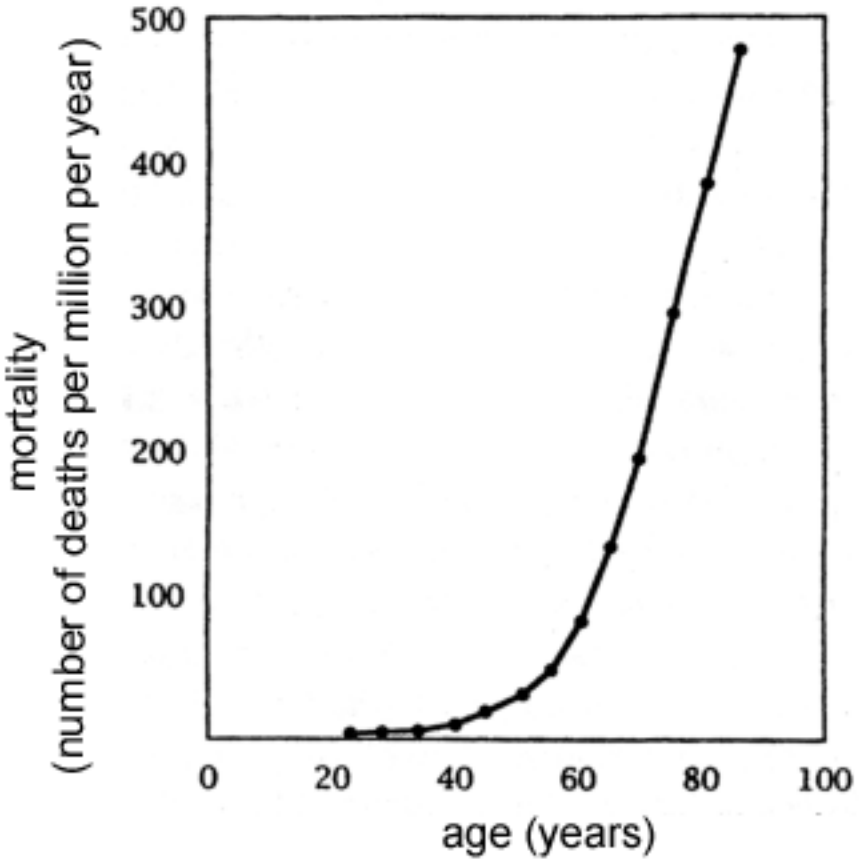
transformed malignant cells

2. Tumor stroma

- resident and non-resident cell types: endothelial cells, pericytes, fibroblasts, lymphocytes, macrophages
- for mechanical and nutritional support
- Transport function (signal tranduction, chemoattactants, storage of growth factors)
- crucial for angiongenesis and metastasis



How many and what genes are altered during cancerogenesis?



- If one alteration was enough for tumor development, probability of the disease would be age-independent − there would be linear relationship
- But the disease risk markedly increases with age. Curve of the relationship reflects a complex process when several independent events occur sequentially.

Weinberg A.R.: One renegade cell.

Carl O. Nordling

- 1953: it was first speculated that neoplastic transformation does not occur in a single step
- An architect Carl O. Nordling studied deaths frekvencies for cancer patients of different age (from 25 to 74 years) and found the death rate increased proportionally with the sixth power of the age.
- he deduced that a cancer cell was the end-result of at least six successive mutations

Nordling CO. A new theory on cancer–inducing mechanism. *Br.J.Cancer* 7: 93-112, 1953

Carl O. Nordling

A NEW THEORY ON THE CANCER-INDUCING MECHANISM.

C. O. NORDLING.

Received for publication December 29, 1952.

RECENT research in genetics and pathology has shown an amazing consistency between the agents causing mutations and those causing—or contributing to the development of—cancer. One of the more prominent theories, which since the 1920's has been advocated by Bauer (1949), Strong (1949) and others, claims that the original cancerous cell is nothing but an ordinary cell affected by genetic mutation of some kind. One of the main objections to this theory has been that it does not explain the age variation of the cancer frequency. In reply, Bauer

Nordling CO. A new theory on cancer–inducing mechanism. *Br.J.Cancer* 7: 93-112, 1953

Carl O. Nordling

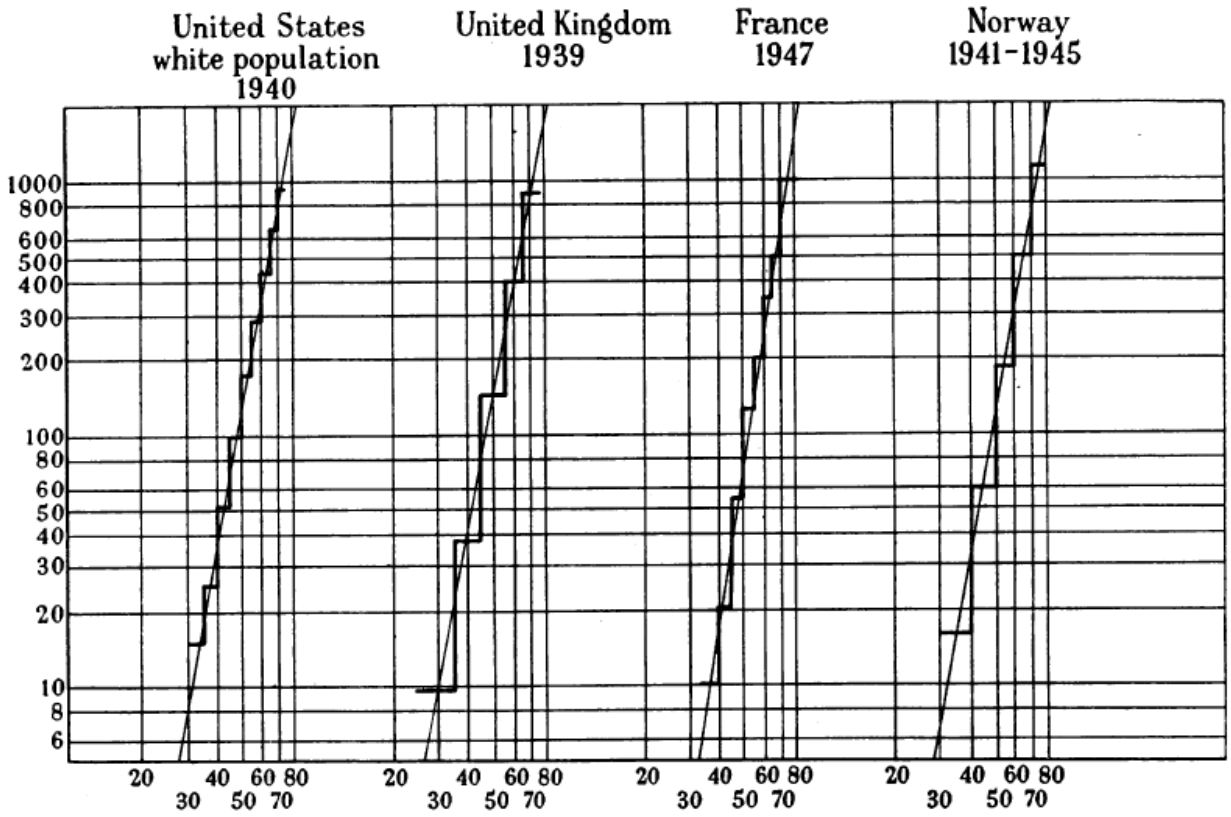


FIG. 1.—Diagram drawn to double logarithmic (log/log) scale showing the cancer death-rate (in the case of the United Kingdom, the carcinoma death-rate) in males at different ages. Deaths per 100,000 males are shown on the vertical scale, age figures on the horizontal scale.

Nordling CO. A new theory on cancer–inducing mechanism. *Br.J.Cancer* 7: 93-112, 1953

Carl O. Nordling

SUMMARY.

The theory is put forward that the cancerous cell contains not one but a number of mutated genes. The occurrence of such accumulations of mutations may be expected to increase according to a certain exponent of age, as well as according to the increase of cell proliferation. Cancer statistics also show that the frequency of carcinoma increases according to the sixth exponent of age in males.

The unexpectedly high incidence of internal neoplasms in childhood is explained by the high frequency of cell division in the foetal stage. Other high cancer incidence rates in particular organs may be explained on the basis of exposure of the tissues either to mutagens or to agents increasing cell proliferation.

Nordling CO. A new theory on cancer–inducing mechanism. *Br.J.Cancer* 7: 93-112, 1953

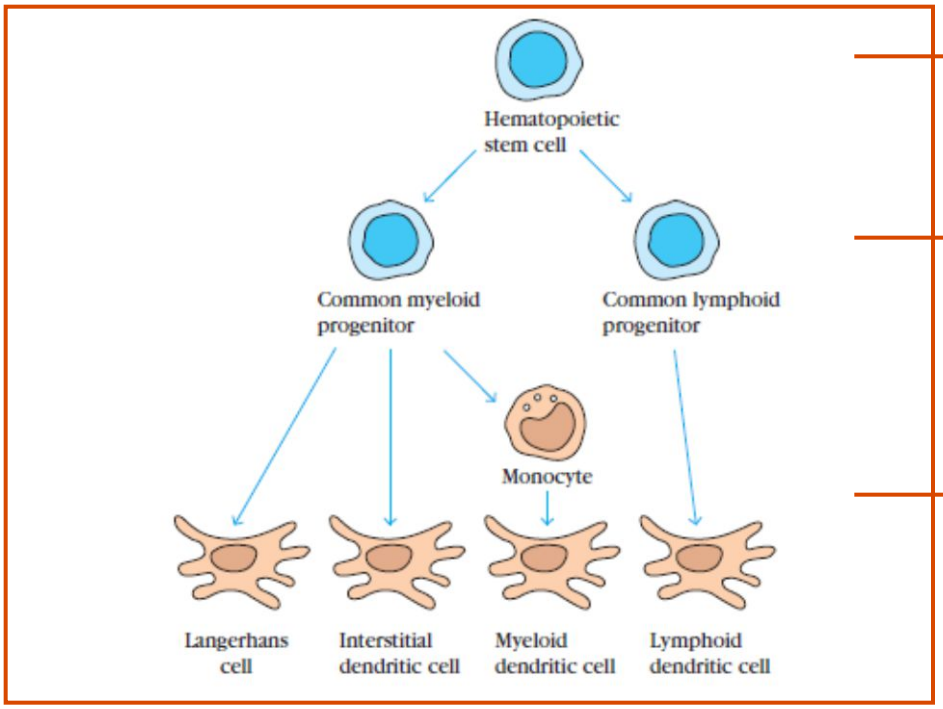
First „renegade“ cell

- What is this cell??

GIT vs. **GIST**
vs. **lymfomas**…

First „renegade“ cell

- What is this cell??



→ Stem cell??

→ Progenitor cell??

→ Differentiated cell??

