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Bi4025en
Molecular Biology

Mgr. Jiří Kohoutek, Ph.D.

Lecture 10

- Molecular basis of carcinogenesis – oncogene versus suppressors

Tumor associated facts

- Every third person in the Czech Republic experiences cancer.
- One in four people dies because of it.
- In 2015, there were almost 542,000 people living in the Czech Republic who had been diagnosed with an oncological disease in that year or earlier.
- Every year, about 27,000 people die of cancer in the Czech Republic.
- Mortality from malignant tumors shows stagnation in absolute numbers!
- Increase in prevalence (number of patients with a given tumor living in a particular year).

Incidence of malignancies in Czech Republic

- Every 20 minutes, one person dies of cancer in the Czech Republic.
- In the number of oncological patients - leading places in Europe.
- The most common newly diagnosed malignant diseases in 2011-2015 were skin tumors (excluding melanoma), colorectal cancer, breast cancer in women, and malignant tumors of the prostate and lung.
- Main factors responsible for high incidence of malignant disease in Czech Republic:
  - Significant demographic aging of the Czech population.
  - Eating habits.
  - Subsequent malignancies in oncological patients.

Dusek et al 2018

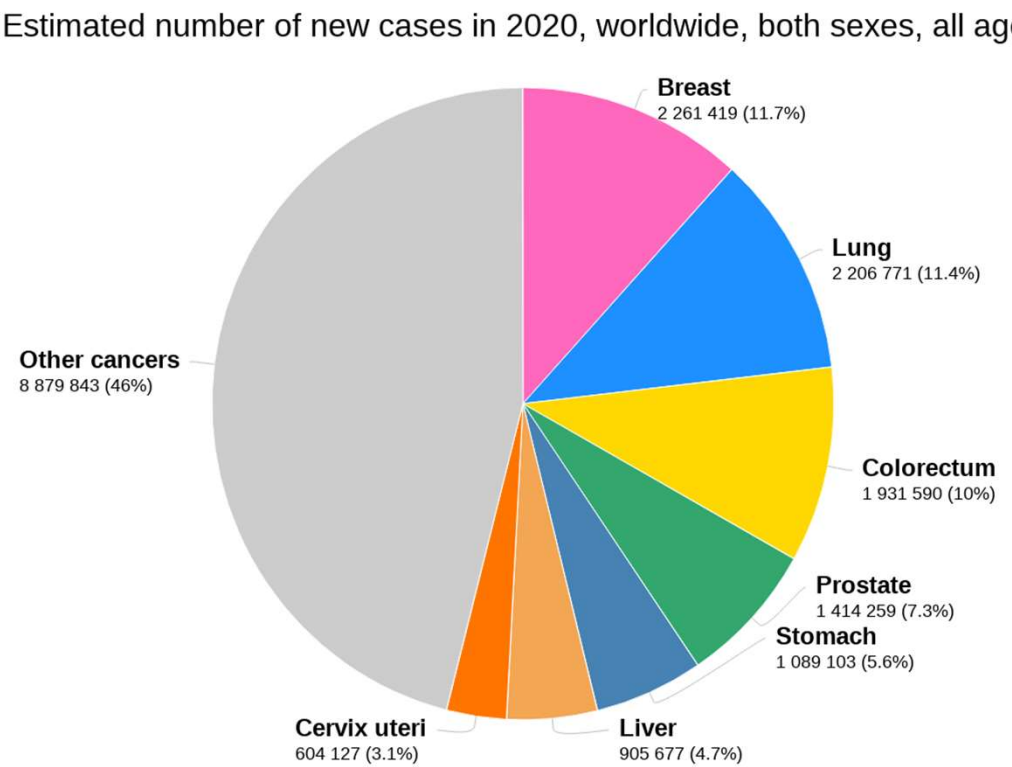
NOR 2017

Incidence of malignancies as a global problem

- Tumors 12% of all deaths (56 million in 2000).
- In 2000 - 5.3 million men and 4.7 million women were diagnosed, 6.2 million died of tumor.
- Prediction – 10 million new cases in 2000 to 15 million in 2020 – aging population – smoking and poor lifestyles.

Update: http://www.who.int/topics/cancer/en/

Incidence of malignancies as a global problem



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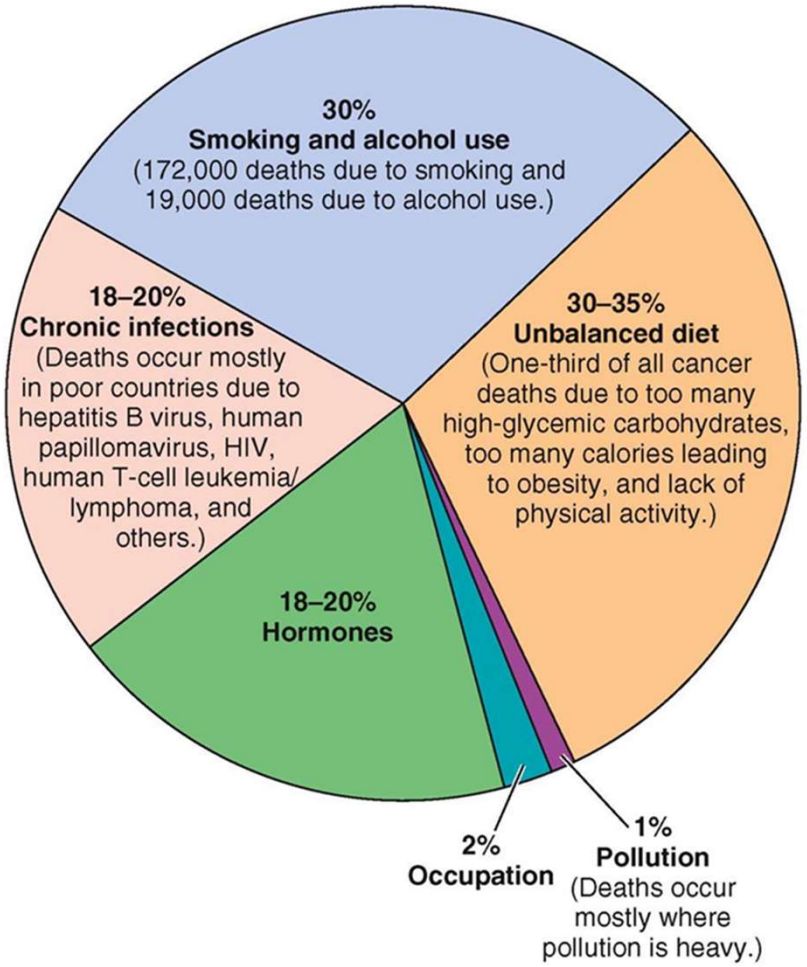
Important facts

- About 1/3 of tumors are associated with obesity, poor diet and low physical activity.
- Smoking – 30% of all deaths from tumors, 87% of deaths from lung tumors.
- Obesity increases the risk of breast tumors in postmenopausal women by 50% and 40% in bowel tumors in men.



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Cancers and rates

Global cancer rates

The ten worst countries and the UK cancer rate, per 100,000 of the population

1	Denmark	326.1
2	Ireland	317
3	Australia	314.1
4	New Zealand	309.2
5	Belgium	306.8
6	France	300.4
7	US	300.2
8	Norway	299.1
9	Canada	296.6
10	Czech Republic	295
22	UK	266.9

SOURCE: WORLD CANCER RESEARCH FUND

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Tumor

- Uncontrolled growth of cells in the tissue of higher organisms.
- Does not have a physiological function.

- Clonal expansion characteristics.

- Disrupts the balance in the body.

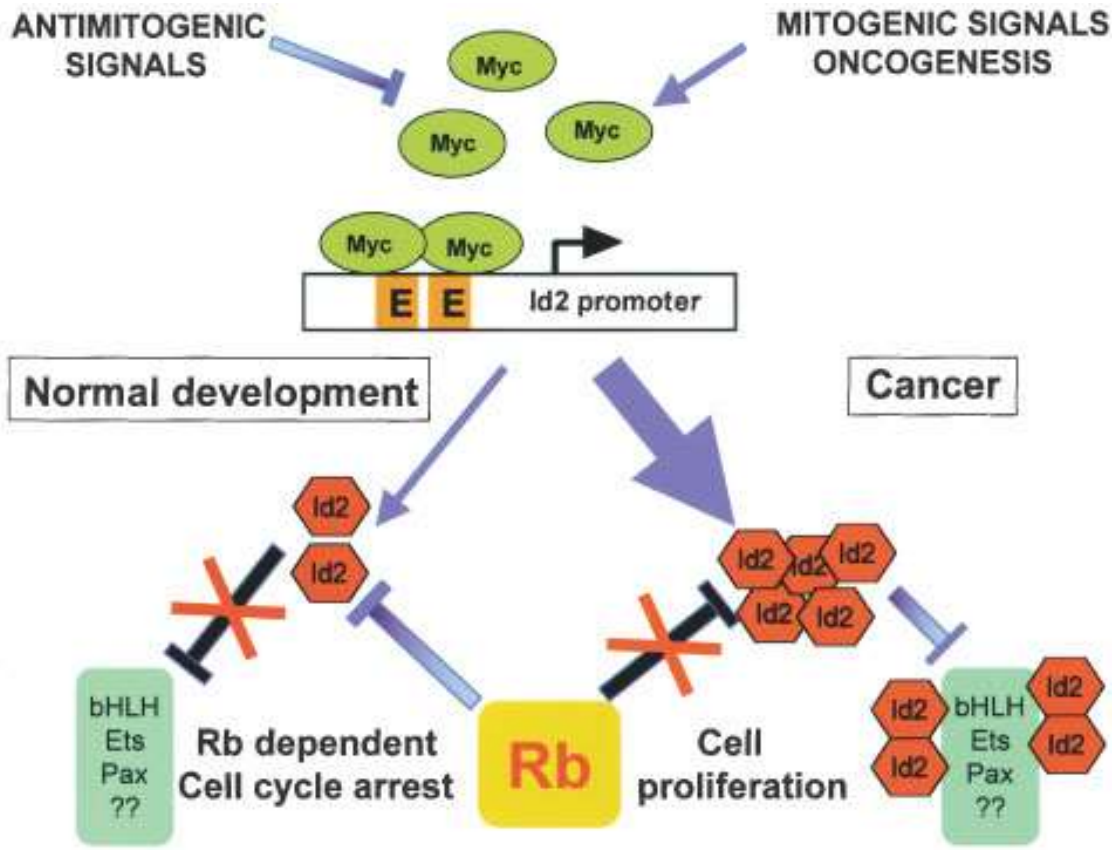
- Critical two types of genes:
- Oncogenes (gain of function).
- Tumor suppressors (loss of function).

  - All of these genes generally have other primary functions.
  - Genes may be tumor suppressors or oncogenes in one tissue, at one time point.
  - Effects of such genes are subject to tradeoffs with other functions.

Tumor oncogenes

- 1970 - *src* – chicken retrovirus Rous sarcoma virus (Dr. Martin, UC Berkley).
- 1976 - Stehelin, Vermus, Bishop – oncogenes are activated proto-oncogenes.
- Proto-oncogene – a gene that encodes proteins that affect growth, differentiation and/or, signal transmission.
- Proto-oncogene activation – is the conversion of proto-oncogene into oncogene. Mutation or increased expression or amplification – oncogene *Ras*, *Myc*, *ERK*.
- Proto-oncogene mutations are:
- Activating.
- Dominant.
- Occur in somatic cells and rarely in germ cells.

Tumor oncogenes



https://blogs.shu.edu/cancer/2014/12/23/merck-acquires-oncoethix-for-novel-myc-blocker/

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Tumor suppressors

- The products of genes for tumor suppressors (antioncogenes) in normal cells do not cause proliferation, but, on the contrary, suppress it and keep the cells at rest (G0). Their loss is manifested by unregulated proliferation.

- Mutations of tumor suppressors are:

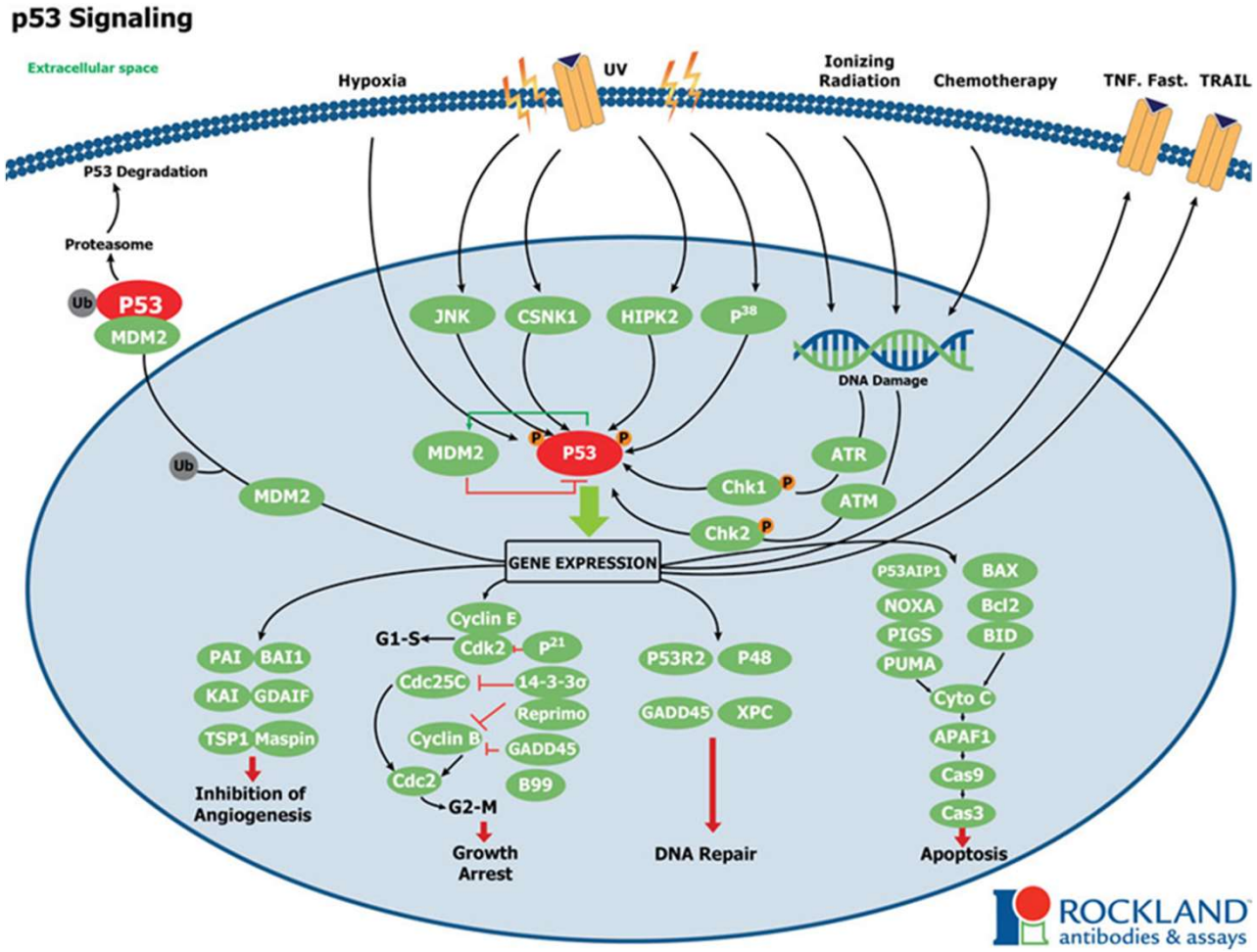
- Inactivating.

- Recessive (associated with Loss Of Heterogeneity) ("recessive oncogenes").
- Occur in somatic and also in germ cells.

Tumor suppressor

- p53 – guardian of the genome.
- 17p13.1, 53 kDA.
- Proliferation, apoptosis, DNA repair, angiogenesis, replication, cell division...
- Rapid degradation.
- Regulated MDM2 protein – transfer to cytoplasm, inactive.
- Induction of expression after cell exposure to stress.
- Cell cycle arrest, apoptosis.
- Mutations in more than 50% of human tumors.
- Li Fraumeni syndrome – hereditary tumor syndrome, germ mutation p53.
- Mutations of inactivating, recessive, somatic and germ cells.