Stem cells

- Self-renewal capacity and production of cells undergoing differentiation
- Relatively rare
- Quiescent
- Resistant to toxins and chemicals
- Intense DNA repair

Cancer stem cells

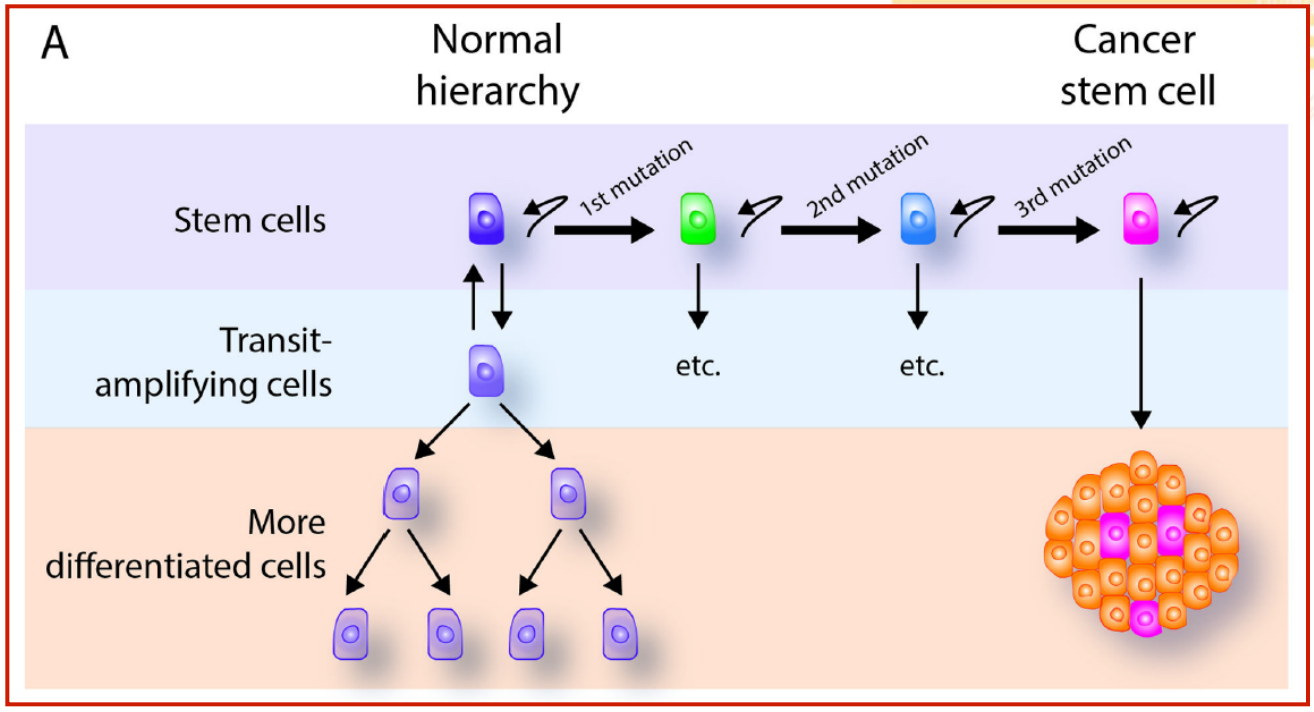
- Both normal and tumor tissues are hiearchically organized; include subpopulation of stem cells

- **Cancer stem cells** (CSCs):
  - Have potential to form new heterogeneic tumor, when implated in a suitable host
  - Portion of CSCs in tumor tissue associates with prognosis

- There are two models explaining presence of CSCs

Chaffer CL a Weinberg RA: How does multistep tumorigenesis really proceed? *Cancer Discov.* 5(1): 22-24, **2015**

Cancer stem cells: model A



- CSCs result from transformation of <u>normal stem cells</u>

Chaffer CL a Weinberg RA: How does multistep tumorigenesis really proceed? *Cancer Discov.* 5(1): 22-24, **2015**

Cancer stem cells: model A

Against:

1. low probability that rare, random and advantageous mutations occur in such a small target population

2. mutations occur more frequently in highly proliferating cells (the growth rate of the population of epithelial SC is much slower than their progeny − progenitor cells that have high mitotic activity and grow exponentially)

3. clonal expansion depends on advantageous phenotype; undifferentiated SCs are less likely to develop such phenotype

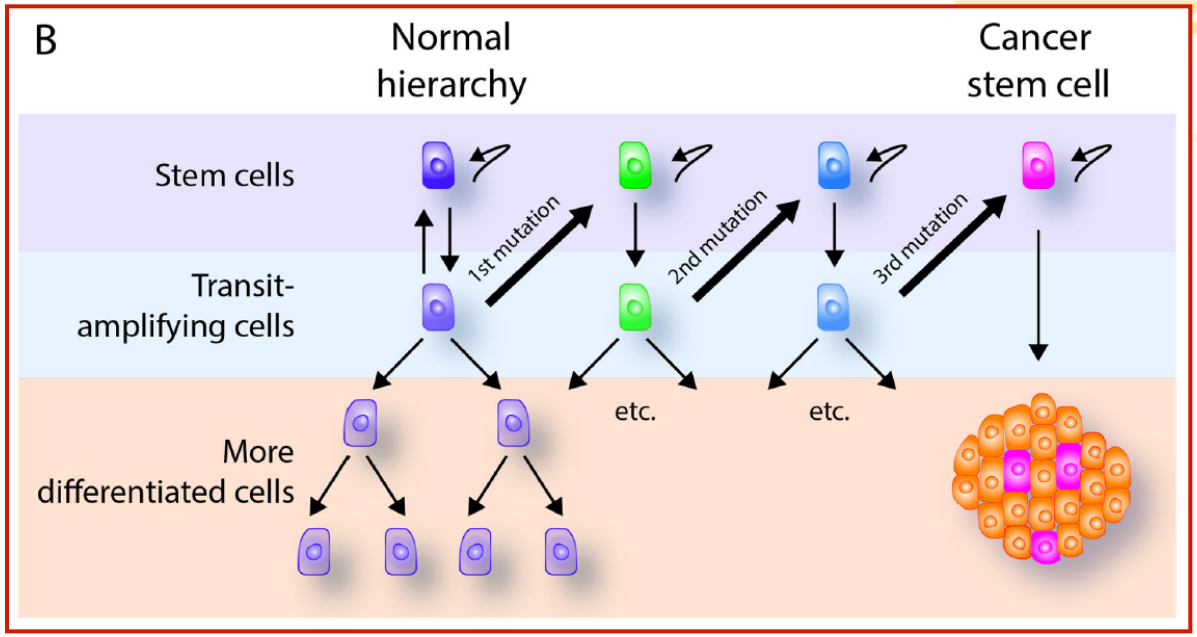
Chaffer CL a Weinberg RA: How does multistep tumorigenesis really proceed? *Cancer Discov.* 5(1): 22-24, **2015**

Cancer stem cells: model A

- Model according to which a founder cell of carcinomas is normal epithelial SC is matematically and biologically improbable

Chaffer CL a Weinberg RA: How does multistep tumorigenesis really proceed? *Cancer Discov.* 5(1): 22-24, **2015**

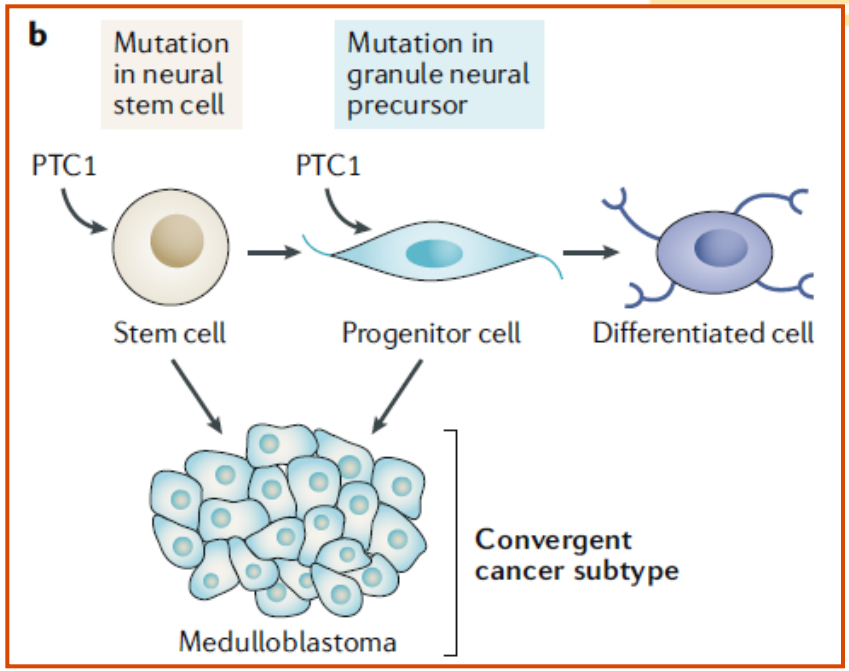
Cancer stem cells: model B



- Transforming genetic (and epigenetic) changes occur in progenitor cells that dedifferentiate into SCs (CSCs).

Chaffer CL a Weinberg RA: How does multistep tumorigenesis really proceed? *Cancer Discov.* 5(1): 22-24, **2015**

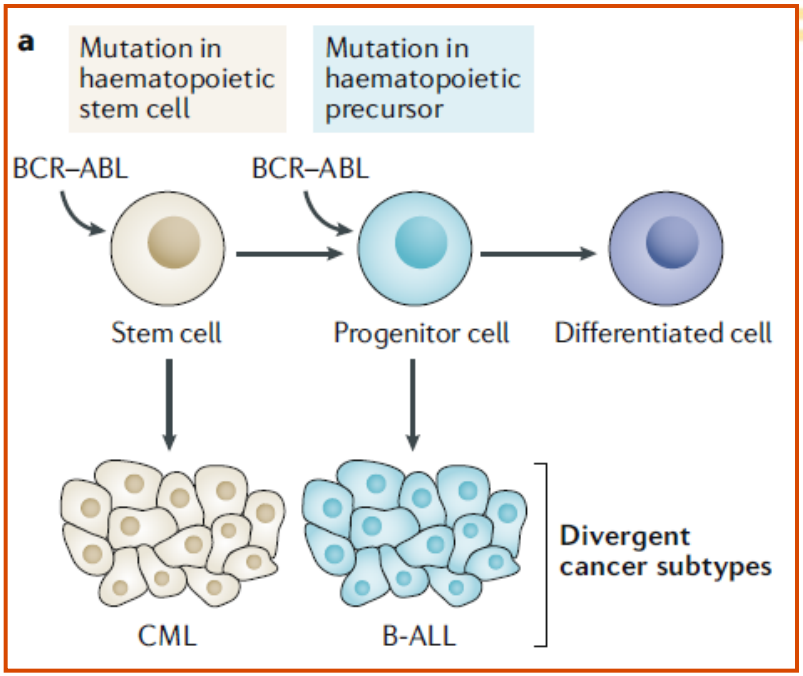
Cancer stem cell: „model A/B“



- Transforming mutations in different founder („renegade“) cells may result in the same type of tumor (convergence) ⇒ some driving mutations are so strong in determing the fate of the cell that they override transcriptional context and origin of cells

Lytle NK et al: Stem cell fate in cancer growth, progression and therapy resistance. *Nat Rev Cancer. 18*: 669-680, **2018**

Cancer stem cells: „model A/B“



- Transforming mutations may lead to the different types of tumors (divergence) depending on the identity of the founder cell, i.e. on the transcriptional and epigentical profile

Lytle NK et al: Stem cell fate in cancer growth, progression and therapy resistance. *Nat Rev Cancer. 18*: 669-680, **2018**

How many and what genes are altered during cancerogenesis?