Tumor suppressor



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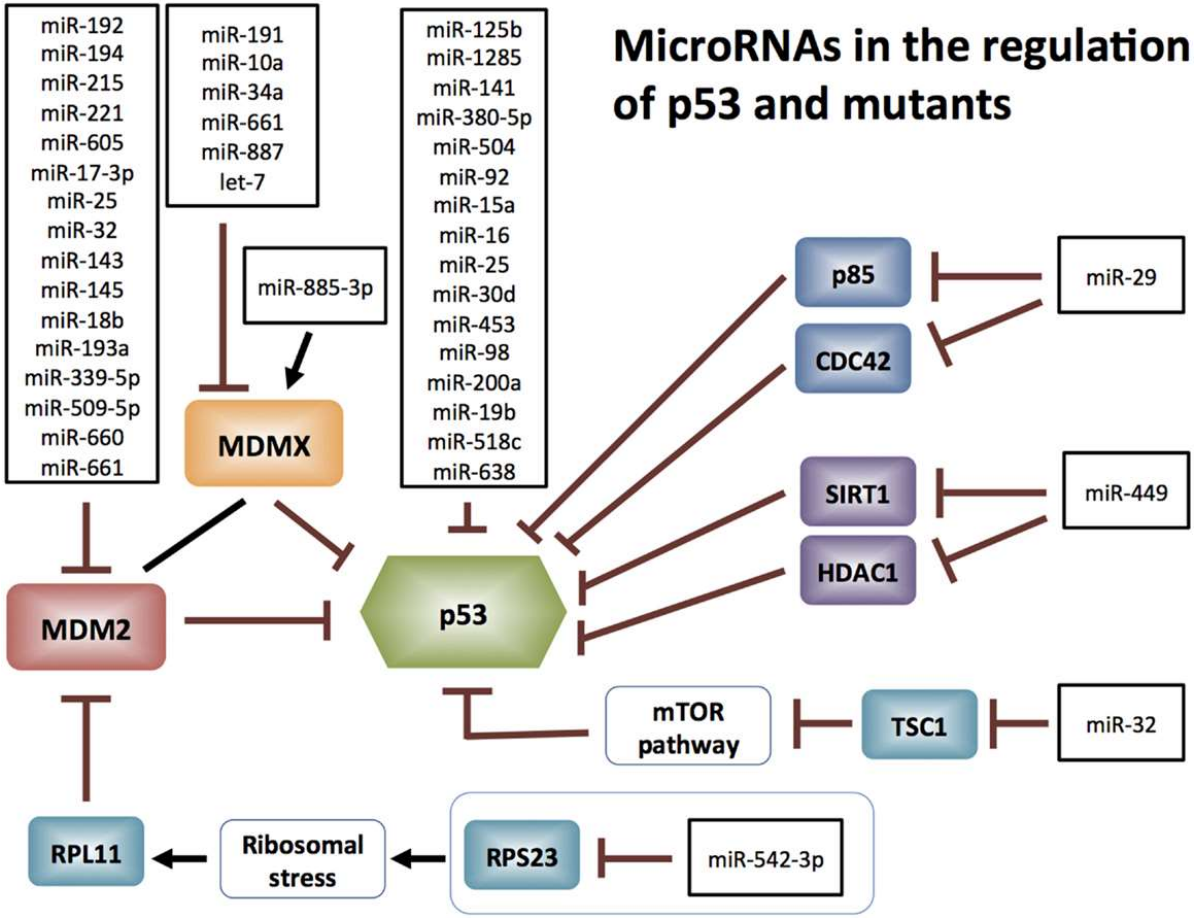
Tumor suppressor



https://www.frontiersin.org/articles/10.3389/fonc.2015.00284/full

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Types of genes mutated in cancers

- Gatekeeper genes - genes that regulate growth and differentiation; include oncogenes and tumor suppressor genes.

- Caretaker genes - genes that help to maintain genetic integrity; their loss of function mutations lead to:
  - Microsatellites instability (due to mismatch repair deficiency).
  - Chromosomal instability (gain or loss of chromosomes or parts thereof).

- Landscaper genes - genes that when mutated lead to abnormal extracellular or intracellular environment that contributes to carcinogenesis.

Risk factors

- The risk of developing a tumor increases with increasing age.
  - Exposure to chemicals.
  - Viruses.
  - Mutations.
  - Lifestyle?

- Early diagnosis and treatment important.

- Identification of persons at risk.

Journal of Nucleic Acids, January 2010, (6551):592980

Chemicals increase risk of cancer

- Asbestos.
- Pesticides.
- Herbicides.
- Benzene.
- Radioactive substances.
- Chemicals in food – herbicides, pesticides, E-numbers.

Oncogenic (Cancerogenic) viruses

- Retroviruses (RNA viruses): contain an oncogene (acutely transforming viruses) in their genome or activate the proto-oncogene next to which they have integrated (slowly transforming).

- DNA oncogenic viruses use a different transformation strategy: they do not contain oncogenes, but encode proteins that interact with tumor suppressors (RB, p53, p300/CBP) of the host cell and thus push the host cell into the S phase:
- SV40 - large T antigen interacts with p53, RB, p300/CBP through different domains.
- Adenovirus - E1A interacts s RB a p300/CBP; E1B associates s p53.
- Papilomavirus HPV-16, HPV-18 - E6 interacts with p53, p300/CBP; E7 interacts with RB.

Oncogenic viruses and human diseases

- RNA viruses:
- human lymphotropic virus type I (HTLV-1) causes adult T-cell leukemia (ATTL)

- DNA viruses:
- Epstein-Barr Viruses (EBV) – Burkitt´s lymphoma (BL), Hodgkin lymphoma (HD), Lymphomas, Nasopharyngeal carcinomas (NPC).
- Hepatitis B virus (HBV) - Hepatocellular carcinoma (HCC).
- Human papillomaviruses (HPV 16, 18,..) - anogenital tumors, oral tumors, warts.
- Human herpesvirus type 8 (HHV8) - Kaposi sarcoma (KS).

Historical perspective

- 400 BC Hippocrates described tumors as long prominences.
- Grece: karkinos = crayfish; onkos = crab
- Latin cancer = crayfish.

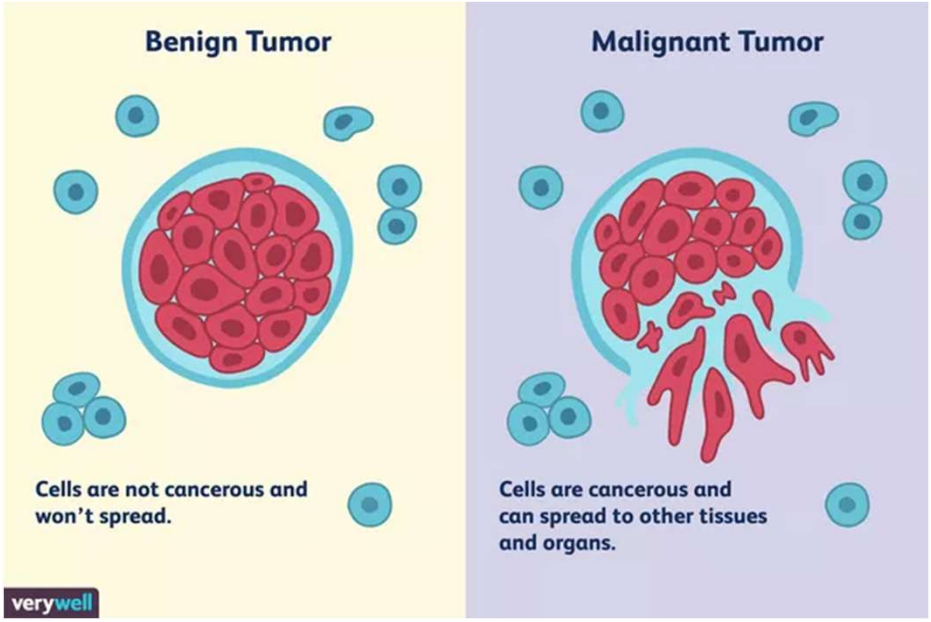
- Descriptive knowledge:
- 1848 - increased incidence of breast cancer in nuns (childlessness, non-breastfeeding).
- 1902 - Connection of X-rays and cancer induction .
- 20th century - occurrence of familiar type of tumors.

Classification of tumors

- Based on their ability to invade other tissues.
- Benign - they remain at the place of their origin, do not migrate, do not invade other tissues.
- Malignant - they invade into surrounding tissues and, through the blood and lymphatic system, throughout the body, in new tissues they provoke the formation of secondary tumors (metastases).
- From this point of view, tumors can be classified into primary and secondary as well.

Classification of tumors

- Based on their ability to invade other tissues.



Benign Tumors	**Malignant** Tumors

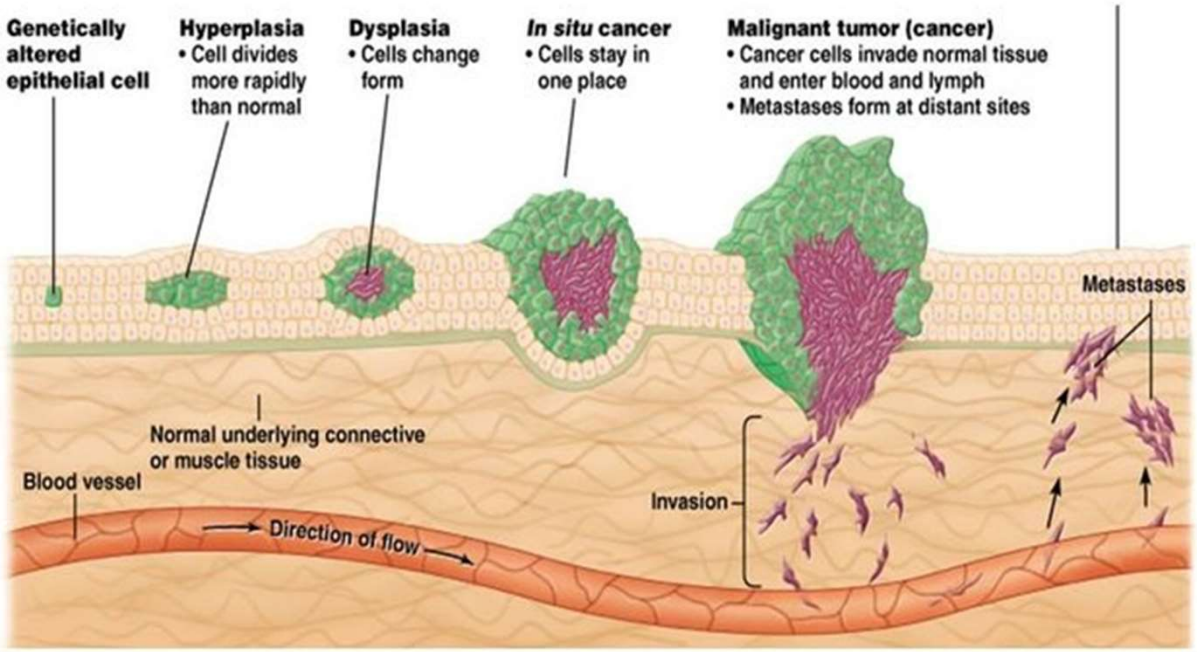
• Small
• Slow-growing
• Non-invasive
• Well-differentiated
• Stay localized
  • Stay where they are.
  • Can't invade or metastasize.
	• Large
	• Fast-growing
	• Invasive
	• Poorly-differentiated
	• Metastasize
	  • Infiltrate, invade, destroy surrounding tissue.
	  • Then metastasize to other parts of body.

https://diffzi.com/benign-tumor-vs-malignant-tumor/

https://www.verywellhealth.com/what-does-malignant-and-benign-mean-514240

Stages of tumor development

- Hyperplasia - is the stage where genetically altered or abnormal cells show uncontrolled and rapid growth.
- Dysplasia - stage of tumor development where overgrowing cells change their original form. It consists of more immature cells than mature.
- In situ cancer - represent neoplastic lesion where cells do not go into the process of maturation, lost their tissue identity and grow without regulation.
- Malignant tumor - overgrowing cells invade other areas by rupturing basal membrane.
- Metastases occur when cancer cells reach to the distant parts through lymphatic system and blood circulation.



https://www.researchgate.net/deref/https%3A%2F%2Ftel.archives-ouvertes.fr%2Ftel-00912341

Classification of tumors

- Based on their origin – from which cell types and tissues.
- Carcinomas – tumors of epithelial cells (about 90% of human tumors).
- Sarcomas – solid tumors of connective tissues – muscles, bones, cartilage.
- Leukemia and lymphomas - derived from hematopoietic cells and cells of the immune system.
- Gliomas - tumors derived from nervous tissue.

Classification of tumors

- Based on the invading organ.

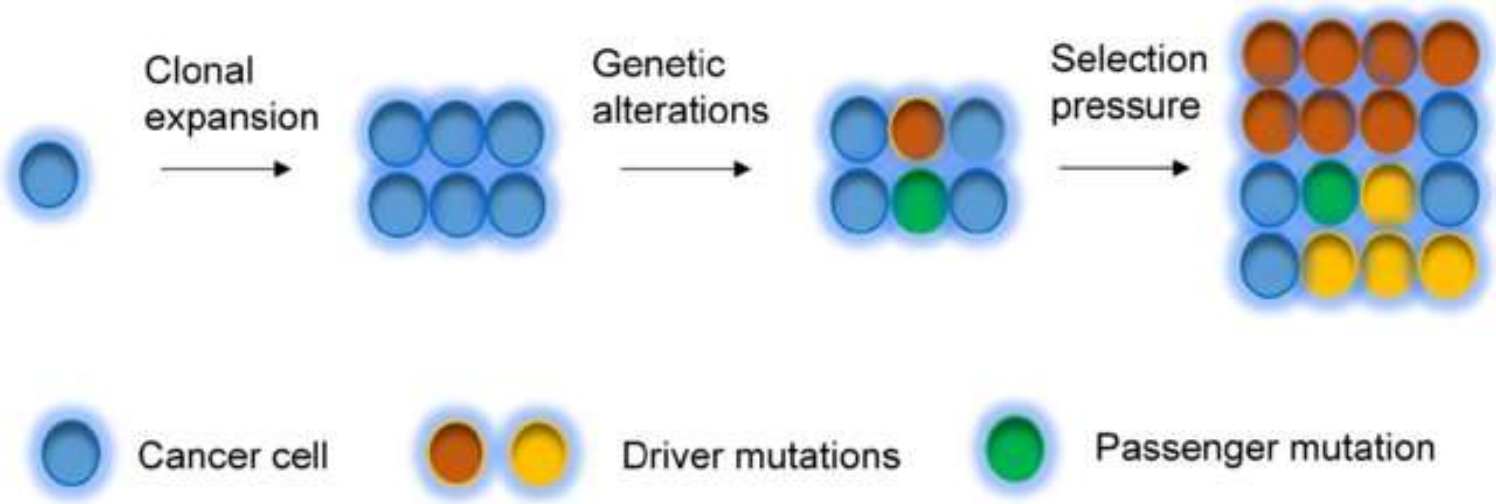
- lung cancer
- colorectal cancer
- breast tumor
- acute myeloid leukemia
- and many other types

Cancerogenesis

- Cancerogenesis or Carcinogenesis - is the process of tumor formation and development. It could be also defined as the process of tumor onset and progression.
- The essence of carcinogenesis is the gradual accumulation of genetic (and epigenetic) changes – mutations.
- It is multistage process.
- Neoplastic transformation - is the transformation of a somatic cell into a tumor cell.

Cancerogenesis

- Carcinogenesis - accumulation of genetic mutations and epigenetic changes.
- Under selection pressure, earlier driver mutations, and also passenger mutations obtain advantage for outgrowth of clones and drive tumors grow.

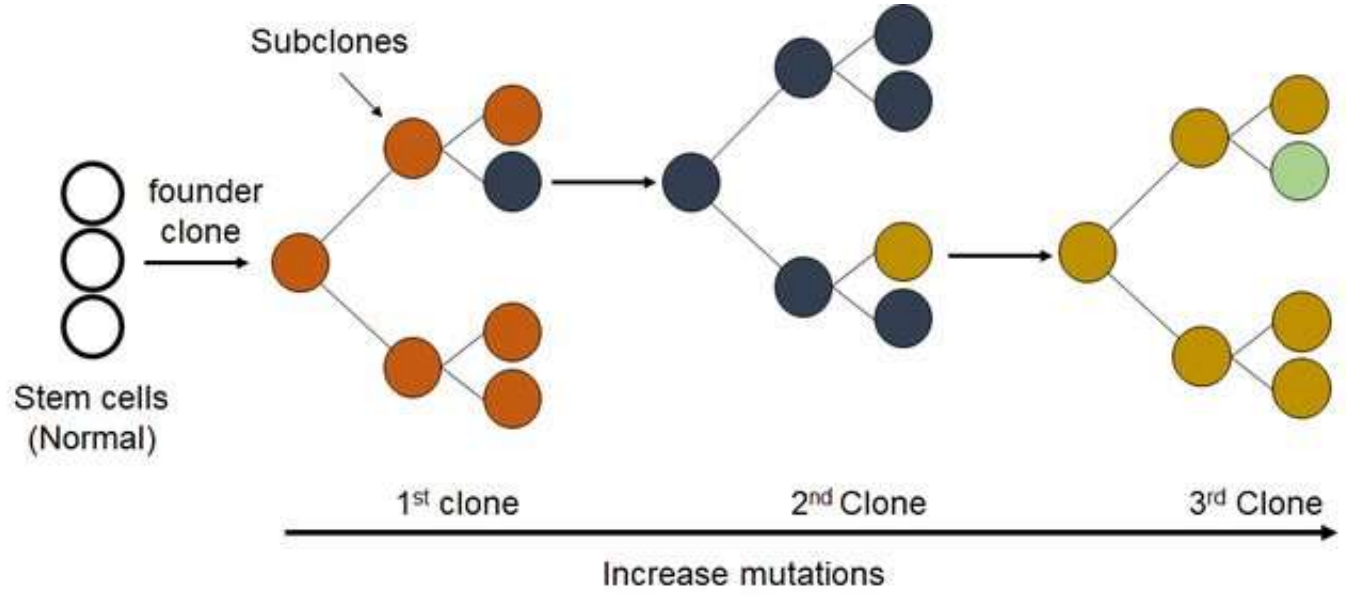


International Journal of Biological Sciences, July 2017, 13(8), 949-960.

Cancerogenesis

- The clonal expansion of a population of mutated cells (cancer cells) from an individual single-cell causes tumor heterogeneity in pathology and molecular profiles.