- Cancer is not a monogenic disease
- It is estimated that **4-7 events (hits)** are required for cancer development
- There are hundreds of distinct genes that may be altered during cancerogenesis

Cancer Genome Atlas consortium

- Genome sequencing of tumors
- 2009: sequencing of the genomes of ovarian carcinomas, pancreatic and lung cancer, melanomas, and some types of leukemia finished
- thereby the complete catalogue of mutations in these cancer types was acquired

Wood LD et al. *Science* 318: 1108-1113, 2007

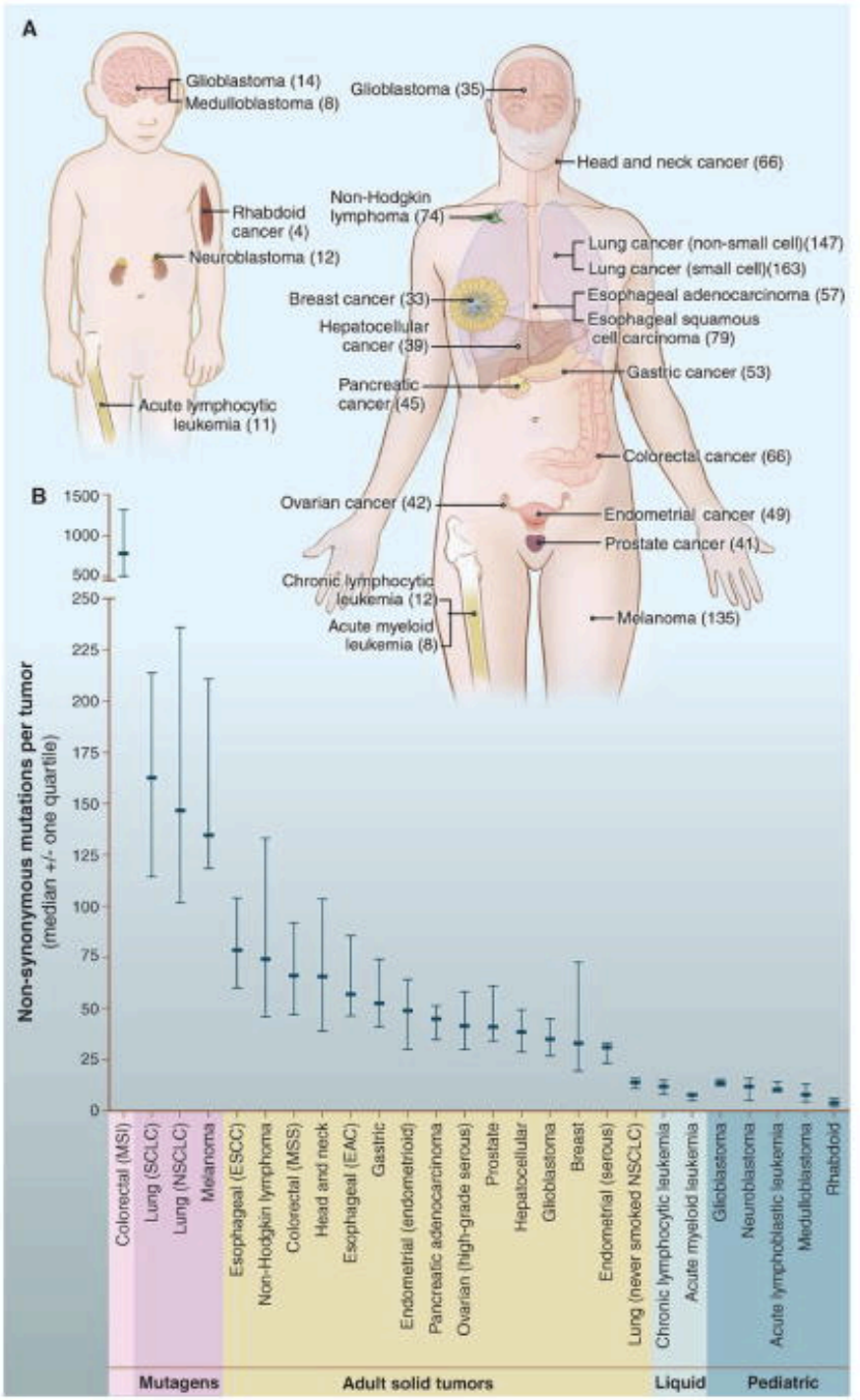
Mukherjee S: Vládkyně všech nemocí. *Nakladatelství MU* 2015

Cancer Genome Atlas consortium

- In individual samples of breast and colon cancer 50 to 80 genes were mutated, in pancreatic cancer 50 to 60 mutations were found
- Even in brain tumors that often develop in relatively young age (thus lower number of mutations would be expected) were found 40 to 50 mutated genes
- In one sample of breast carcinoma of 43y old patient **127** genes were mutated
- There are just few cancer types that are exceptions to this rule and carry only few mutations; e.g. Acute lymphoblastic leukemia (ALL) has only 5 to 10 mutated genes

Wood LD et al. *Science* 318: 1108-1113, 2007

Mukherjee S: Vládkyně všech nemocí. *Nakladatelství MU* 2015



Cancer Genome Atlas consortium

Vogelstein B et al. *Science* 339: 1546–1558 , 2013

Cancer Genome Atlas consortium

- In one type of tumor there is almost „depressing“ mutation heterogeneity (B. Vogelstein)
- Comparing two samples of breast tumors showed that set of mutated genes is not the same
- Genome sequencing confirmed hundreds of years of clinical observations: cancer of each patient is unique, because every cancer genome is unique

Wood LD et al. *Science* 318: 1108-1113, 2007

Mukherjee S: Vládkyně všech nemocí. *Nakladatelství MU* 2015

Genomic landscapes of human cancers

Bert Vogelstein: mutations in tumors are of two types:

- (1) **passenger (bystander, passive)**: during the course of cancer divisions they accumulate mutations due to the DNA replication errors, but they do not affect the biology of tumor, they are just passively transfered during somatic cell division, they do not have significance, just bystanders
- (2) „**driver**“: i.e. directly stimulating growth and behaviour of cancer cells, they determine biology of cancer cell

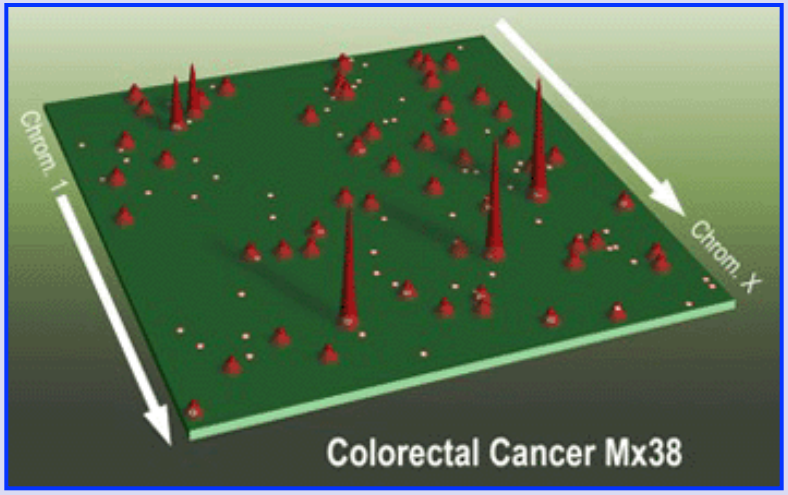
- Passenger mutations occur accidentally, and their genomic location is also accidental; on contrary driver mutations affect key oncogenes and tumor suppressors, there is limited number of such genes in genome

Wood LD et al. *Science* 318: 1108-1113, 2007

Vogelstein B et al. *Science* 339: 1546–1558 , 2013

Genomic landscapes of human cancers

- „passenger“ mutations occur accidentally
- „driver“ mutations affect key oncogenes and tumor suppressor (e.g. *ras, myc* a *RB*) are repeatedly found in samples; on „Vogelstein‘s map“ they represent high mountains, whereas passenger mutations form valleys



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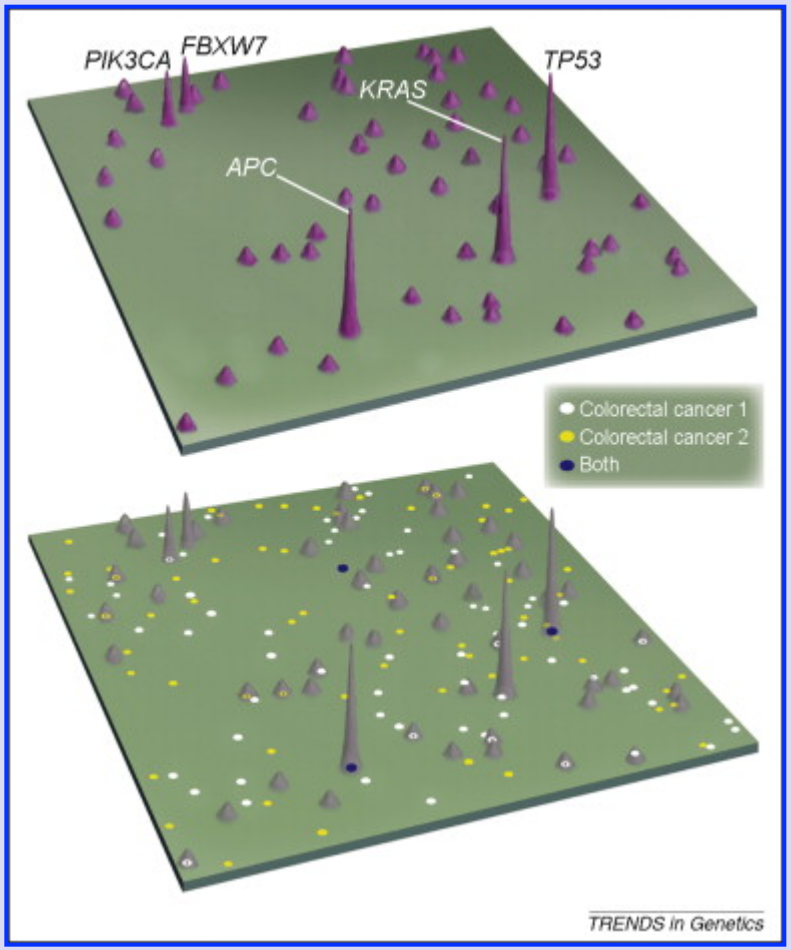
Genomic landscapes of human cancers

- „**mountains**“ in cancer genomes– i.e. the most frequently mutated genes of a single cancer type, may form distinct signaling pathways (e.g. Ras-Mek-Erk)
- How many pathways are usually deregulated in cancer? Vogelstein found that typically 11 to 15, in average **13**



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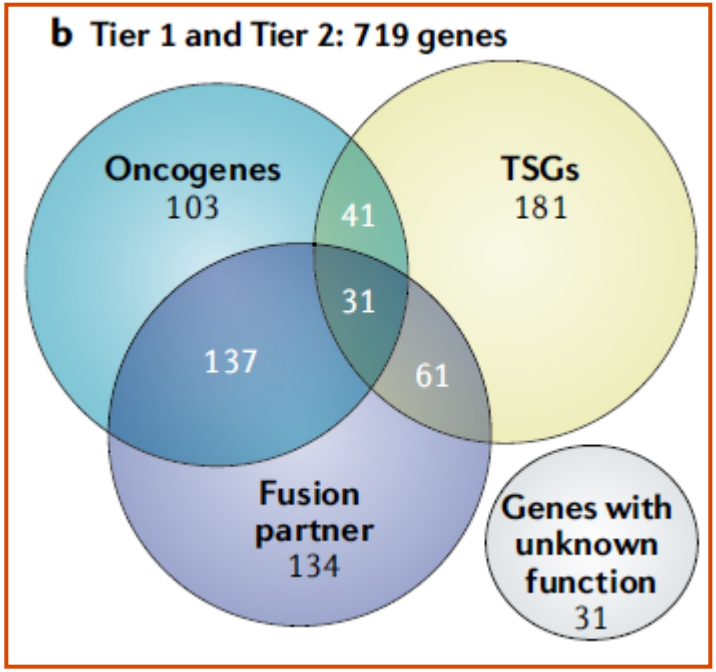
There are hundreds of distinct genes that may be altered during cancerogenesis

- Catalogue of Somatic Mutations in Cancer (COSMIC) Cancer Gene Census (CGC)
- version 86, August 2018: **719** „cancer-driving“ genes: oncogenes + tumor suppressors (554 genes), fusion genes

- level 1 (554 genes): known function and evident impact on transformation
- level 2: (i) less clear mechanism of transformation; (ii) partner genes in fusions with unknown function, yet oncogenic function of respective partner gene was confirmed



Sondka Z et al. *Nat Rev Cancer* 18: 696-705, **2018**; Tate JG et al. *Nucl Acid Res* 47: D941-D947, **2019**

There are hundreds of distinct genes that may be altered during cancerogenesis

- Catalogue of Somatic Mutations in Cancer (COSMIC) Cancer Gene Census (CGC)
- Version 95, November 2021: **733** „cancer-driving“ genes: oncogenes + tumor suppressors, fusion genes
  - level 1 (579 genes): a documented activity relevant to cancer, along with evidence of mutations in cancer which change the activity of the gene product in a way that promotes oncogenic transformation
  - level 2 (154 genes): genes with strong indications of a role in cancer but with less extensive available evidence

Sondka Z et al. *Nat Rev Cancer* 18: 696-705, **2018**; Tate JG et al. *Nucl Acid Res* 47: D941-D947, **2019**

https://cancer.sanger.ac.uk/cosmic

How many and what genes are altered during cancerogenesis?

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- There are **hundreds** (733) of distinct genes that may be altered during cancerogenesis
- In general there are 10 **hallmarks of malignant cell**

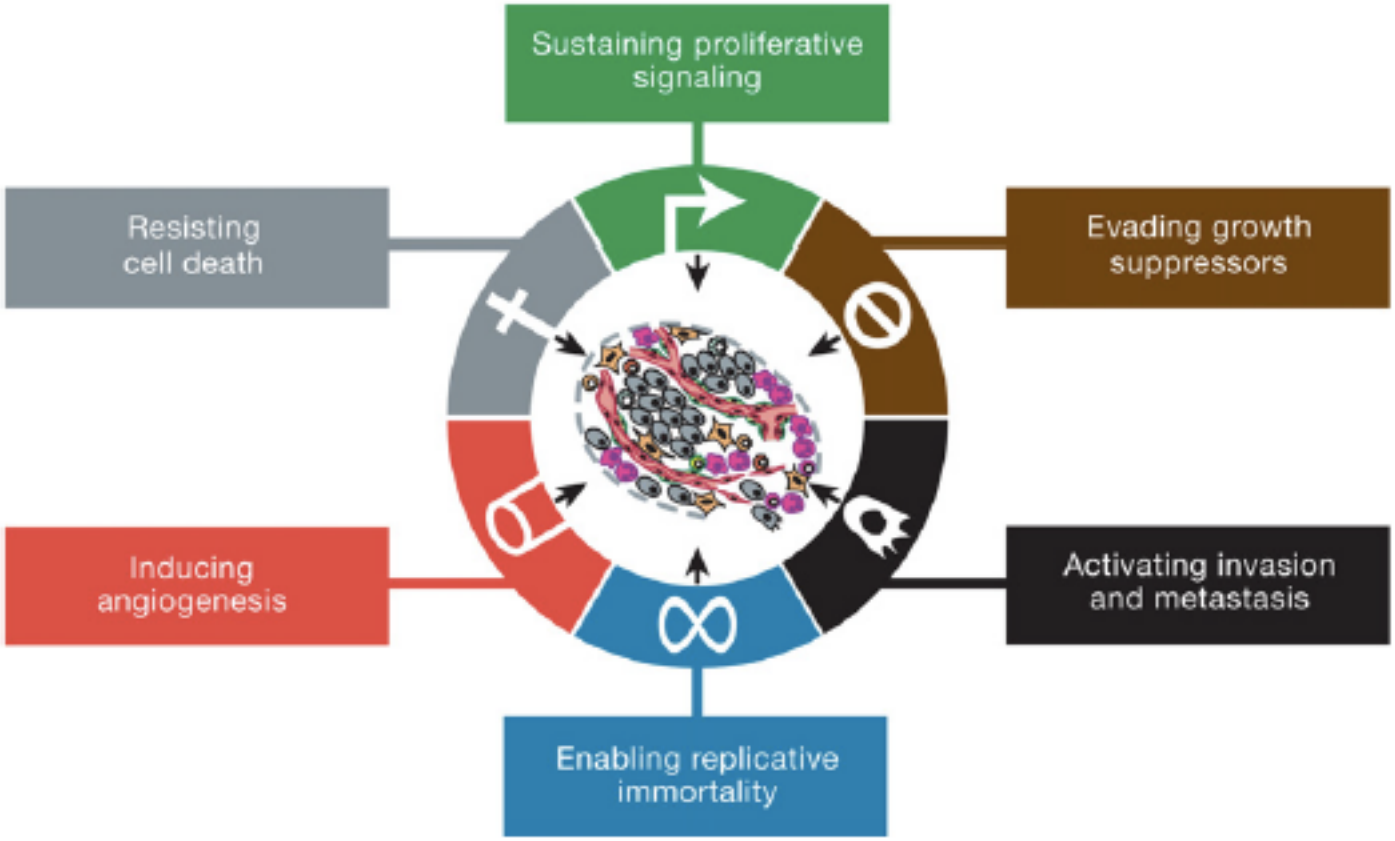
Six hallmarks of malignant tumor

Component	Acquired Capability	Example of Mechanism
	Self-sufficiency in growth signals	Activate H-Ras oncogene
	Insensitivity to anti-growth signals	Lose retinoblastoma suppressor
	Evading apoptosis	Produce IGF survival factors
	Limitless replicative potential	Turn on telomerase
	Sustained angiogenesis	Produce VEGF inducer
	Tissue invasion & metastasis	Inactivate E-cadherin

Genomic instability is required for all necessary mutations to accumulate.

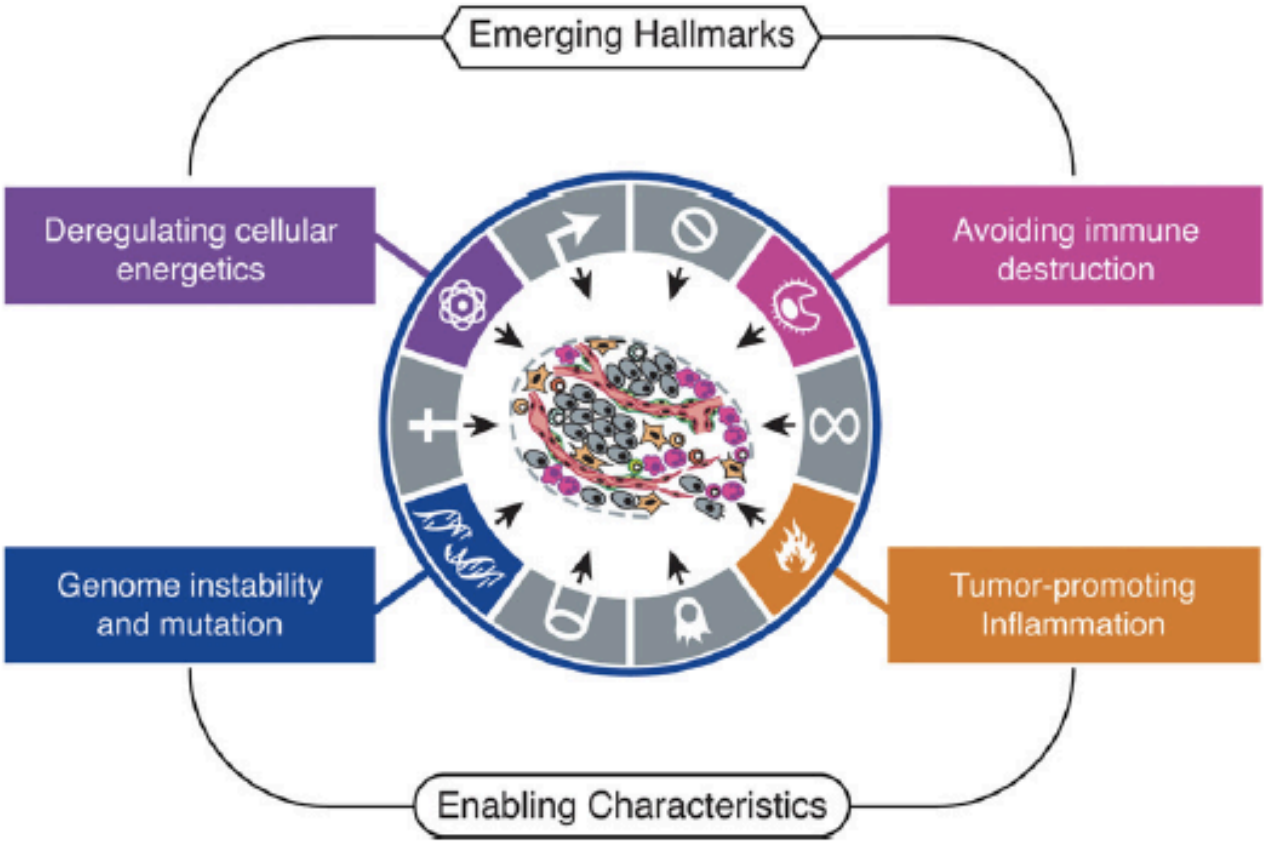
Hanahan D and Weinberg RA. *Cell* 100: 57-70, **2000**