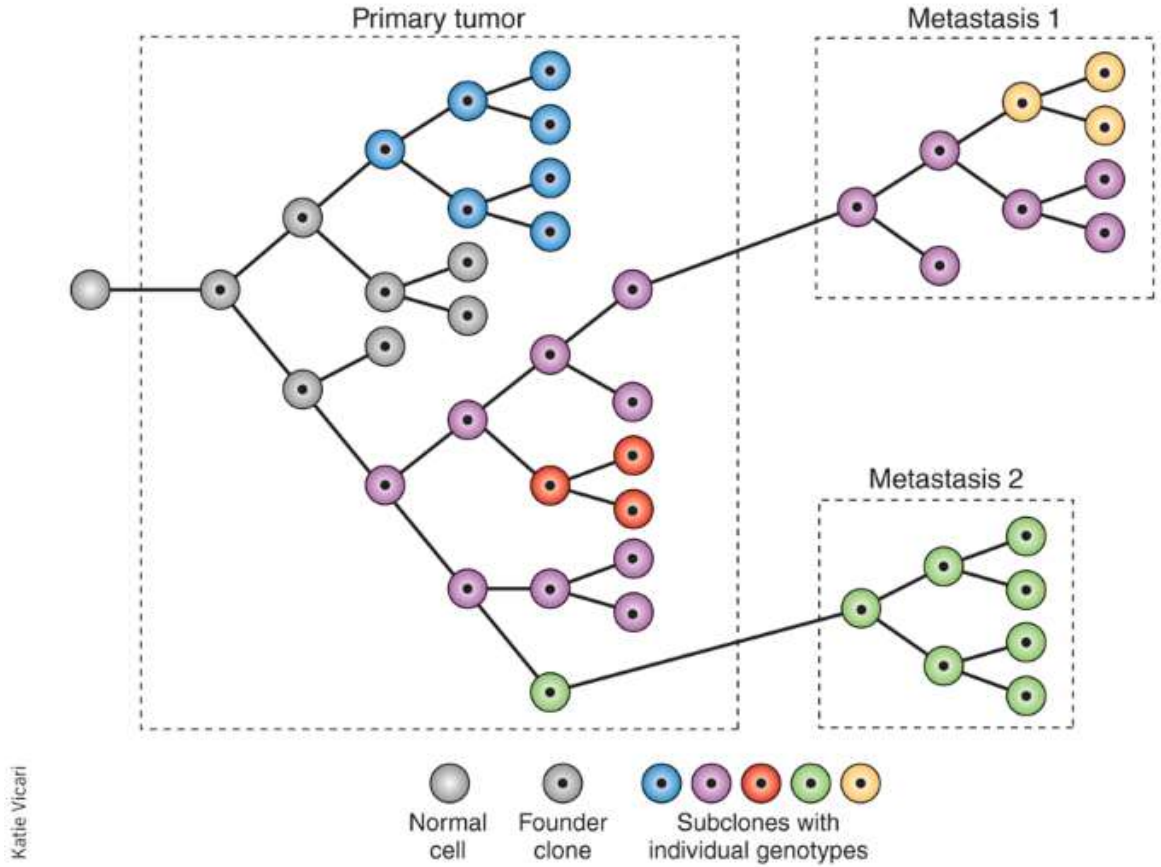
- Tumor heterogeneity within tumors is created by genetic and epigenetic changes.



Cancers, April 2020, 12(1050)

Cancerogenesis

- Nowell's theory of tumor evolution.
- The tumor arises from a single cell.
- Dominance of one clone.
- Aggressive clones spread, passive ones perish
- Selection, clonal expansion.
- Clonal diversity is accompanied by genetic heterogeneity.



Nature Biotechnology volume 30, pages408–410 (2012)
Science. 1976 Oct 1;194(4260):23-8.
Blood. 2012 Aug 2;120(5):927-8.

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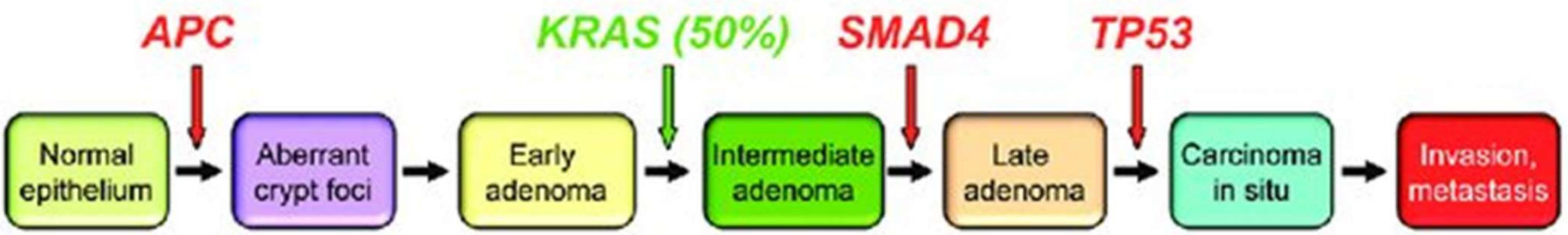
Cancer multistage process

- Colonic epithelial cells undergo a histologic transition from normal to malignant state that is driven by specific genetic events including inactivation of tumor suppressors (APC, SMAD4 and TP53) and activation of the KRAS oncogene.

- The three stages of adenomas represent tumors of increasing size, dysplasia, and villous content.



Tumor – histology

- All tumors, benign and malignant, have two basic components:

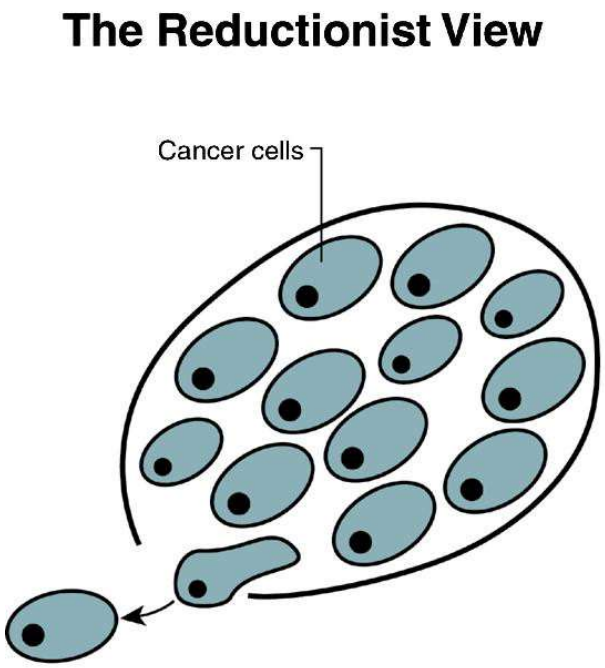
- Stroma - supporting, host-derived, non-neoplastic, made up of connective tissue, blood vessels, and host-derived inflammatory cells.
  - Crucial to the growth of the neoplasm.
  - Carries the blood supply.
  - Provides support for the growth of parenchymal cells.

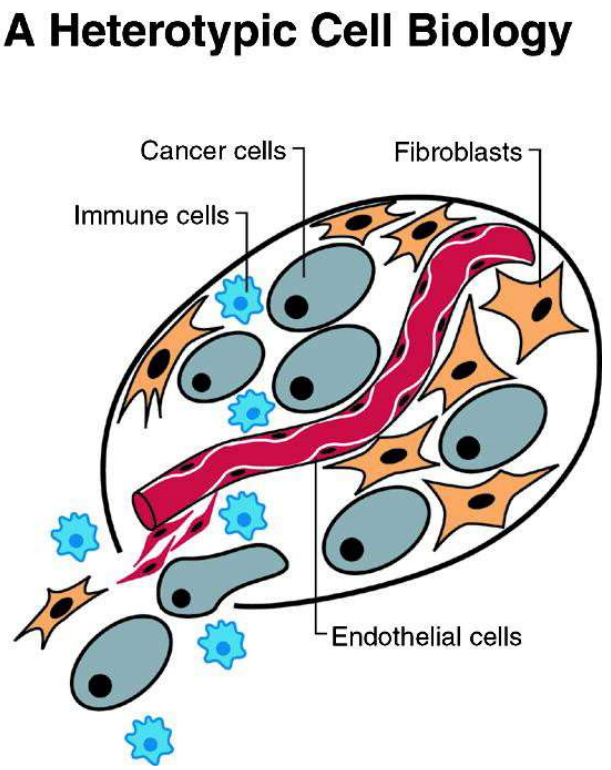
- Parenchyma - made up of transformed or neoplastic cells.
  - Largely determines its biologic behavior.
  - Tumor derives its name.

https://www.slideshare.net/DeepakKumarGupta2/general-aspect-of-neoplasia

Tumor is a complex tissue

- The interactions between the genetically altered malignant cells and these supporting coconspirators proves to be critical in cancer onset and progression – microenvironment.





https://www.nature.com/subjects/double-strand-dna-breaks

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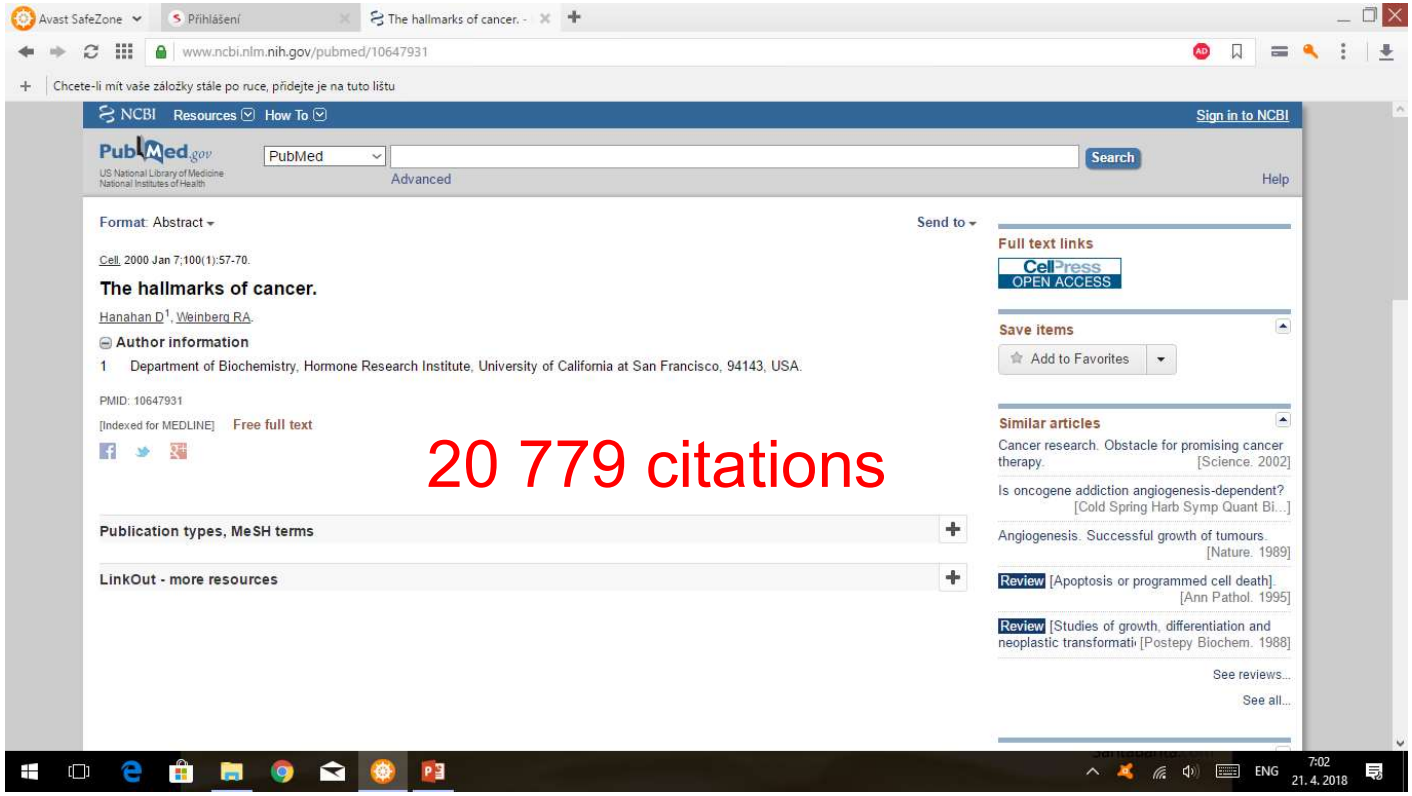
- The Hallmarks of Cancer

The hallmarks of cancer

- Hanahan and Weinberg



Cell. 2000 Jan 7;100(1):57-70.

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The hallmarks of cancer

- Robert Weinberg
  - 1982 – Discovered Ras, the first human oncogene
  - isolated Rb for the first time in his lab
  - MIT

Robert Weinberg

- Dough Hanahan
  - MIT, UCSF
  - Director of École Polytechnique Fédérale de Lausanne
  - 1983- SOB medium for bacteria

Douglas Hanahan

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What genes are altered in cancer?

- A tumor is not a monogenic disease.
- It is estimated that 4 - 7 events (interventions) are necessary for the development of the tumor.
- There are dozens of specific genes that can be altered during carcinogenesis.
- In general, there are six (seven?) basic acquired features of a fully malignant tumor.

Tumors must acquire the same hallmarks capabilities

- Weinberg & Hanahan

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

Cell. 2000 Jan 7;100(1):57-70.

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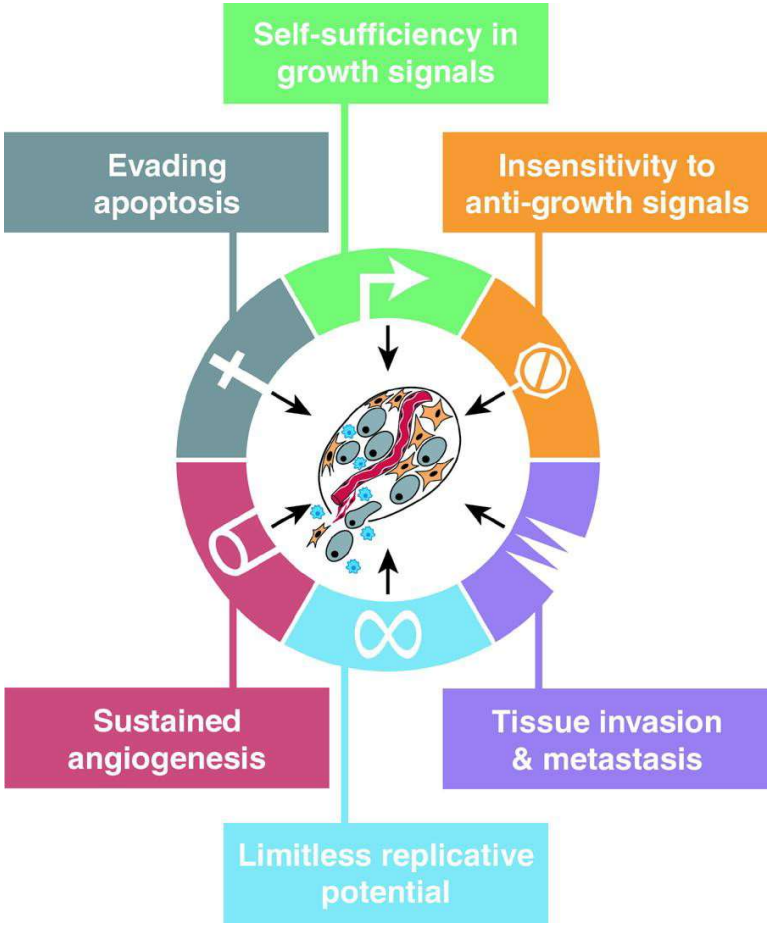
Tumors must acquire the same hallmarks capabilities



- Genome instability as a necessary condition for the accumulation of all necessary mutations?

Cell. 2000 Jan 7;100(1):57-70.

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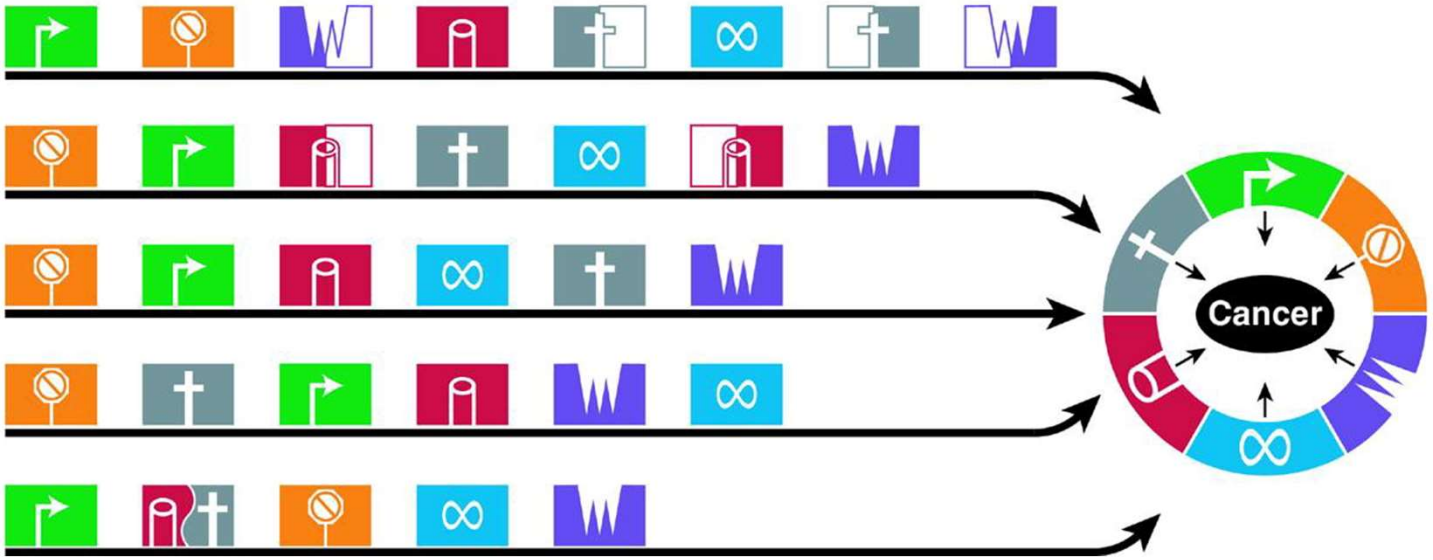
Cancerogenesis has an individual course

- Thus, the order in which these capabilities are acquired seems likely be quite variable across the spectrum of cancer types and subtypes.