6 hallmarks of cancer cells



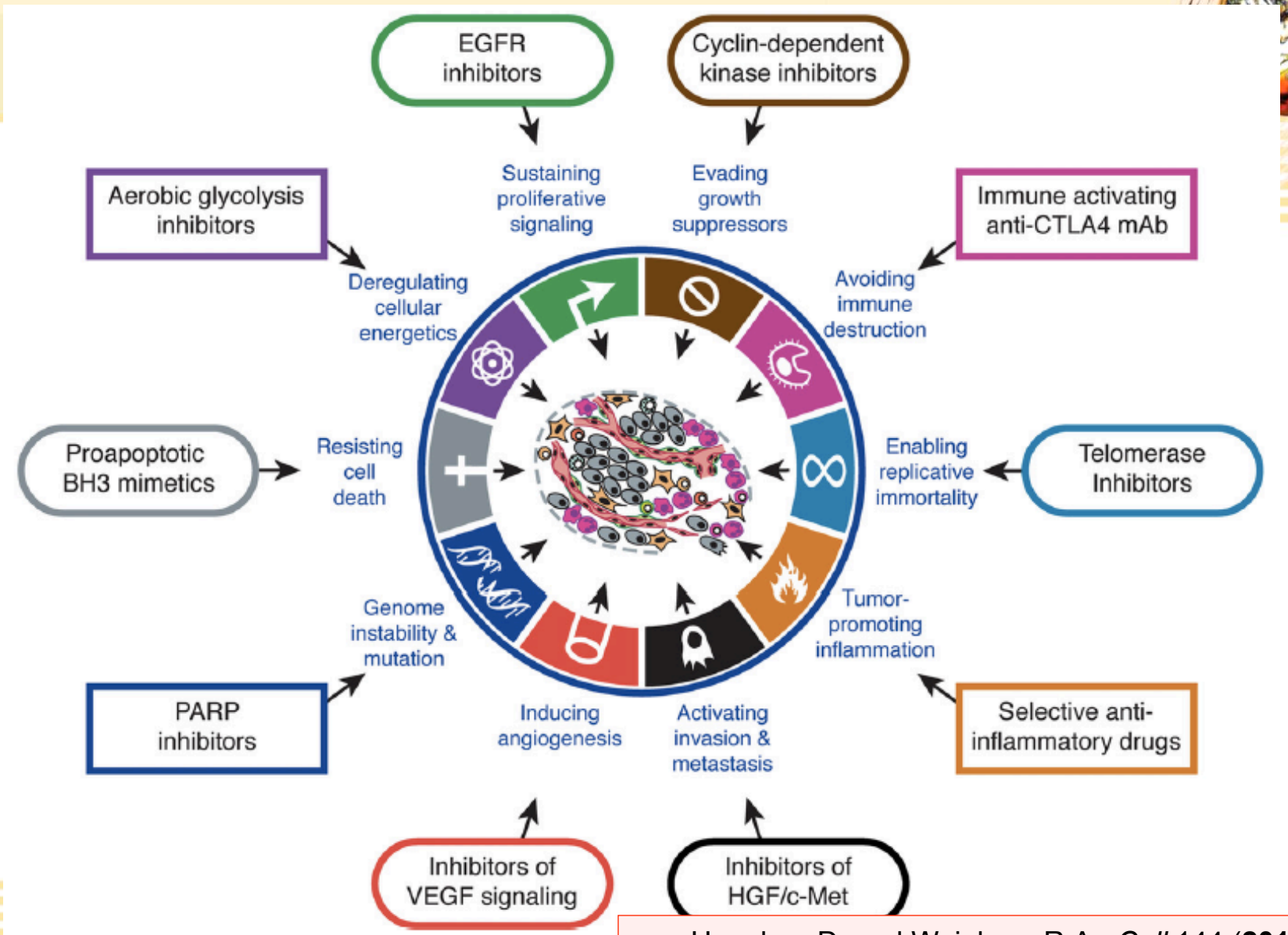
Hanahan D. and Weinberg R.A., *Cell* 100 (**2000**) 57-70

10 hallmarks of malignant tumor



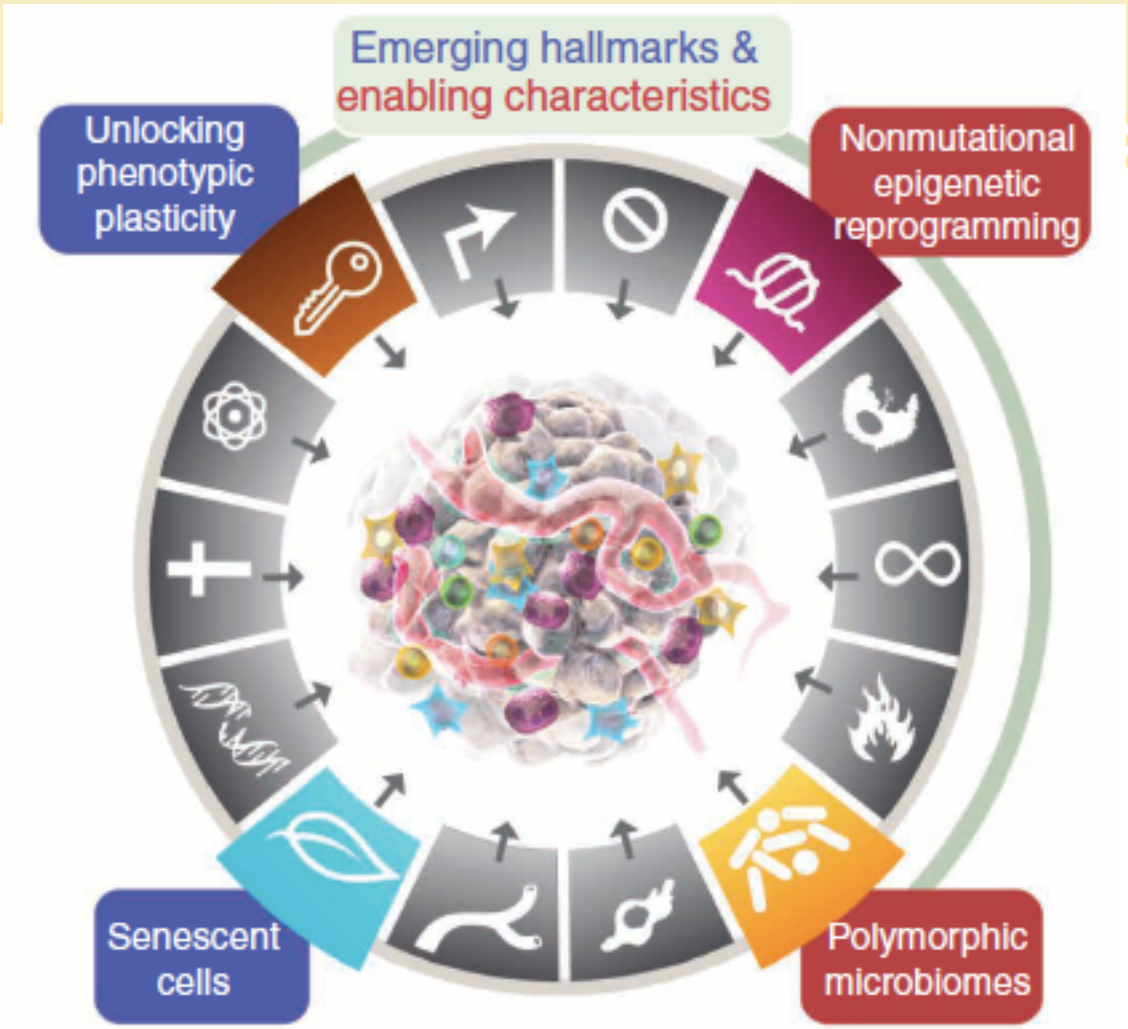
Hanahan D. and Weinberg R.A., *Cell* 144 (**2011**) 646-674

10 hallmarks of cancer



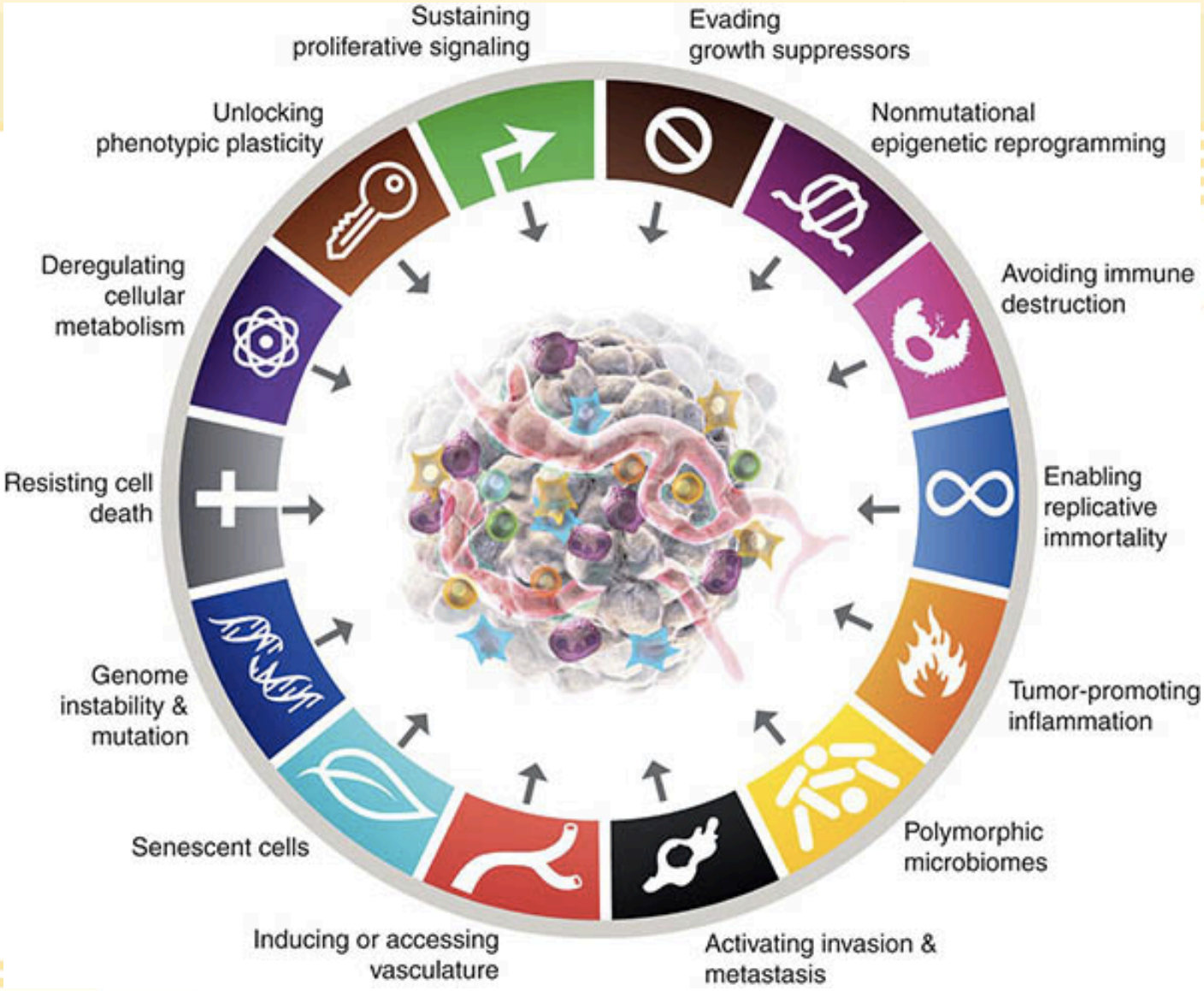
Hanahan D. and Weinberg R.A., *Cell* 144 (**2011**) 646-674

14 hallmarks of cancer



Hanahan D., *Cancer Discov* (**2022**) 12 (1): 31–46.