- In some tumors, a particular genetic lesion may confer several capabilities simultaneously, decreasing the number of distinct mutational steps required to complete tumorigenesis.



Cell. 2000 Jan 7;100(1):57-70.

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Tumors must acquire six hallmarks capabilities

Component	Acquired Capability	Example of Mechanism
	Self-sufficiency in growth signals	Activate H-Ras oncogene

Sustainig cell signaling

- Healthy cells – carefully control growth and division. The signal, binding to receptor, triggering of the signaling pathway.

- Tumor cells deregulate these signals. They do not need stimulation by growth factors to proliferate.

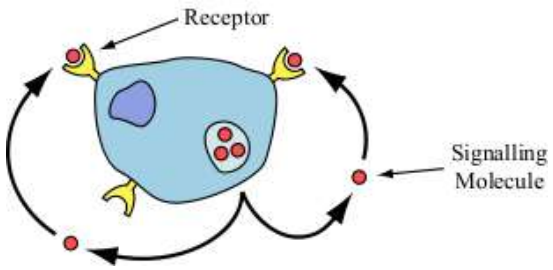
- Tumor cells can produce:
  - Their own growth factors, such as PDGF – glioblastomas.
  - Receptor overexpression - EGFR – breast and stomach tumors.
  - Change the receptor structure – receptor does not need a ligand to activate.

Growth factors and Receptor-tyrosine kinases

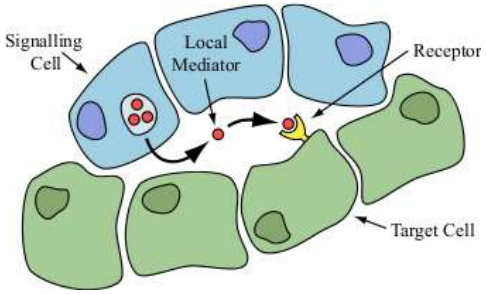
- Growth factors: Polypeptides, which are produced by cells and induce signaling to start or stop proliferation, differentiation, survival,... by activating its specific receptors on the cell surface.

- Stimulation: Three types of communication systems in biological systems differs manly by the distance that the communication operates over.
  - Autocrine - growth factor stimulates the producing cell.
  - Paracrine - stimulation of a neighboring cell.
  - Endocrine - stimulation of distant cells.

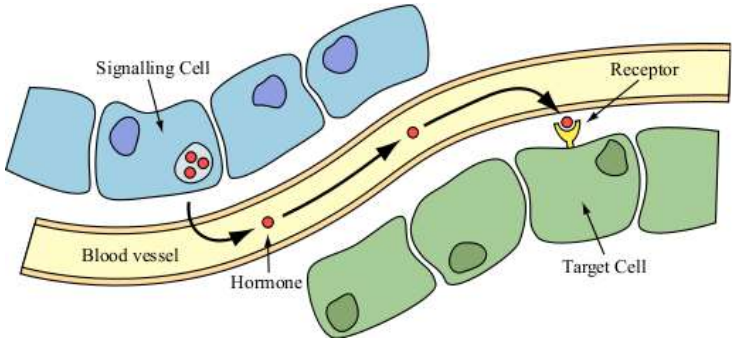
Autocrine



Paracrine



Endocrine



http://www.labbookpages.co.uk/research/bioNode.html

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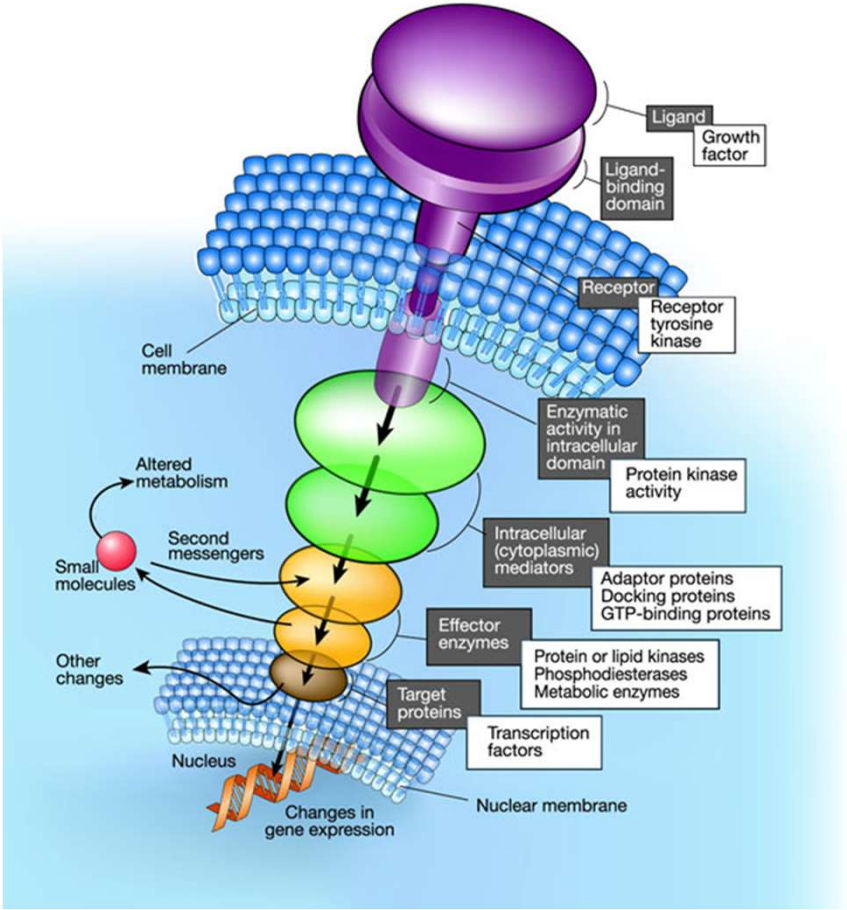
Cell signaling

- This distribution is related to the structure of the signal pathway:
  - Growth factor binding to receptor.
  - Activation of receptor protein kinase.
  - Signal transmission to the nucleus by a cascade of protein kinases.
  - Activation of the transcription factor.



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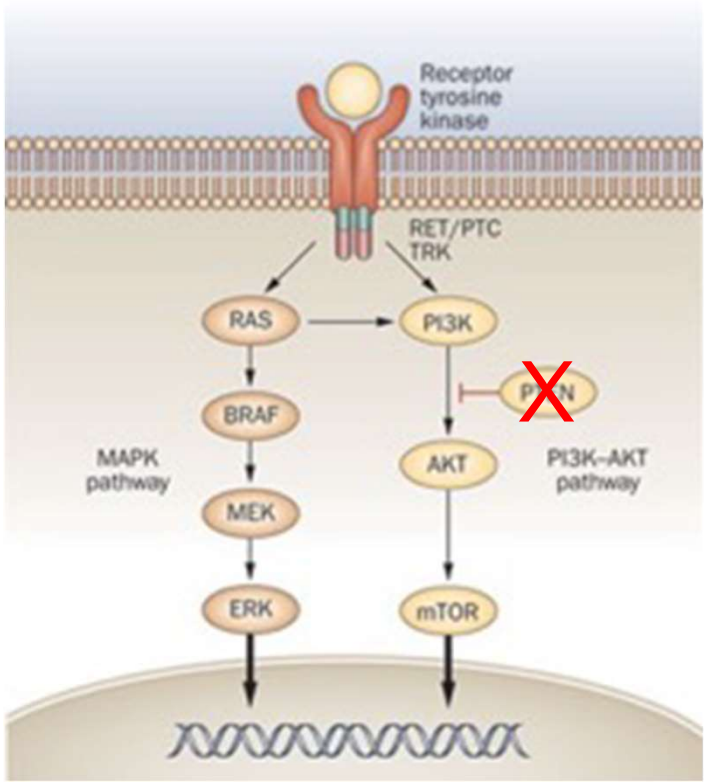
Sustainig cell signaling

- Cellular pathways critical for cell survival are deregulated in tumors.
- Mutation – activation/inactivation of the pathway leads to:
  - Uncontrollable proliferation.
  - Invasiveness.
  - Resistance to signals.
  - Angiogenesis.
  - Metastasizing.
  - Resistance to apoptosis.



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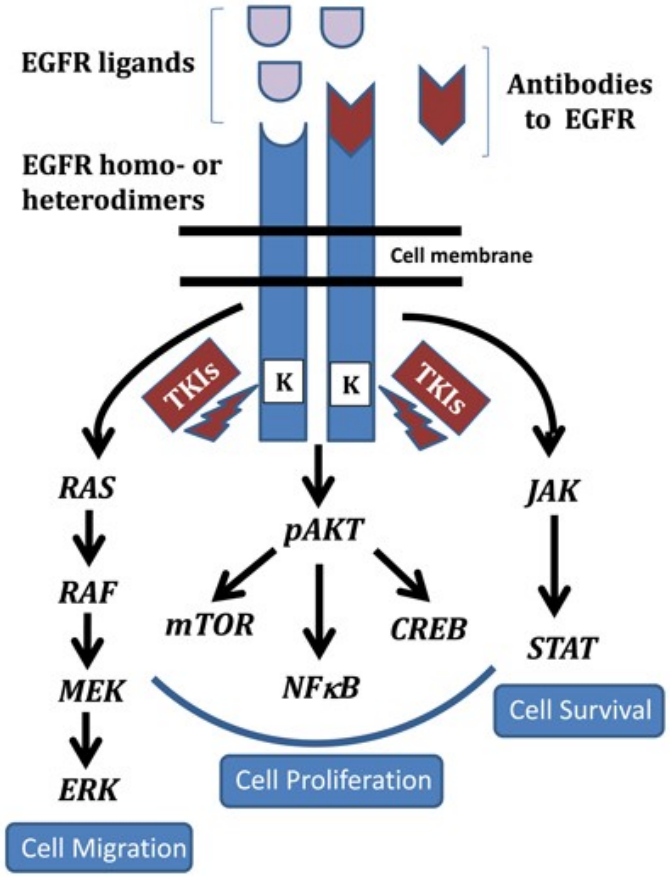
Sustainig cell signaling



- Activation of the EGFR pathway induces cellular survival and anti-apoptosis signals by activating transcription of genes associated with cell survival - NFκB and JAK/STAT.
- EGFR is activated either as a homo- or heterodimer resulting in regulation of multiple pathways.
- In particular the RAS/RAF/MAPK, AKT, and JAK/STAT pathways downstream of EGFR play integral roles in cell migration, proliferation, and survival, respectively.
- Anti-EGFR antibodies are targeted to the external ligand binding domains while the small molecule inhibitors or tyrosine kinase inhibitors (TKIs) target its cytoplasmic kinase domains.

https://www.aacc.org/cln/articles/2013/october/egfr-mutations

Tumors must acquire six hallmarks capabilities

Component	Acquired Capability	Example of Mechanism
	Insensitivity to anti-growth signals	Lose retinoblastoma suppressor

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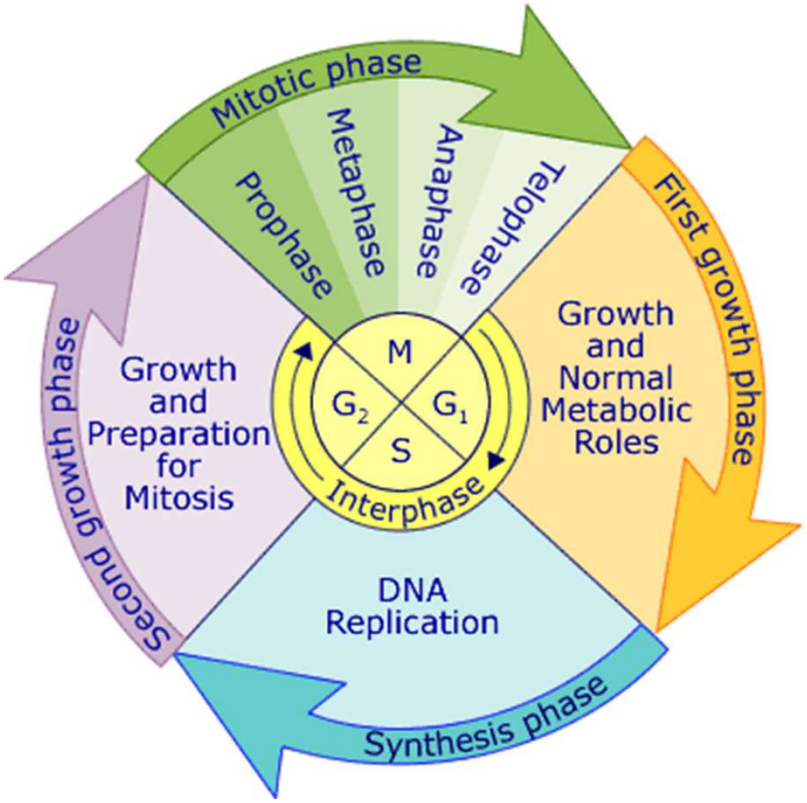
Tumor as disease of the cell cycle

- Loss of the cell cycle regulation is a critical part of cell transformation.
- Loss of the cell cycle regulation is not the only part of cancerogenesis.
- When altered it is not fully transformative itself.

Cell cycle

- Cell cycle:
  - Interphase
  - Mitosis.



https://quizizz.com/admin/quiz/606682e1825246001f488abd/dna-structure-function-and-replication-basics

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Cell cycle

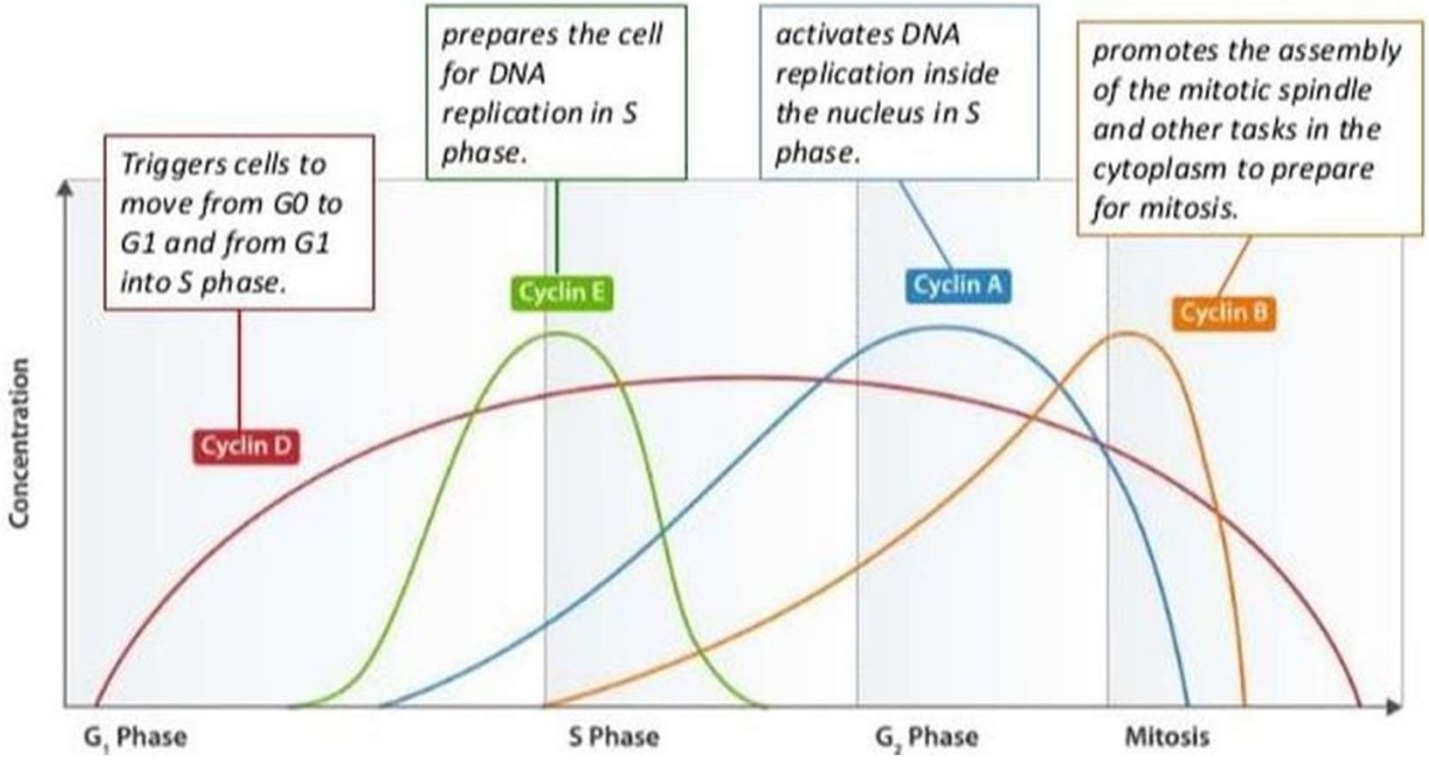
- Each phase of the cycle is catalyzed by specific cyclin-dependent kinase associated with cyclin.