14 Hallmarks of cancer



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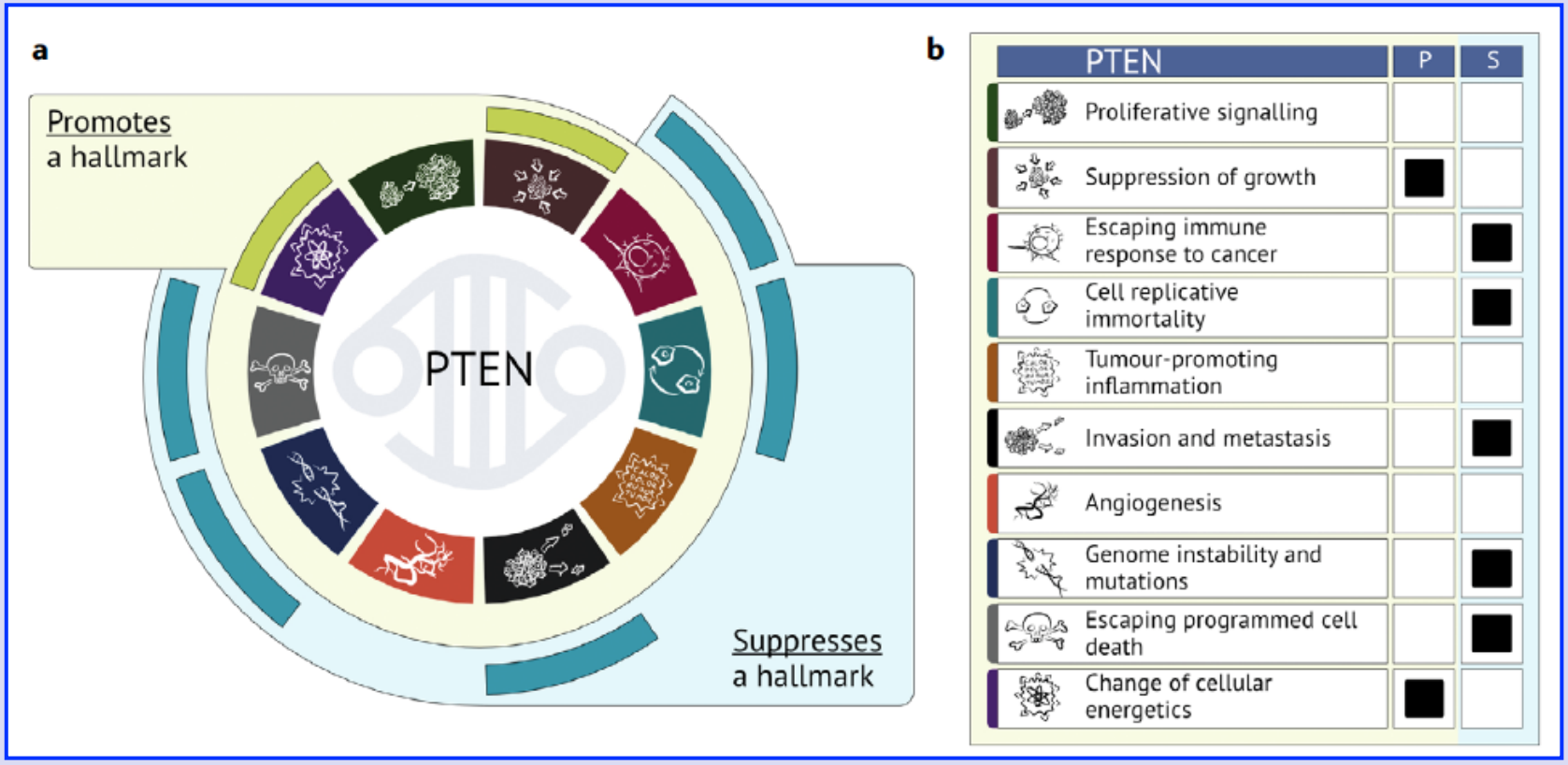
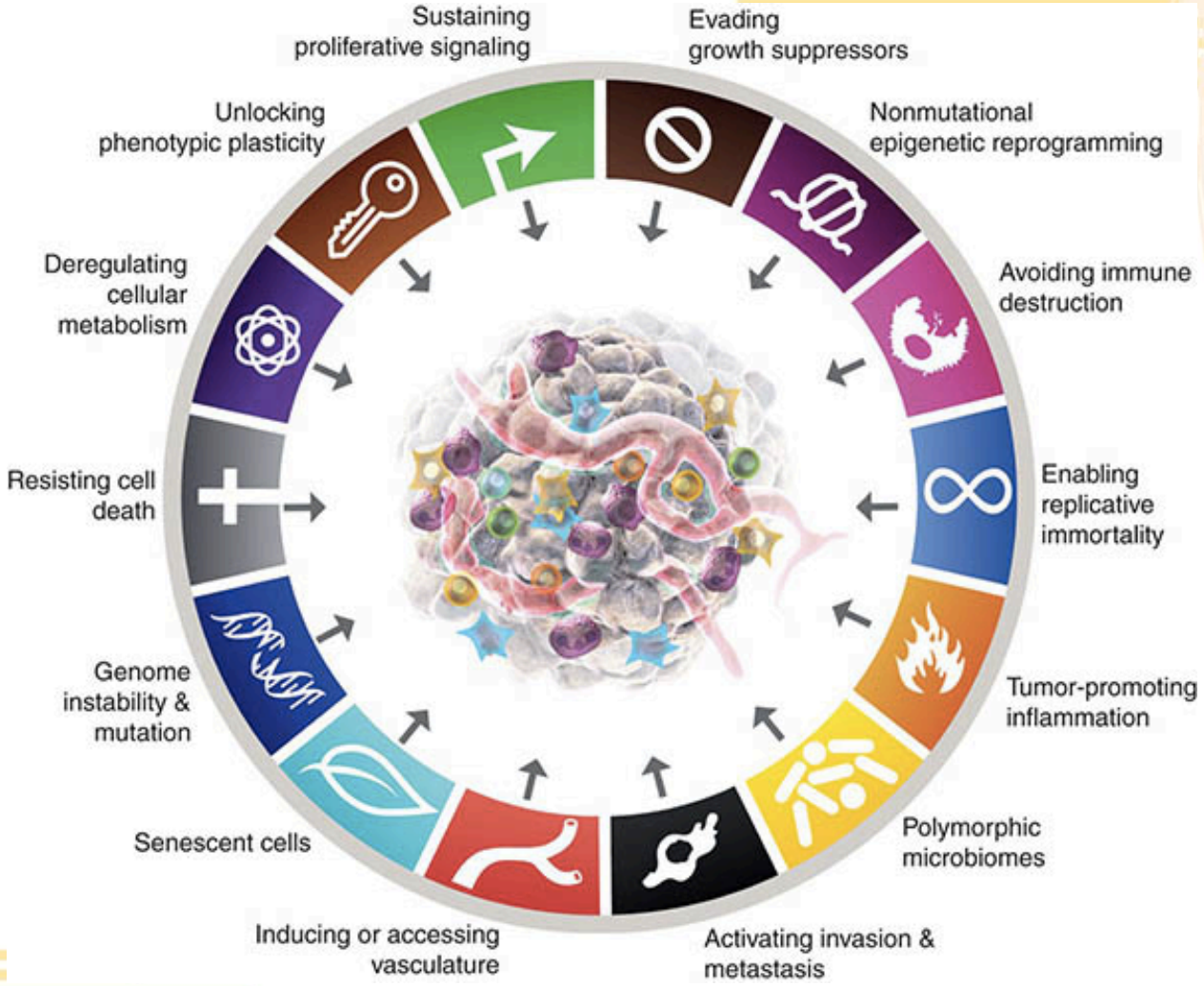
There are hundreds of genes that may be altered during cancerogenesis

- Catalogue of Somatic Mutations in Cancer (COSMIC) Cancer Gene Census (CGC)



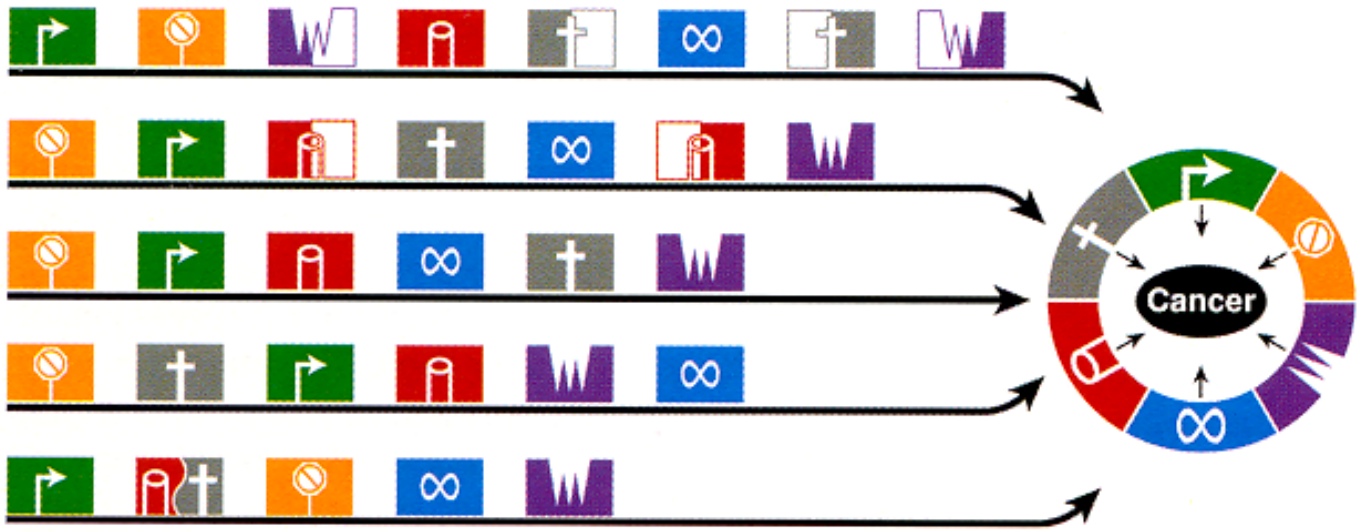
Sondka Z et al. *Nat Rev Cancer* 18: 696-705, 2018; Tate JG et al. *Nucl Acid Res* 47: D941-D947, 2019

Cancerogenesis has general features ...



Hanahan D., *Cancer Discov* (**2022**) 12 (1): 31–46.