- Cyclin-dependent kinase (CDK):
  - Phosphorylates its substrates.
  - Typically have a catalytic and regulatory subunit, activity always dependent on cyclin binding.

- Cyclins:
  - Their level fluctuates depending on the phase of the cell cycle.
  - They activate the appropriate CDK and direct it to its substrates, then they are quickly degraded.

Cell cycle

- Cyclins level fluctuates depending on the phase of the cell cycle.



https://mysciencesquad.weebly.com/ib-hl-16u5.html

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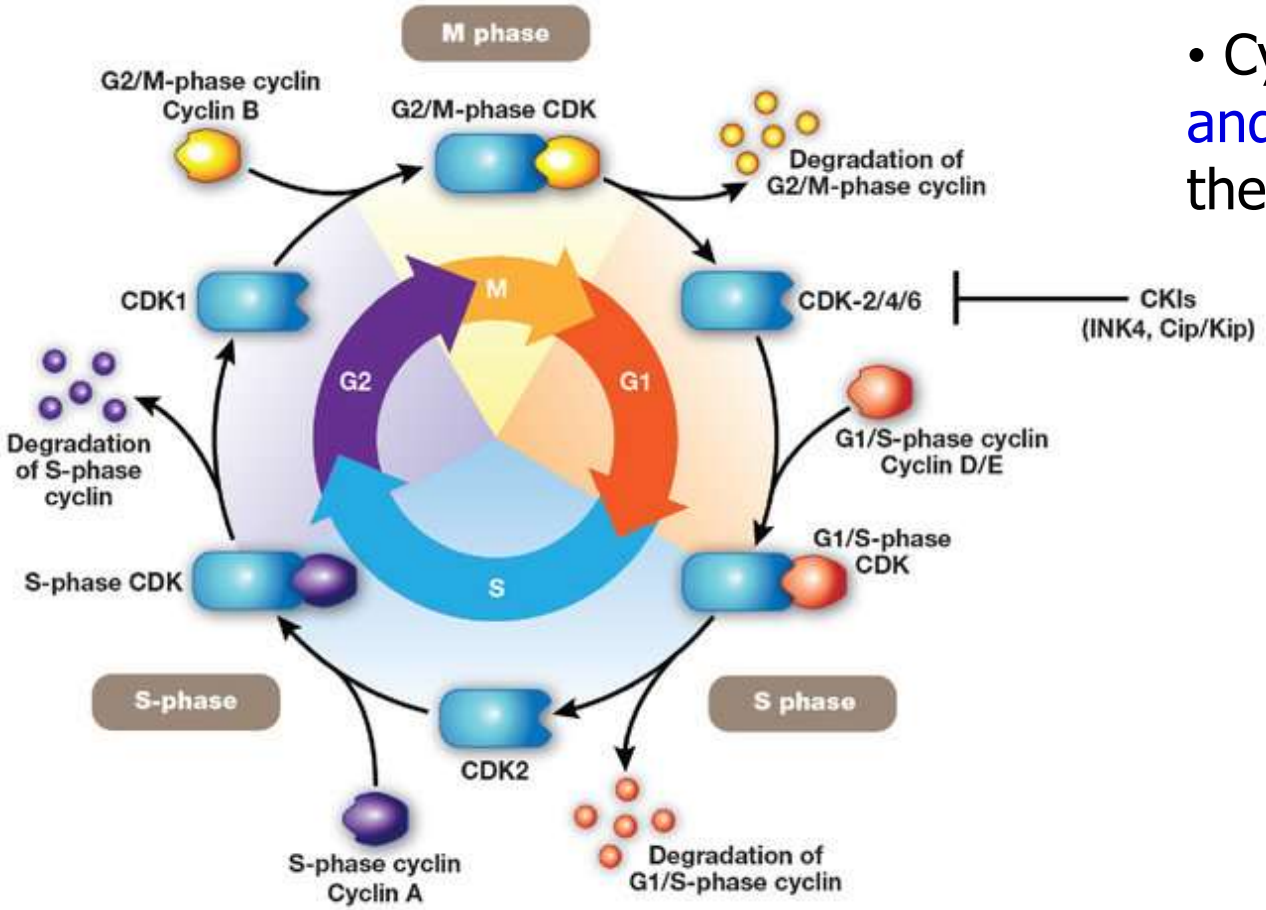
Cell cycle



- Cyclins activate appropriate CDK and direct it to its substrates, then they are quickly degraded.

https://mysciencesquad.weebly.com/ib-hl-16u5.html

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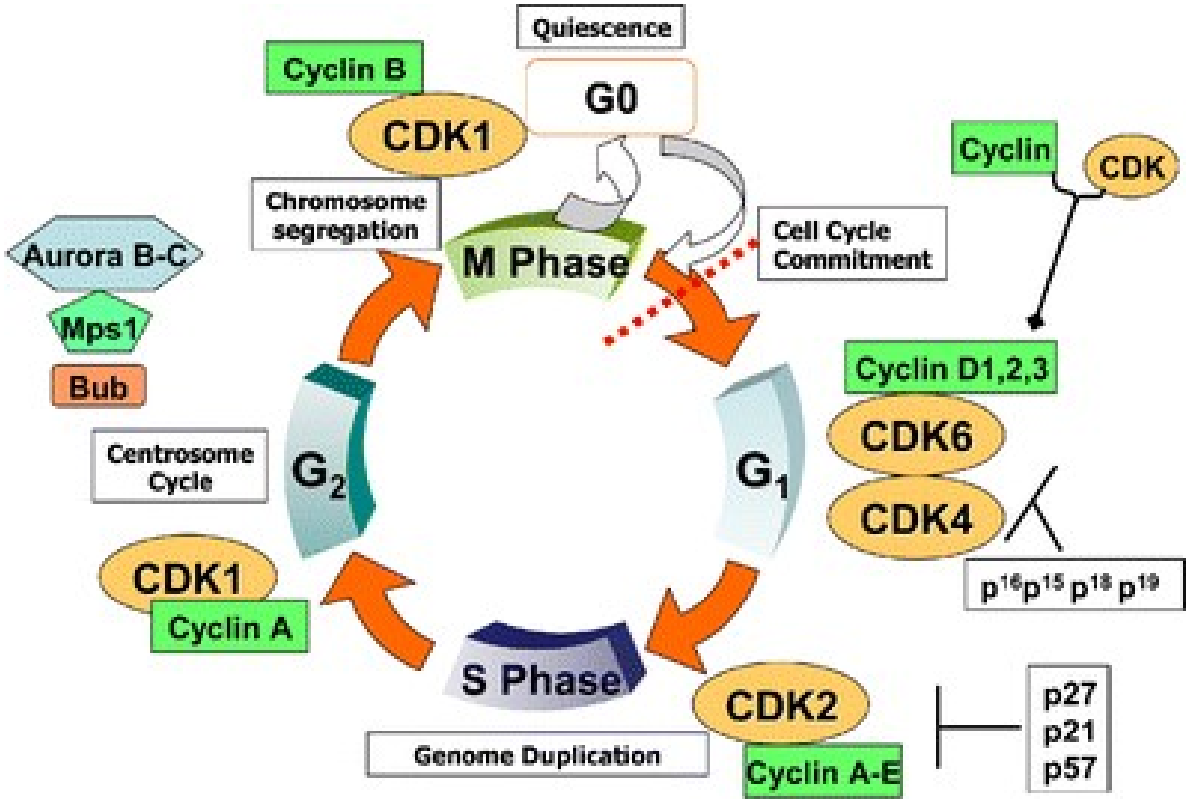
Cell cycle

- Cell cycle:
  - Interphase
  - Mitosis.



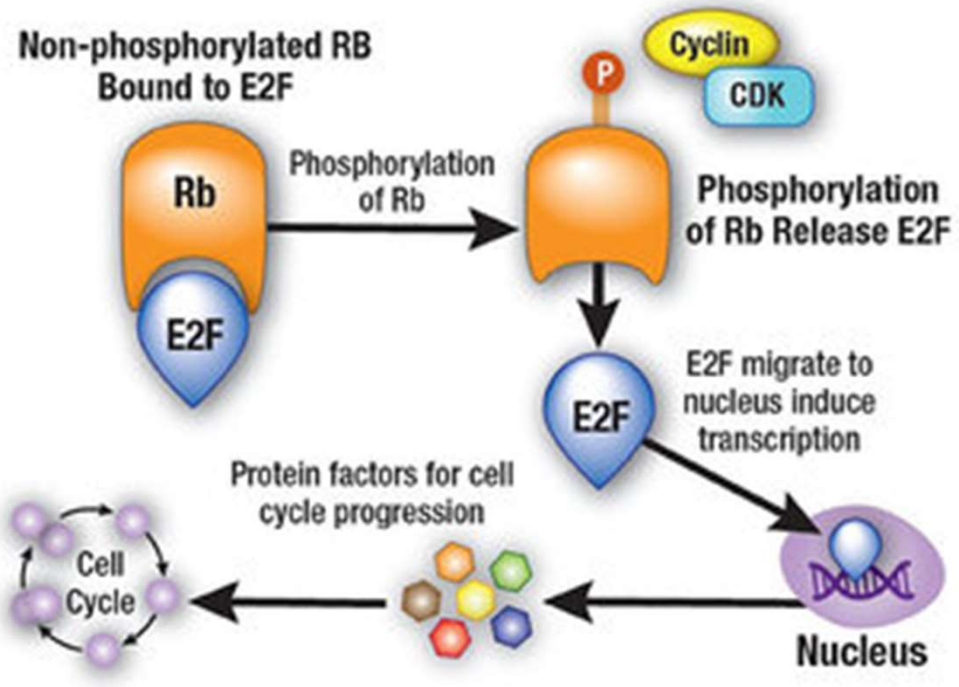
https://link.springer.com/article/10.1007/s10637-009-9236-6/figures/1

MUNI SCI

pRb a principal regulator of cycle

- Retinoblastoma protein - pRB when non-phosphorylated or unphosphorylated state it blocks the passage through the G1 – S restriction point:
  - Interacts with TF family E2F.
  - Blocks their ability to transactivate their target genes - necessary for the S phase.
- Phosphorylation of pRB leads to its inability to bind E2F and passage through the restriction point is thus possible.
- Regulation of passage through the restriction point = regulation of pRB phosphorylation.

Regulation of pRb activity



Oncotarget, February 2019 10(12).

MUNI SCI

Regulation of pRb activity

- The activity of pRB is positively affected:
  - Cyclins D (D1, D2 and D3) with CDK4/CDK6.
  - Cyclin E and CDK2 complexes.

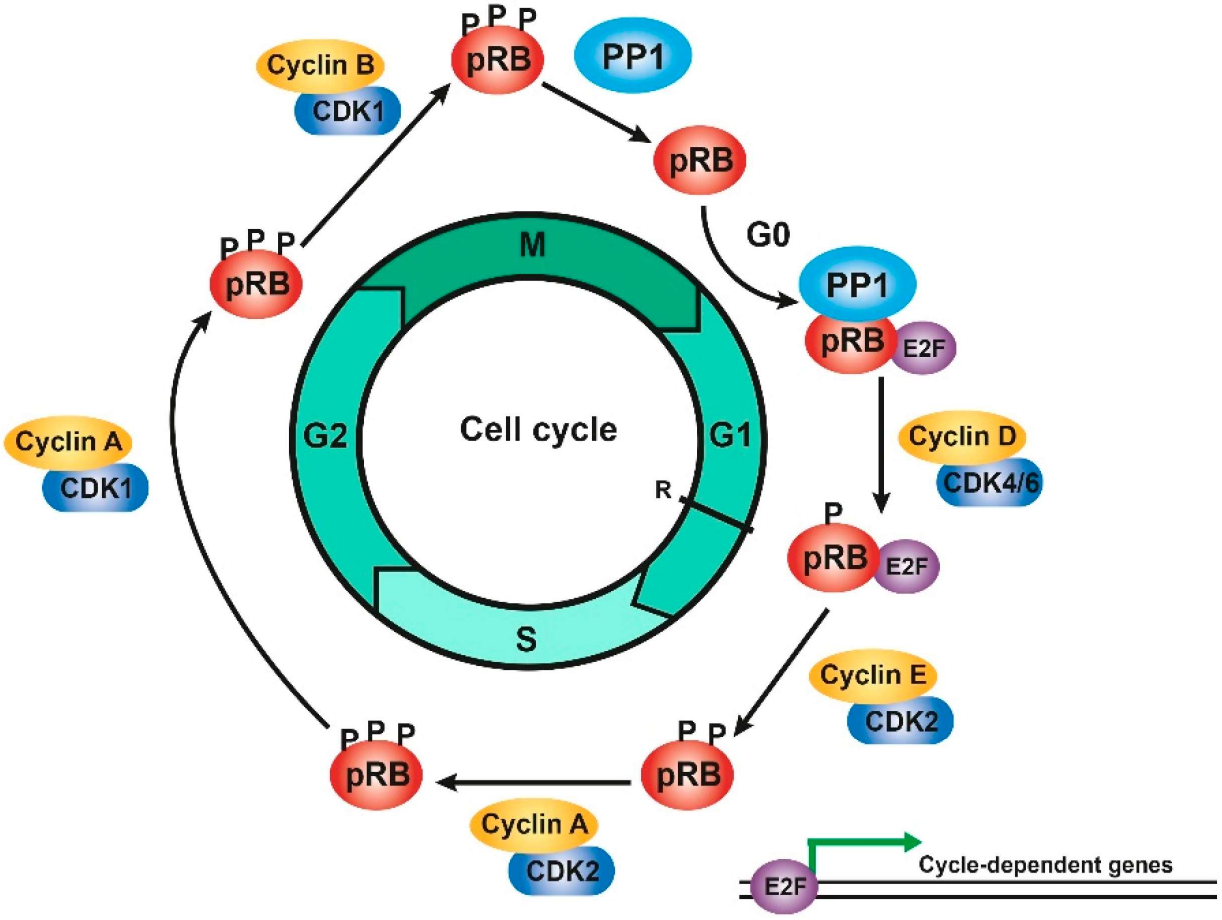
- Negatively affected by cellular CDK/cyclin inhibitors:
  - Cip/Kip family proteins - p21WAF1 a p27KIP1.
  - INK4 family proteins- p15INK4B a p16INK4A.

- Mitogenic signaling leads to increased expression of cyclins D and decreased levels of inhibitors.

- Antimitogenic signaling leads to a decrease in the level of cyclins D and induction of the CDK/cyclin inhibitors.

- Mutation in Rb – often leads to an uncontroled division due to hyperphosphorylated status of pRb.

pRb a principal regulator of cycle



Cancers 2021, 13(9), 2226.

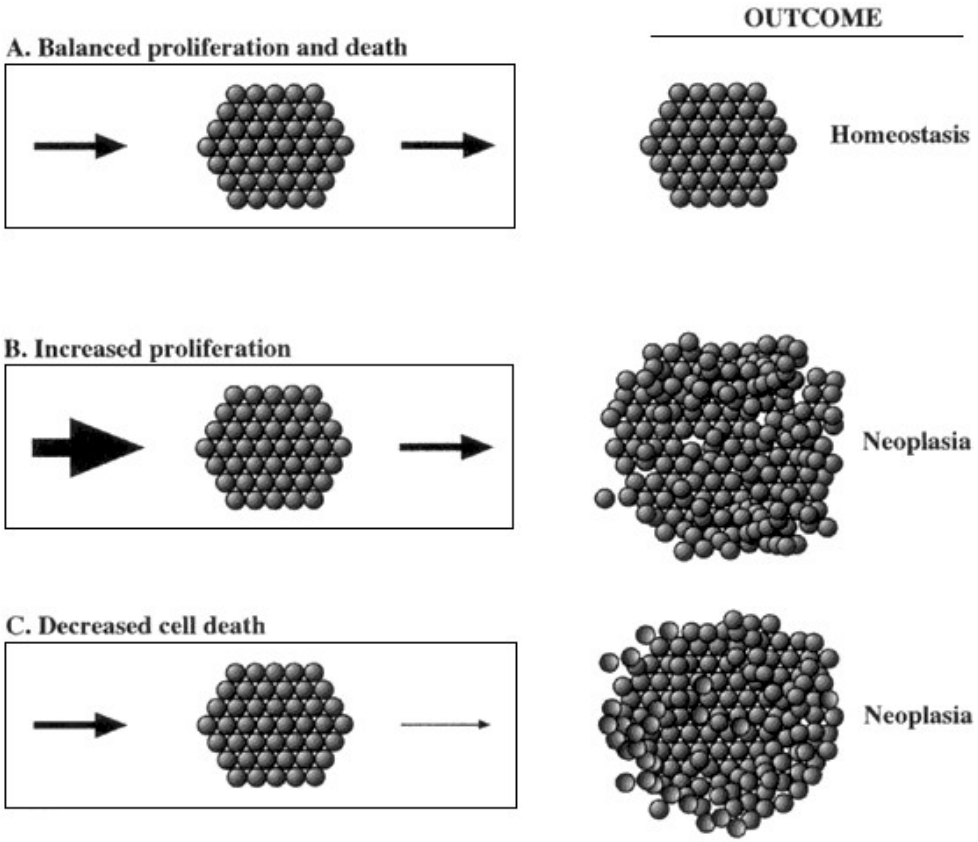
MUNI SCI

Tumors must acquire six hallmarks capabilities

Component	Acquired Capability	Example of Mechanism
	Evading apoptosis	Produce IGF survival factors

MUNI SCI

Cell death and tumors progression

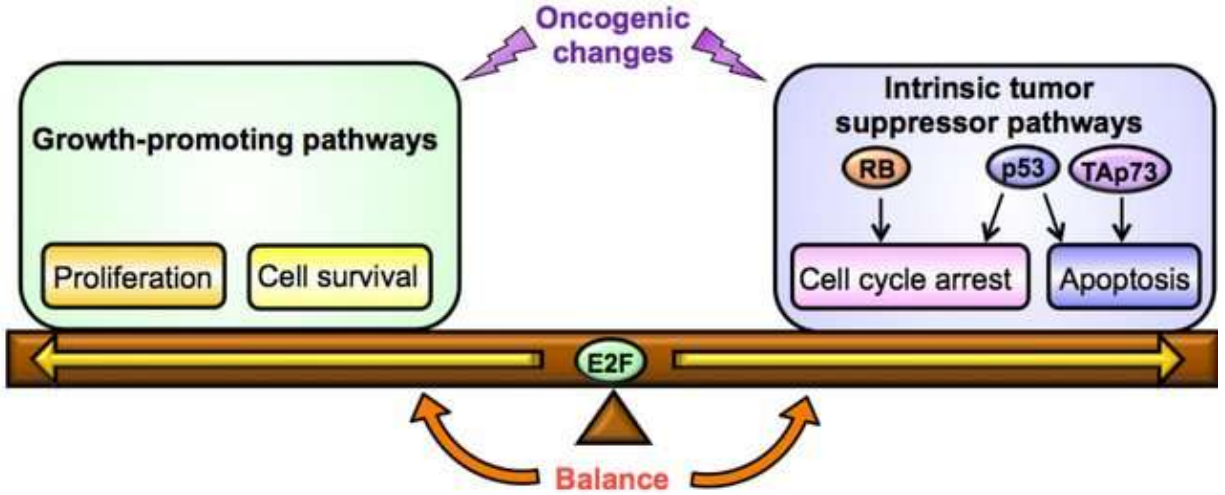


- The rate of tissue growth is determined by the rate or equilibrium of cell division and the rate of cell death.
- During homeostasis, both processes are in balance.
- In a tumor, the balance between cell division and cell death is broken.