Cancerogenesis has individual course



Individual is − sequence of hits

- number of hits

- actual mutated genes

Hanahan D and Weinberg RA. *Cell* 100: 57-70, 2000

Oncogenes

Protooncogene is a protein coding gene of eukaryotic cell whose translational product participates in regulation (stimulation) of cell division

Oncogene is altered (activated) protooncogene that causes neoplastic transformation

Activation of protooncogene is a conversion of protooncogene into oncogene

Mutations of protooncogenes are:

- activating
- dominant
- occur in somatic cells and only rarely in germ cells

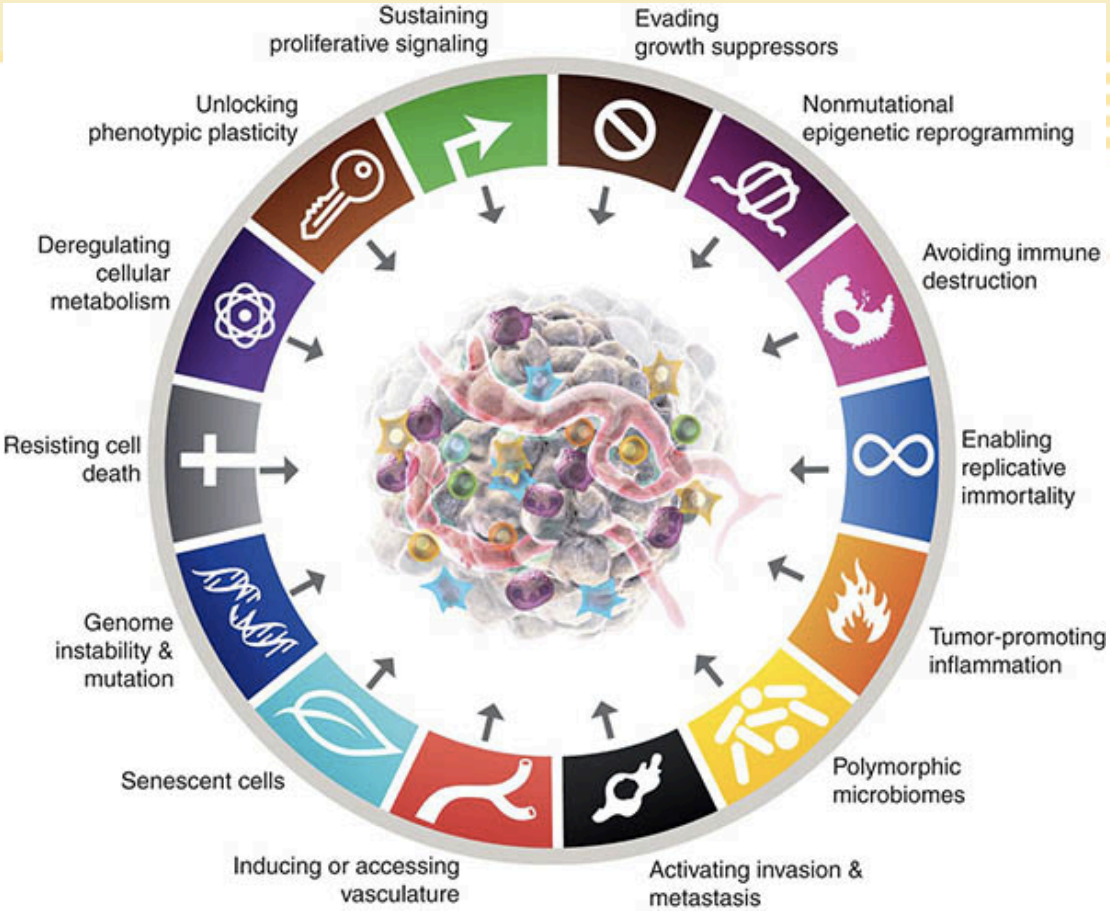
Tumor suppressors

Proteins coded by tumor suppressor genes slow down proliferation and keep cells in quiescent phase of cell cycle. Their loss results in unregulated proliferation

Mutation of tumor suppressor gene are:

- inactivating
- recessive (connection with **LOH, L**oss **o**f **h**eterozygosity**)**
- occur both in somatic and germ cells

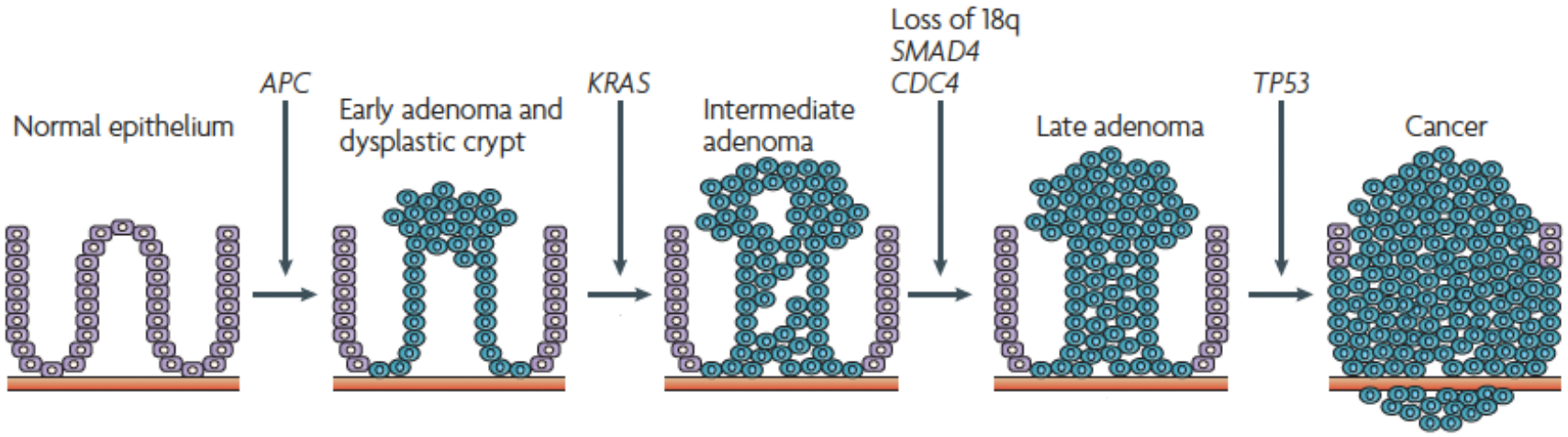
14 Hallmarks of cancer ...



... multistep cancerogenesis − models:

Model of multistep cancerogenesis

Development of colorectal carcinomas



Walther A et al. *Nat Rev Cancer* (2009) **9**: 489–499

Oncogenic (cancer) viruses

- **Retroviruses** (RNA viruses)**:** have an oncogene in their genomes (acutely transforming) or they activate eukaryotic protooncogene by insertion mutagenesis (slowly transforming)

- **DNA cancer viruses:** have different „strategy“ of transformation, they code viral oncoproteins that interact with host tumor suppressors (**RB**, **p53**, **p300/CBP**) and thereby promote transition of the cell cycle to the S phase

Oncogenic (cancer) viruses

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- **DNA cancer viruses:** have different „strategy“ of transformation, they code viral oncoproteins that interact with host tumor suppressors (**RB**, **p53**, **p300/CBP**) and thereby promote transition of the cell cycle to the S phase:

  SV40: large T antigen interacts with p53, RB, p300/CBP (via different domains)

  adenovirus: E1A interacts with RB and p300/CBP; E1B interacts with p53

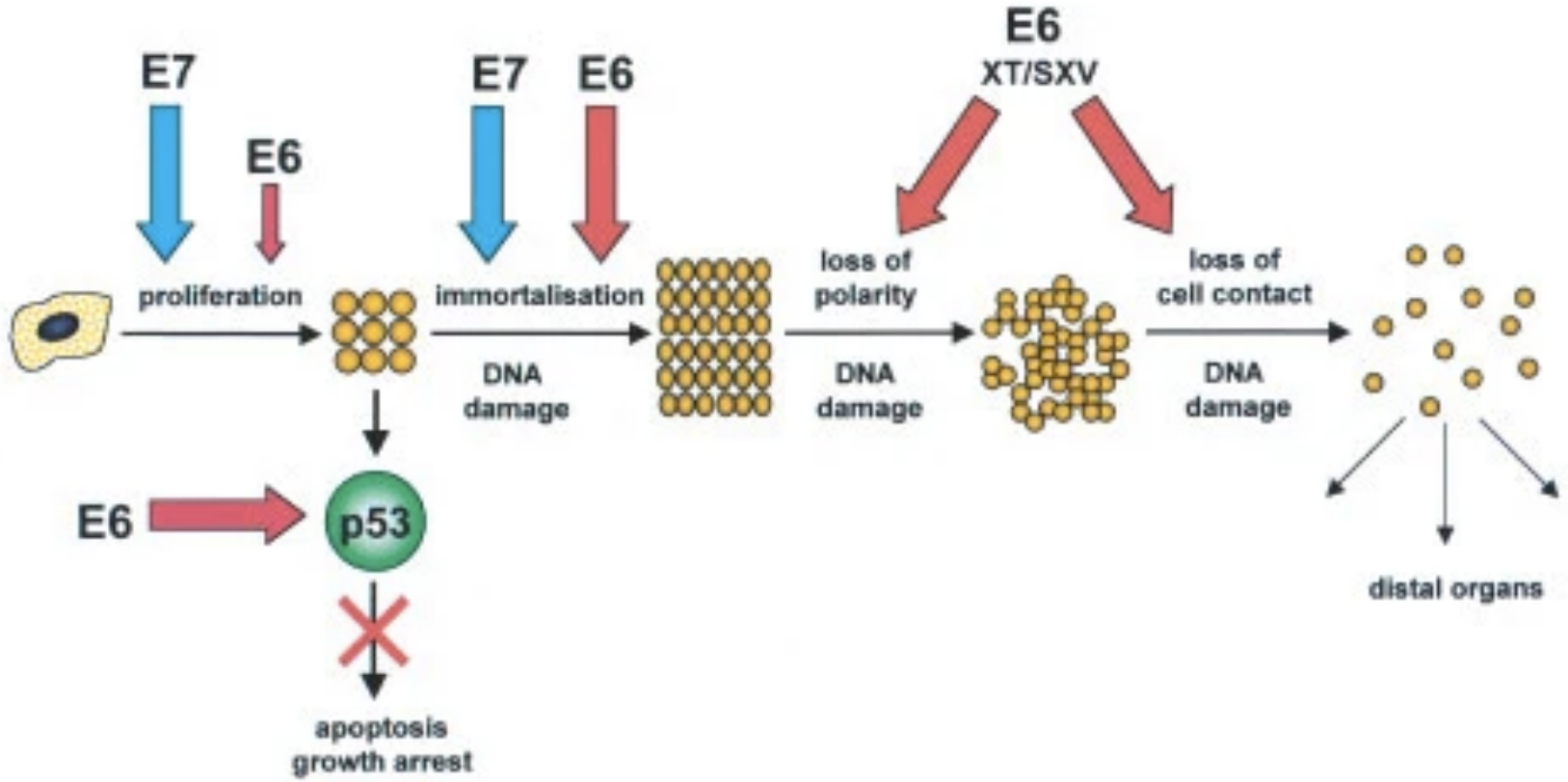
  papillomavirus HPV-16, **HPV-18**: E6 interacts with p53, p300/CBP; E7 interacts with RB

Some ways of p53 inactivation by viral oncoproteins

- Inactivation of tumor suppressor p53 is a crucial for transformation by DNA viruses:

- **Large TAg** (SV40) − interacts with DNA binding domain of p53 and prevents binding of p53 to DNA
- **E1B** (adenoviruses) − interacts with transactivating domain of p53 thereby hinders transactivation of its target genes
- **E4orf6** (adenoviruses) - causes degradation of p53
- **HBV X** (hepatitis B virus) − blocks nuclear translocation of p53
- **E6** (papillomaviruses) − induced degradation of p53 (via cellular E6AP ubiquitin ligase); E6 also directly inhibits transactivating function of p53

Contribution of E6 and E7 proteins to transformation by papillomaviruses



viral oncoproteins: stimulate proliferation, inhibit apoptosis, increase replicative potential, change morphology of cells, induce malignant phenotype