MUNI SCI

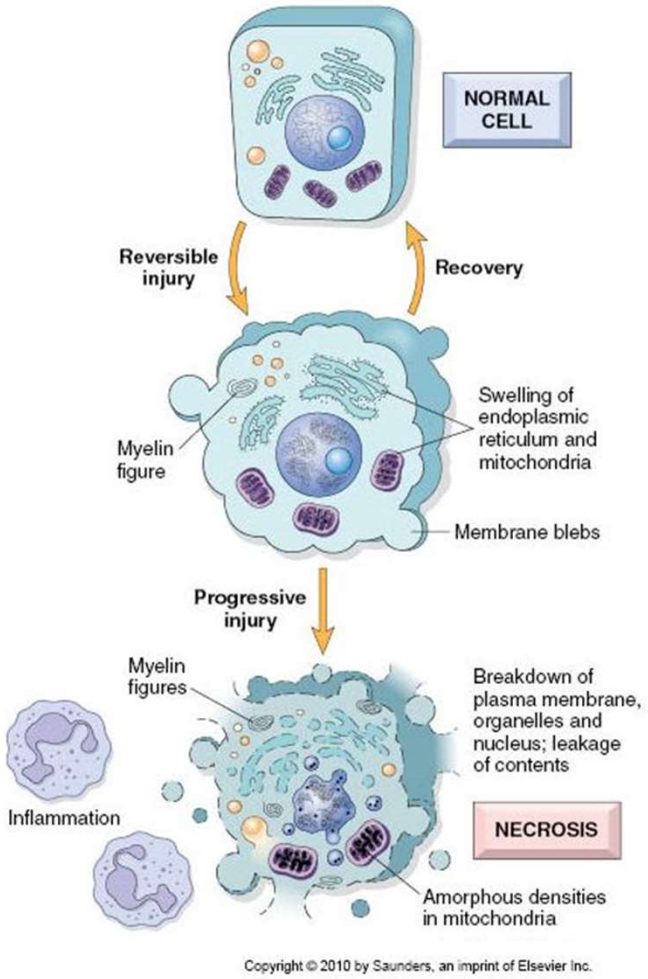
Cell death and tumors progression

- Apoptosis:
  - Cells live for a limited time.
  - Then apoptosis, programmed cell death, is triggered.
  - Tumor cells are resistant to the induction of cell death due to incapability to respond to death signals or they become sensitive to the oncogenic signals.



Future Aspects of Tumor Suppressor Genehttp://dx.doi.org/10.5772/56471

Necrosis



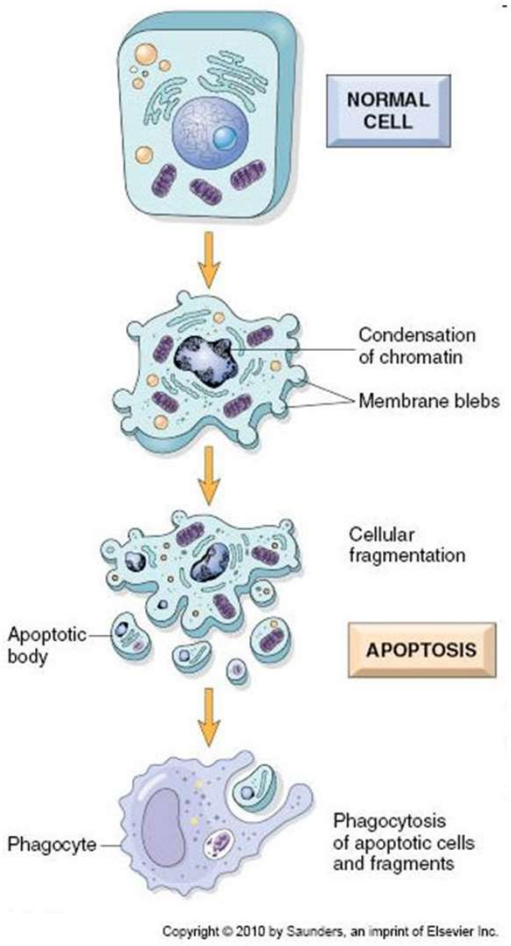
- Necrosis refers to a group of affected cells caused by non-physiological damage (viral infection, hypothermia..), significant inflammatory reaction.

- Morphological features:
  - Begins with swelling of the cytoplasm and mitochondria organelles crumble.
  - Loss of cell membrane integrity.

- Biochemical features:
  - Loss of regulation of homeostasis.
  - Passive process without energy (runs even in 4°C).
  - Random DNA degradation.

MUNI SCI

Apoptosis



- Apoptosis is programmed cell death:
- Affects individual cells.
- Induced by physiological stimuli (lack of growth factors) no inflammatory reaction physiological.

- Morphological features:
  - Condensation of the cell and nucleus.
  - „Blembs“ of cell membranes.
  - Chromatin condensation.
  - Nucleus fragmentation and cell fragmetation into apoptotic bodies.

- Biochemical features:
  - Strictly regulated, active process.

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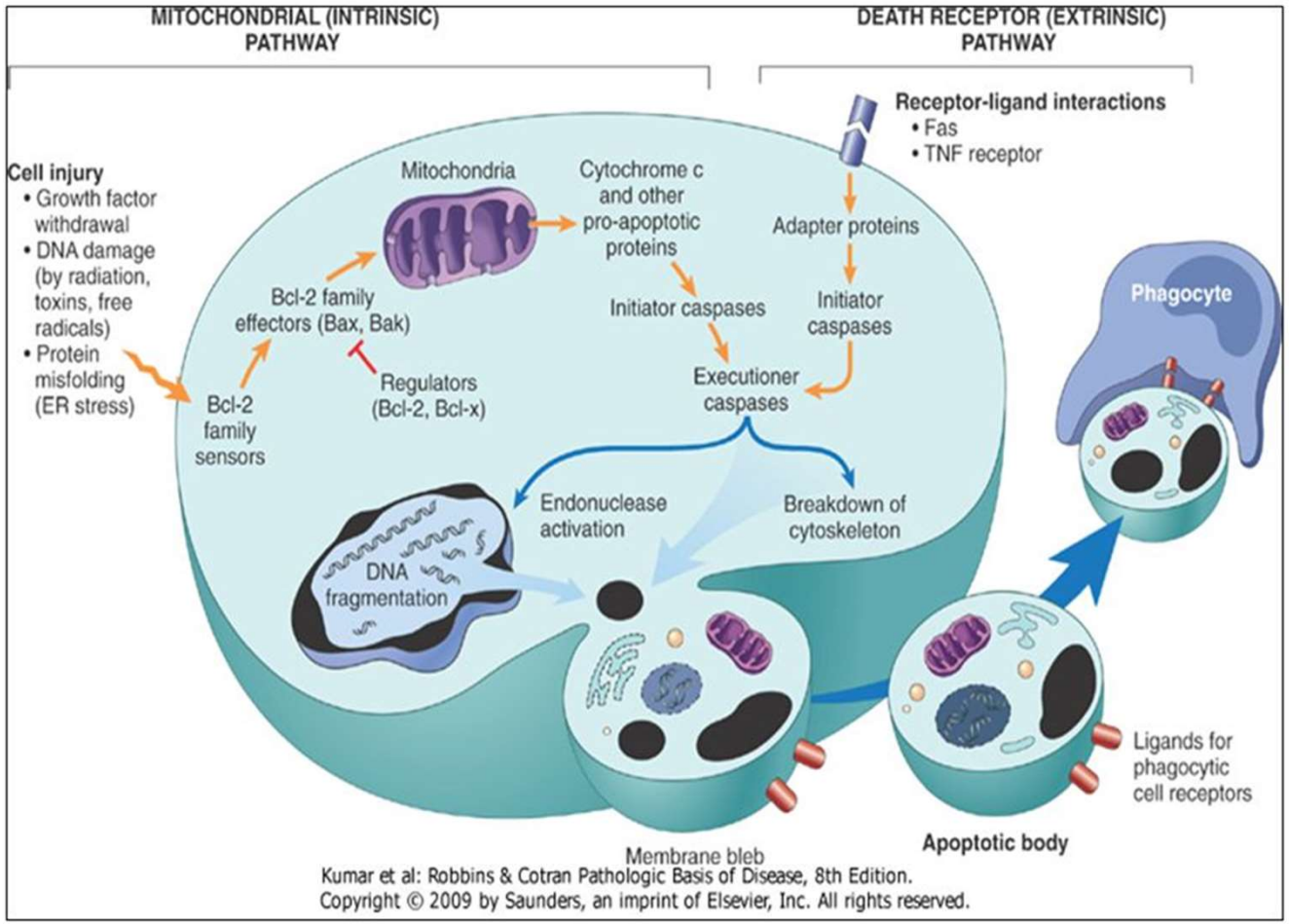
Regulation of apoptosis



- External receptor pathway - ligand binding to the corresponding death receptor produces a proapoptotic signal. This leads to the activation of the domain of death with the cooperation of other proteins. The goal is to activate procaspase 8.
- Internal path - signaling e.g. through p53 initiates an apoptotic process, the component of which is the Bcl-2/Bax system, signaling through mitochondria, the formation of a complex called apoptosome and the goal is to activate procaspase 9.

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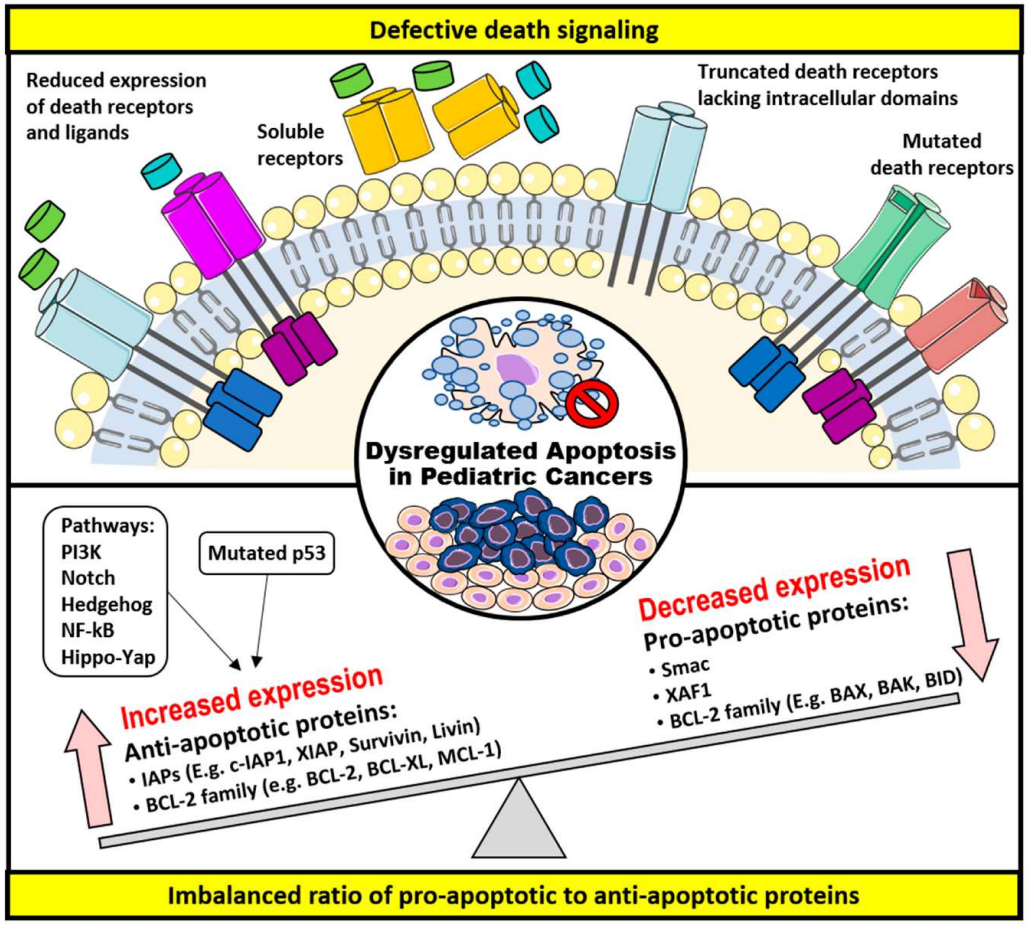
Dysregulation of apoptosis in cancerogenesis

- Apoptosis is dysregulated in cancer leading to disruptions of the delicate balance between cell proliferation and cell death.

- Examples of altered apoptotic proteins in tumors:
  - Upregulation of Bcl-2 (chromosomal translocation) in lymphomas.
  - Upregulation of survival factors IGF-1, IGF-2.
  - Mutation and downregulation of the Fas death receptors.
  - Mutations.
  - Activation of BAX.
  - Inactivation of p53.



Cancers , 2019, 11(11), 1623.

MUNI SCI

Tumors must acquire six hallmarks capabilities

Component	Acquired Capability	Example of Mechanism
∞	Limitless replicative potential	Turn on telomerase

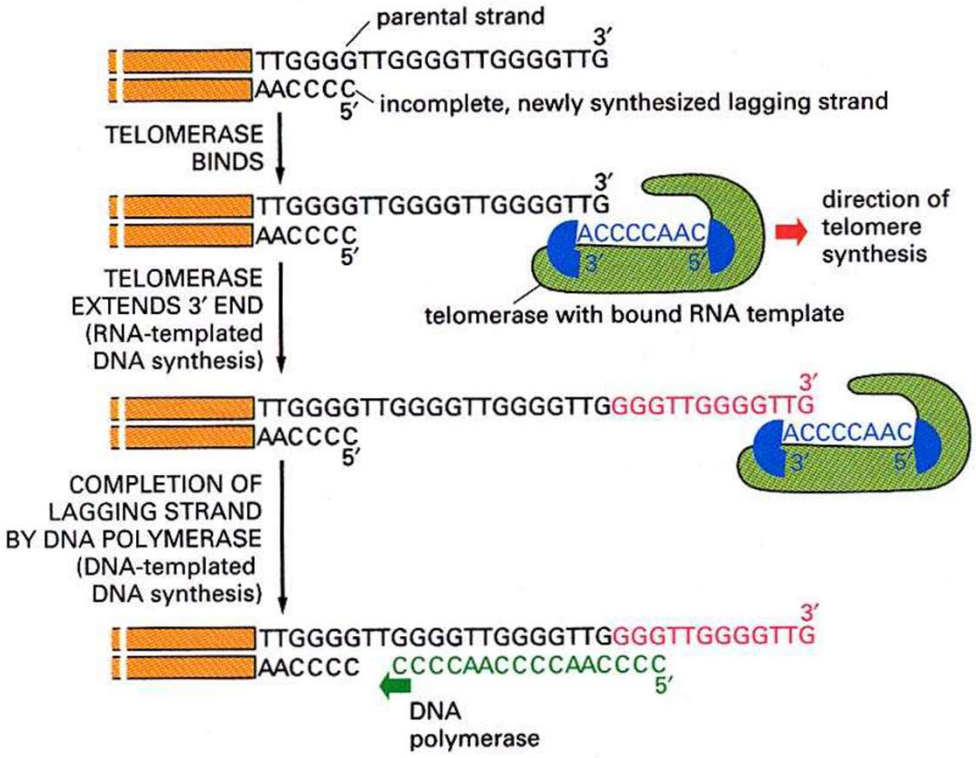
MUNI SCI

Replication potential

- Mammalian cells have a replication potential of 60-70 divisions (Hayflick limit).
- Then they go to the stage of senescence – they change the morphology, metabolically active, do not divide, block cell cyle – p53, Rb.
- Mutation in p53 or Rb – another 30 divisions – crisis, chromosomal aberration – apoptosis.
- If the mutation occurs with probability 10^{-7} – then there is a great chance that immortal cell will appear.
- Most tumor cells are immortal.

Telomerase hypothesis

- When the critical length of the telomeres is reached, signals are triggered that induce a state of senescence.
- With further division (inactivation of p53 and pRB), telomeres are further shortened and cause chromosomal instability, which provokes a crisis.
- Telomere shortening acts as a mitotic counter that determines the proliferative capacity of all cell types that do not have telomerase activity.



Clear view of telomerase at last (acs.org)

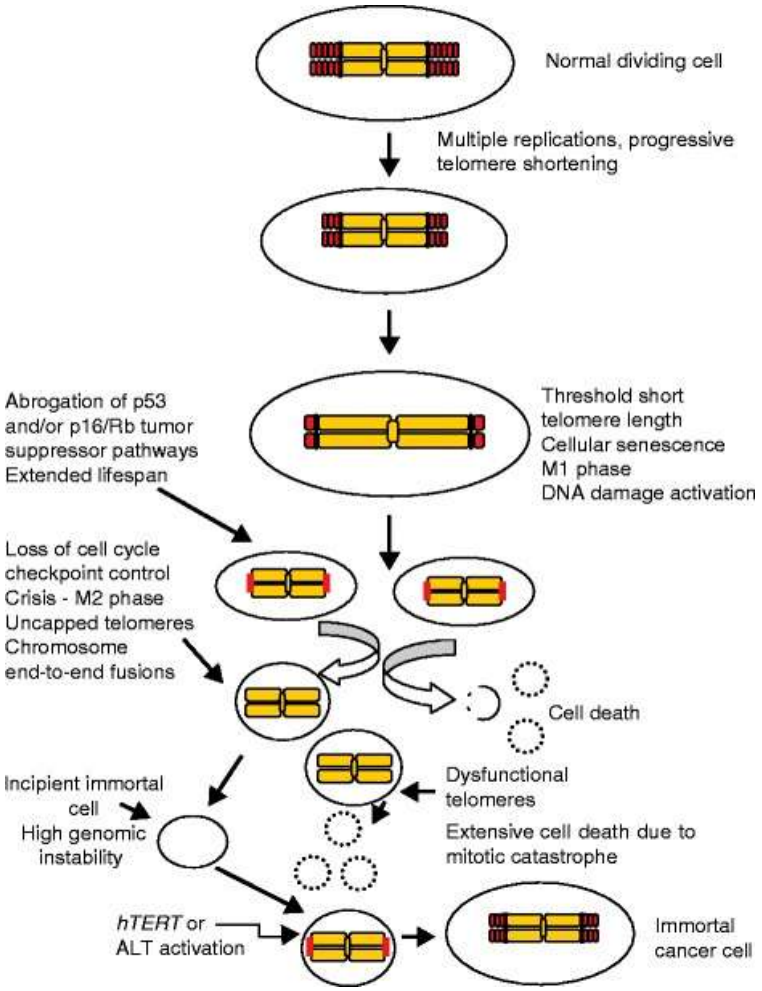
https://cen.acs.org/biological-chemistry/genomics/Clear-view-telomerase-last/96/i18

MUNI SCI

Telomerase

- Germ cells.
- Some stem cells.
- Some somatic cells under specific conditions:
  - Lymphocytes activated by mitogens.
  - Cells in the proliferative zone of intestinal crypts.
  - Cells of the proliferative basal layer of the skin.
  - Cells of the lobular endothelium of the breast during pregnancy.

Telomerase hypothesis



- Telomere shortening is a natural consequence of cell division due to the “end replication problem”.
- In the cells undergoing replicative senescence, the p53 and p16–pRB pathways are often activated leading to essentially irreversible growth arrest.
- Cells that gain additional oncogenic changes (p53 loss) can bypass senescence and continue to divide until chromosome end-to-end fusions.
- Only a rare human cell (one in 10^5 to 10^7) can engage a mechanism to bypass crisis and become immortal.
- This is almost universally accomplished by the upregulation or reactivation of telomerase.

Genome Med. 2016; 8: 69.

MUNI SCI

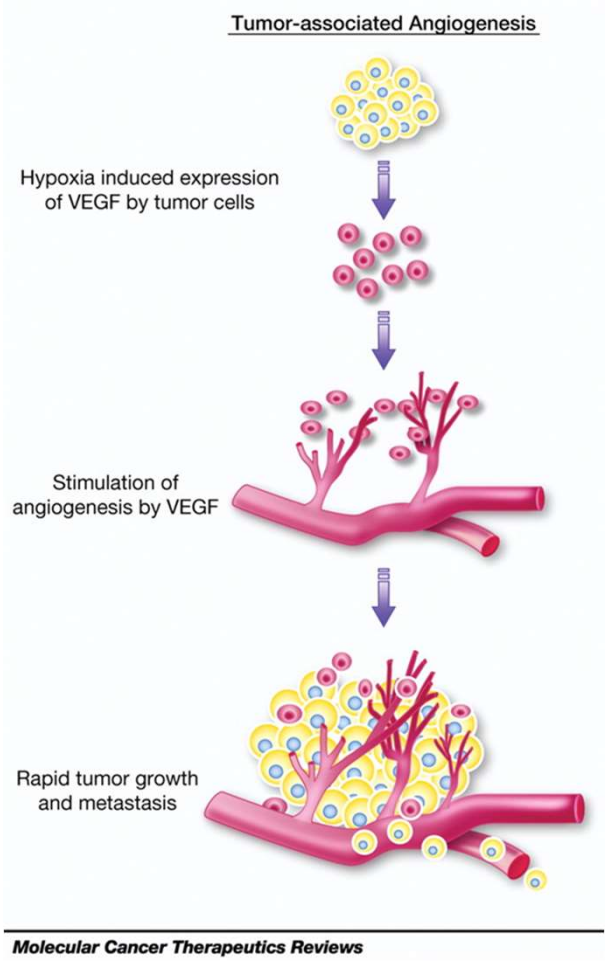
Tumors must acquire the six hallmarks capabilities

Component	Acquired Capability	Example of Mechanism
	Sustained angiogenesis	Produce VEGF inducer

MUNI SCI

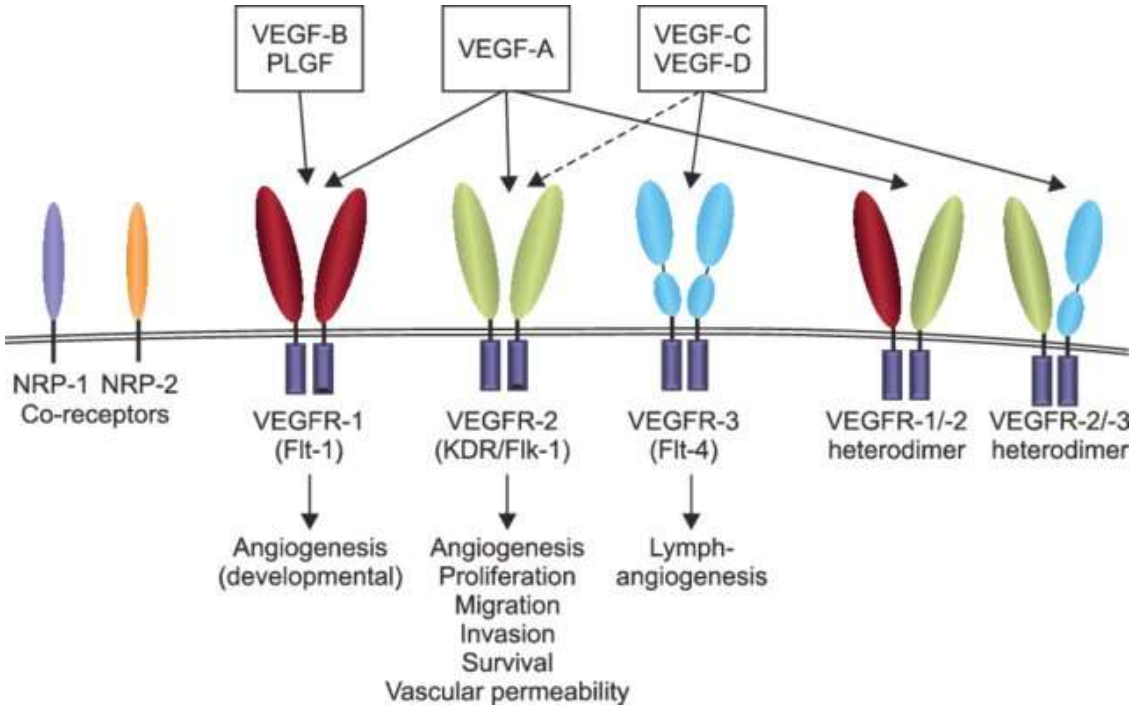
Angiogenesis in cancerogenesis

- Growth of blood capillaries from the existing vasculature.
- An important step - from the dormant state of the tumor to the malignant.
- Tumor – population of rapidly and uncontrollably growing cells.
- Tumors can not grow more than 1 - 2 mm^3 several million cells, lack of nutrients and oxygen.
- HIF-1 activates vascular endothelial growth factor - VEGF.
- Without angiogenesis, the tumor grows slowly and linearly, then exponentially.



MUNI SCI

VEGF



- VEGF was the first characterized factor specific to vasculogenesis - vascular endothelial growth factor
- It is critical for the initiation of vasculorogenesis, rise of the heart and primitive vascular plexus, as well as for angiogenic branching.
- Today described 5 different VEGF factors and 3 different receptors.
- Interactions between VEGF and VEGF receptors orchestrate distinct biological functions.

Biomolecules and Therapeutics, January 2015, 23(1):12-8.

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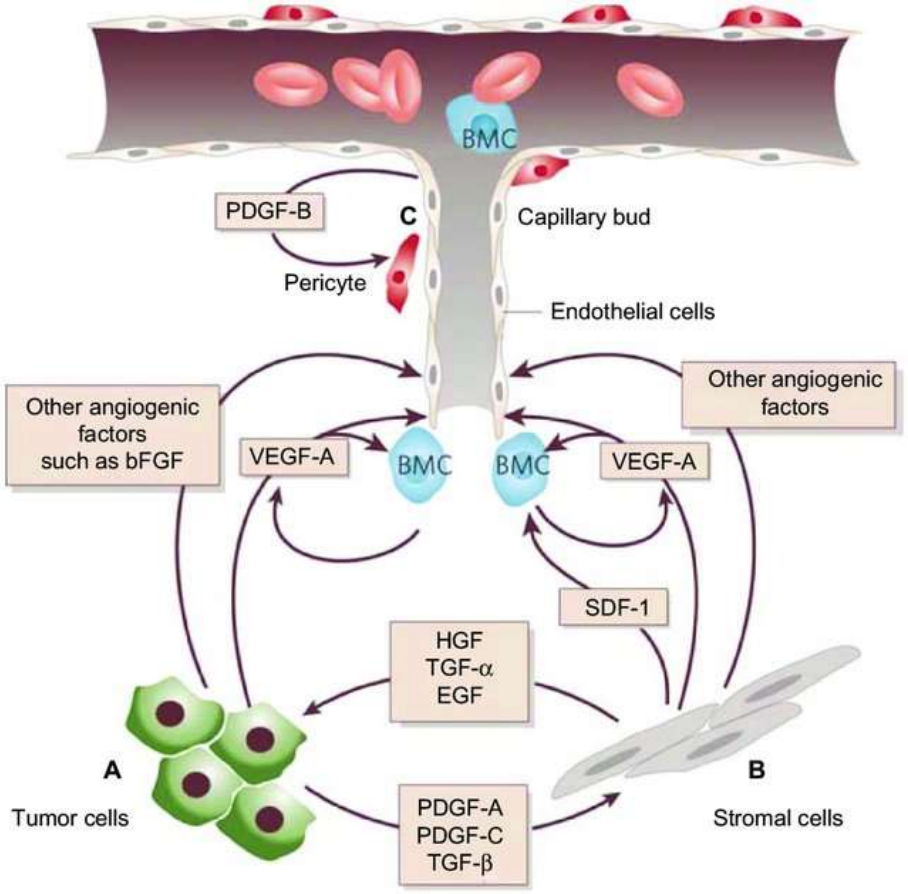
Tumor angiogenesis

- Tumor angiogenesis:
  - (A) Tumor cells produce VEGF-A and other angiogenic factors such as bFGF and angiopoietins. These stimulate endothelial cells to proliferate and migrate.
  - (B) An additional source of angiogenic factors is the stroma. This is a heterogeneous compartment, comprising fibroblastic, inflammatory, and immune cells.
  - (C) Endothelial cells produce PDGF-β, which promotes recruitment of pericytes, cells present at intervals along the walls of capillaries, in the microvasculature after activation of PDGFR-β.

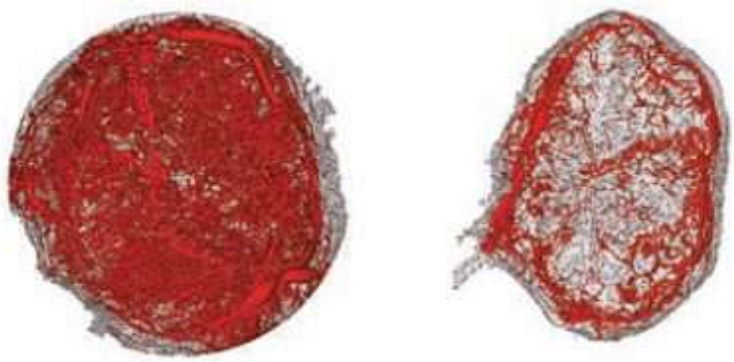


OncoTargets and Therapy, December 2014, 7(default):2237–2248

Tumor angiogenesis

- Tumor vasculature is highly disorganized.
- This can arise as a result of the uneven release of angiogenic regulators.
- The flow of blood is chaotic in different parts of the system.
- Therefore, places with hypoxia and excessive acidity are formed in the tumor.
- This circumstance may affect the effect of therapy; a space is created in which, for example, the selection and clonal expansion of cells that do not respond to hypoxia by apoptosis.

Control +VEGF inhibitor



- Microcomputed tomography image showing effect of VEGF inhibition in a preclinical model.

https://www.genentechoncology.com/pathways/cancer-tumor-targets/vegf.html

Tumors must acquire the six hallmarks capabilities

Component	Acquired Capability	Example of Mechanism
	Tissue invasion & metastasis	Inactivate E-cadherin

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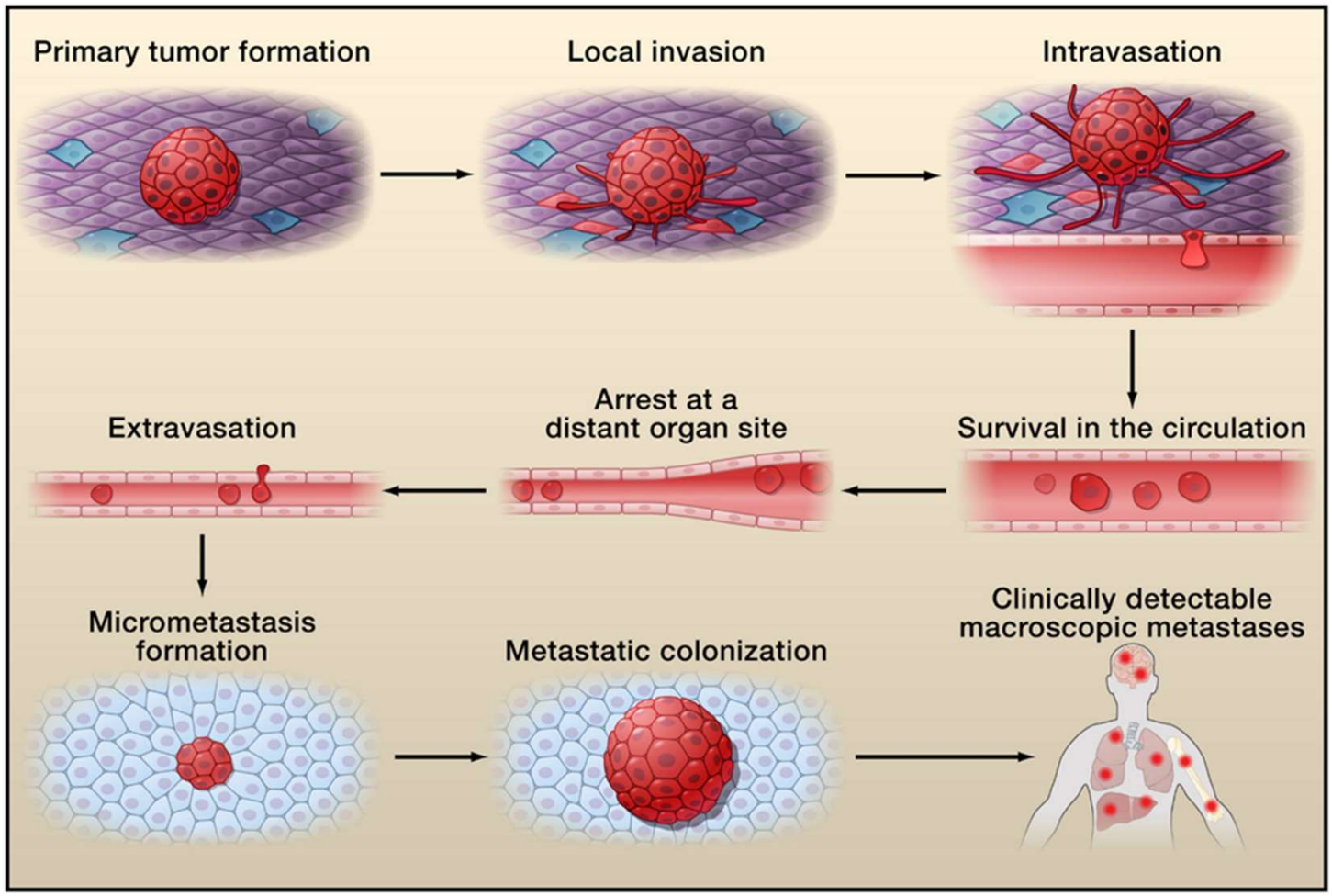
Tumors progression