Mantovani F a Banks L, *Oncogene* 20: 7874-7887, 2001

Contribution of E6 and E7 proteins to transformation by papillomaviruses

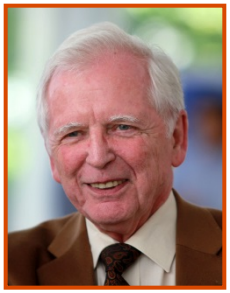
E6 - inactivates **p53** (disrupted: blok G_1, apoptosis, genetic stability)
- interacts with **p300/CBP** (homeostasis pertrubation)
- activates expression of **hTERT** (telomerase activation)
- inactivates **p16^{ink}** (distrubed cell cycle control)
- interacts with **Bak** (inhibition of apoptosis)
- interacts with **E6BP/ERC-55** (inhibits differentiation)
- induces degradation of **hDlg** (and with other proteins with PDZ motif) (change of morphology, induction of motility)

E7 − binds protein **RB**
- inactivation of **p21^{Cip}** and **p27^{Kip}** (disconnected proliferation and differentiation)
- prevent inhibitory effect of **TGF-β** on cell growth
- cause formation of multiple centrosomes

Mantovani F a Banks L, *Oncogene* 20: 7874-7887, 2001

Clinical role of papillomaviruses

- Identified more than 100 of different types of human papillomaviruses (HPV)
- fall into two groups: „high-risk“ and „low-risk“ types according to the prognosis; „low-risk“ viruses cause no disease or benign tumors, „high-risk“ viruses are functionally linked with malignant progression
- cca 30 types of HPV preferetially infects anogenical areas, almost all cervical tumors are associated with infection of „high-risk“ viruses
- 20% of tumors of oral cavity that are not linked with smoking history or alcoholism are associated with HPV infection



2008: Nobel prize - Harald zur Hausen

Oncogenic viruses and human cancer

DNA viruses:

- Epstein Barr virus (EBV) – Burkitt's lymphoma (BL), Hodgkin lymphoma (HD), other lymphomas, nasopharyngeal carcinoma (NPC)
- Hepatitis B virus (HBV) – hepatocellular carcinoma (HCC)
- (Hepatitis C virus (HCV) – HCC a lymphomas; RNA virus!)
- human papillomaviruses (HPV 16, 18,..) – anogenital tumors, oropharyngeal tumors , warts
- human herpesvirus type 8 (HHV8) - Kaposi sarcoma (KS)
- Merkel cell polyomavirus (MCV) – Merkel cell carcinomas (neuroendocrinne skin carcinoma)

Oncogenic viruses a human cancer

RNA viruses:

In general: retroviruses do not cause many cancers, but research on RNA cancer viruses helped to elucidate many aspects of cancerogenesis

(1) acutely transforming, (2) slowly transforming and

(3) Human T-lymphotropic virus type 1 (HTLV-1) − causes adult T cell leukemia/lymphoma (ATL)

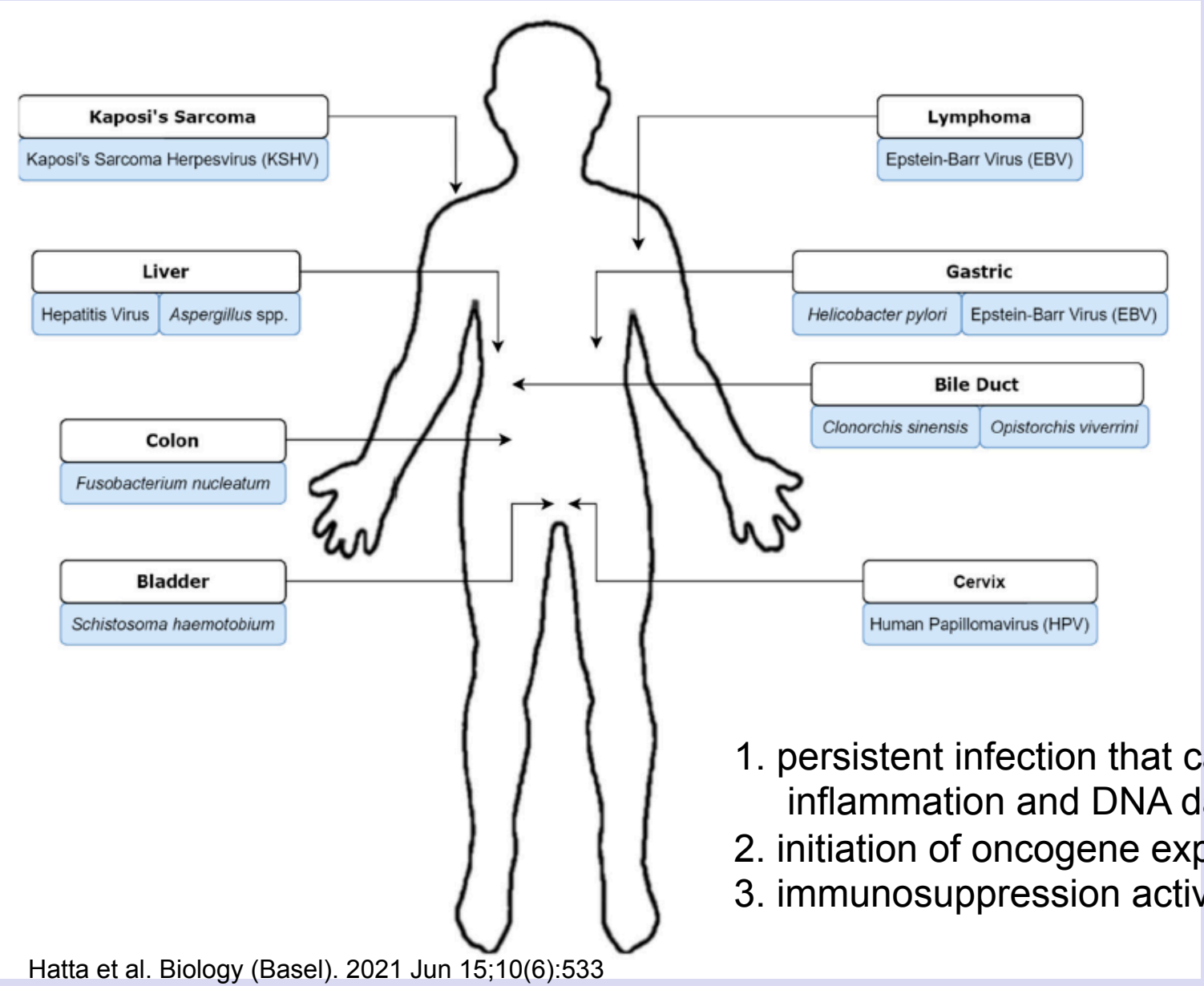
- HTLV-1 is transmitted primarily through infected bodily fluids including blood, breast milk and semen
- Most people with HTLV-1 infection are asymptomatic, but the lifetime risk of developing adult T-cell leukaemia/lymphoma (ATL) among people with an HTLV-1 infection is about 5%
- Mechanism different from insertion mutagenesis: viral gene ***tax*** is responsible for trancription activation of viral DNA and probably also activates transcription of host cell genes: ***IL-2*** and ***GM-CSF***. Stimulation by these cytokines probably causes T-cell neoplasia
- (4) HIV-1 a HIV-2 – do not cause tumors directly, but via immunosuppression

Infectious agents in cancerogenesis

- infectious agents are associated with **20 % of deaths from cancer**

- 6 % of worldwide mortality for liver cancer associated with chronic hepatitis B and C (**HBV**, **HCV**)
- 5 % of worldwide mortality for cervical cancer associated with HPV infection
- 9 % of worldwide mortality for stomach cancer associated with persistent infection of **Helicobacter pylori**

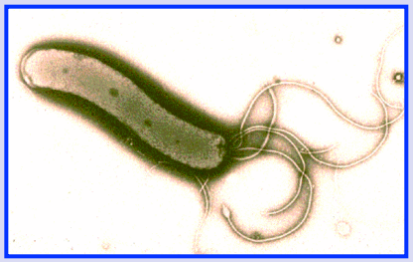
Infectious agents in cancerogenesis



1. persistent infection that cause inflammation and DNA damage,
2. initiation of oncogene expression,
3. immunosuppression activity of the host

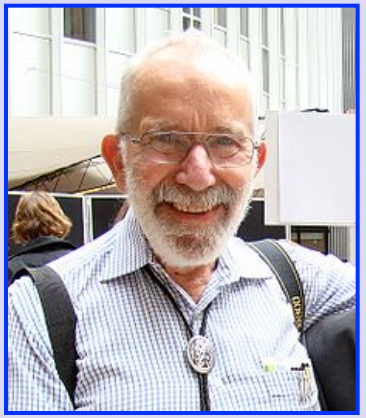
Hatta et al. Biology (Basel). 2021 Jun 15;10(6):533

Helicobacter pylori



- spiral, mikroaerofil, gramnegative bacteria
- found in **1982**
- colonize stomach mucosa
- Prevalence in our population is estimated around 30-55%; increases with age

- Discovery that chronic stomach inflammation is caused by infection by *Helicobacter pylori*: Barry Marshall a Robin Warren – **2005** – Nobel prize for physiology and medicine

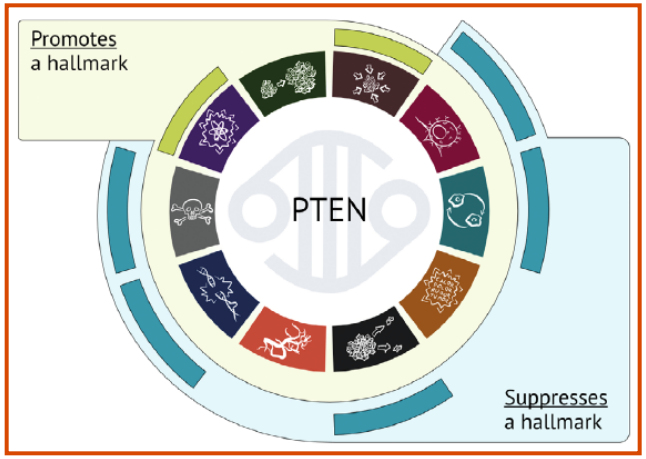


Conclusions of „Introduction“

- Genes‘ functions are not limited to one cellular process (cell cycle + apoptosis + genomic stability etc.)
- Only small number of signaling pathways are inactivated almost universally in tumors (RB, p53)
- Single signaling pathway is ussually damaged in one component only

(COSMIC)
Conclusions of „Introduction“

- Only a few genes affect only one exclusive cancer hallmark, most of the genes involved in cancerogenesis affect multiple hallmarks
- Different mutations of a single gene may have different impact of its function
- Classification of oncogenes and tumor suppressors is not clear-cut
- Tissue specific impact
- Dependent of the phase of tumor development



PTEN	P	S
Proliferative signalling		
Suppression of growth	■	
Escaping immune response to cancer		■
Cell replicative immortality		■
Tumour-promoting inflammation		
Invasion and metastasis		■
Angiogenesis		
Genome instability and mutations		■
Escaping programmed cell death		■
Change of cellular energetics	■	

Sondka Z et al. *Nat Rev Cancer* 18: 696-705, 2018; Tate JG et al. *Nucl Acid Res* 47: D941-D947, 2019

Thank you for your attention!