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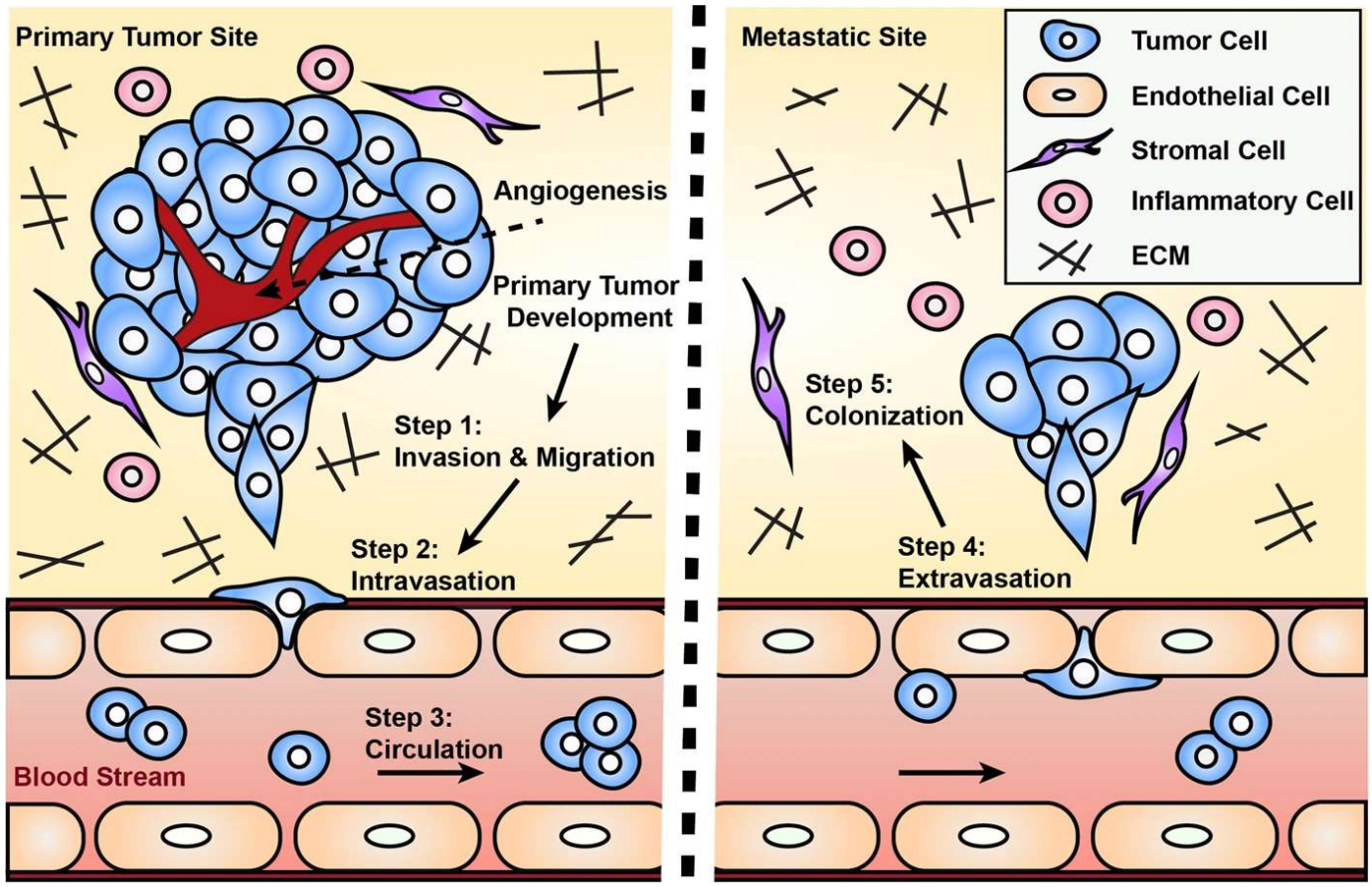
Metastatic cascade



- Overview of the Metastatic Cascade.
  - Step 1: Invade through basement membrane and migration.
  - Step 2: Intravasation into vasculature.
  - Step 3: Circulation of tumor cells in the bloodstream to blood vessels around secondary sites.
  - Step 4: Extravasation through the endothelial barrier.
  - Step 5: Colonization in the metastatic target organ.

npj Precision Oncology, 2019, volume 3, Article number: 20.

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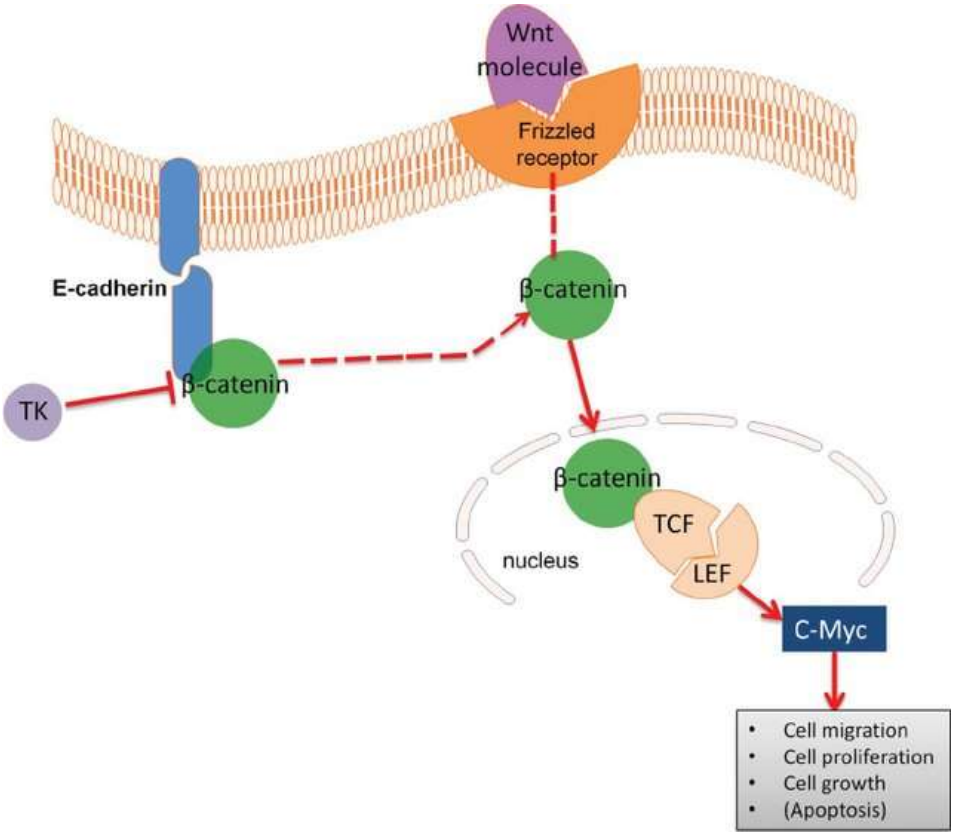
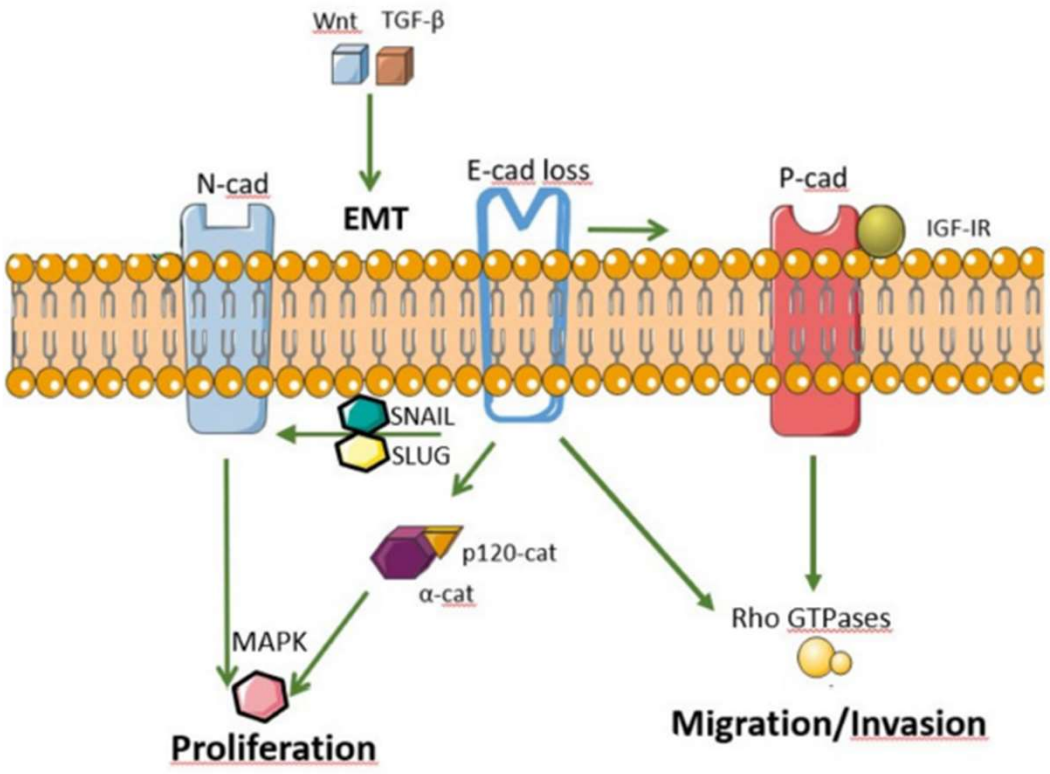
The mechanism of metastasis



- Adhesive molecules – N-CAM – adhesive molecule, increased expression in Wilms tumor, neuroblastoma.
- E-cadherin – on epithelial cells, antiproliferative signals, tumors reduce expression.
- Integrins – changes in expression on migrating cells.

Journal of Clinical Pathology, August 2013, 66(11)

The mechanism of metastasis



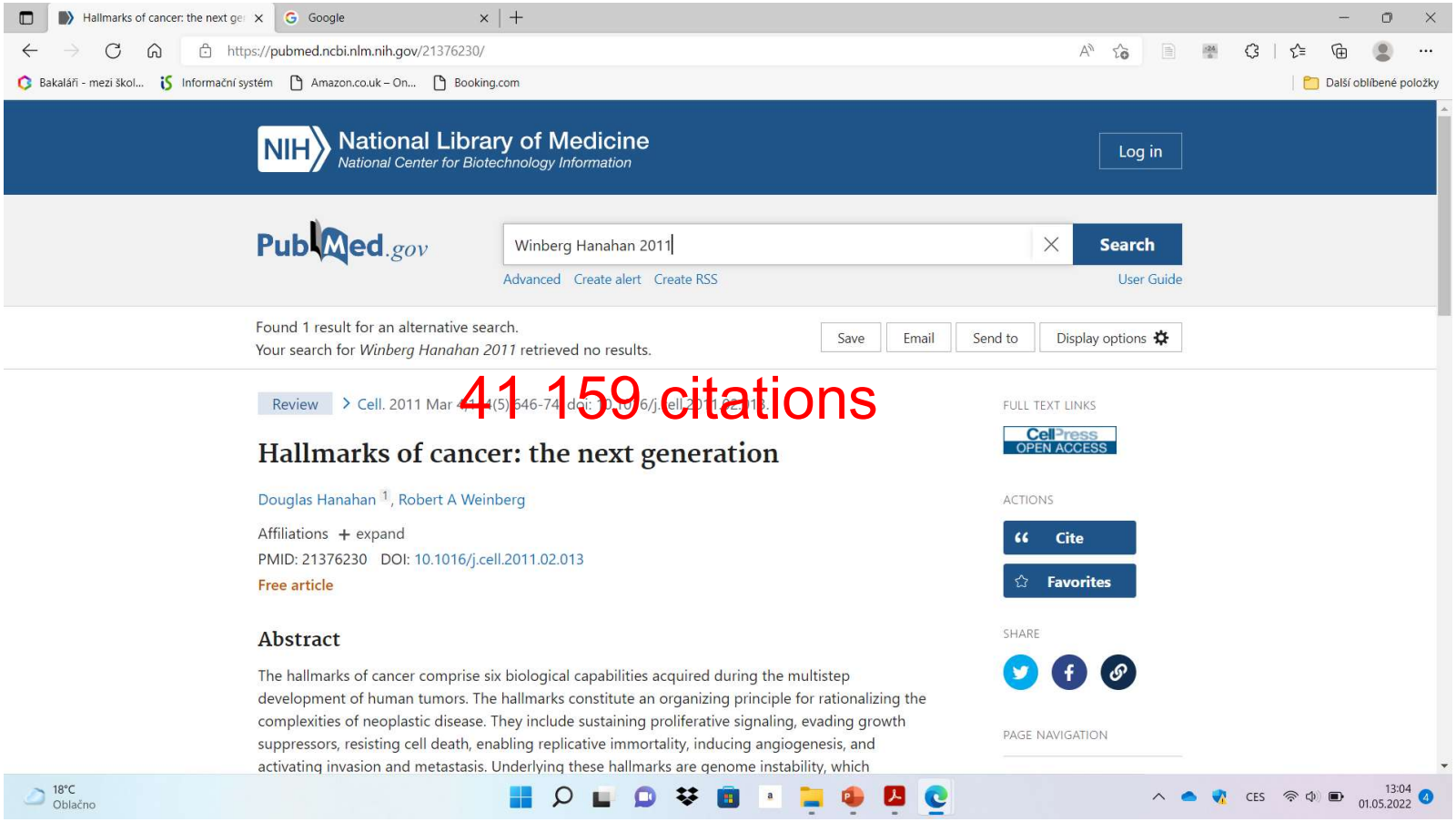
- Glycoprotein important for cell adhesion.
- Differentiation of epithelial cells.
- Loss of expression – epithelial-mesenchymal transition (EMT), an important step in the metastatic progression of human tumors.
- Tumor suppressor.
- Decreased expression in epithelial tumors – invasiveness and worse prognosis.

Int. J. Mol. Sci. 2020, 21, 7624; doi:10.3390/ijms21207624

- The Hallmarks of cancer: The next generation

Hallmarks of cancer: The next generation

- Hanahan and Weinberg



Cell, Volume 144, Issue 5, 4 March 2011, Pages 646-674

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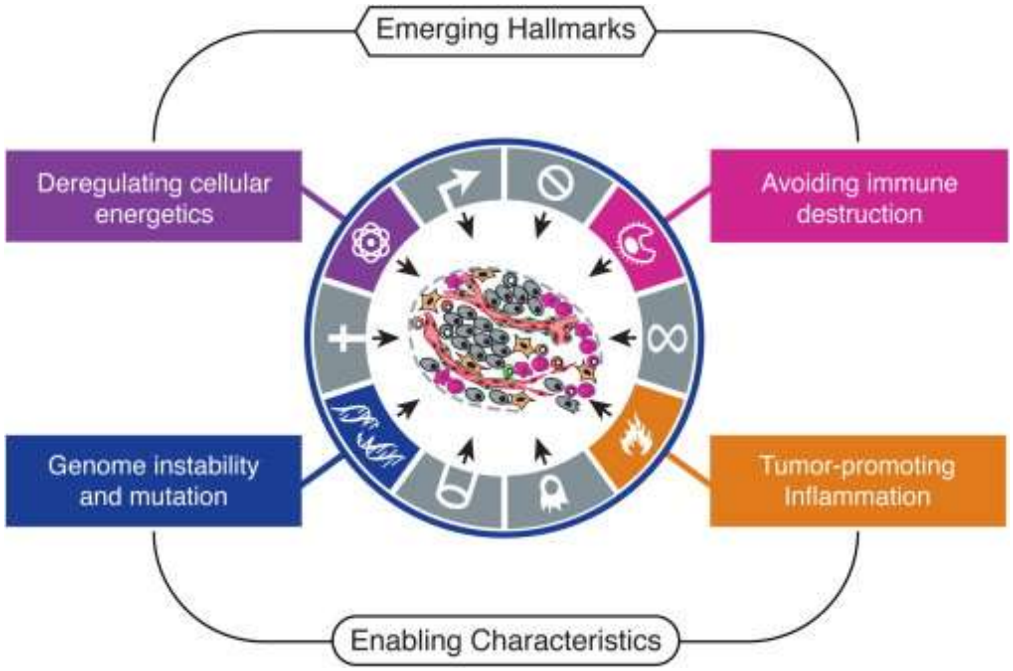
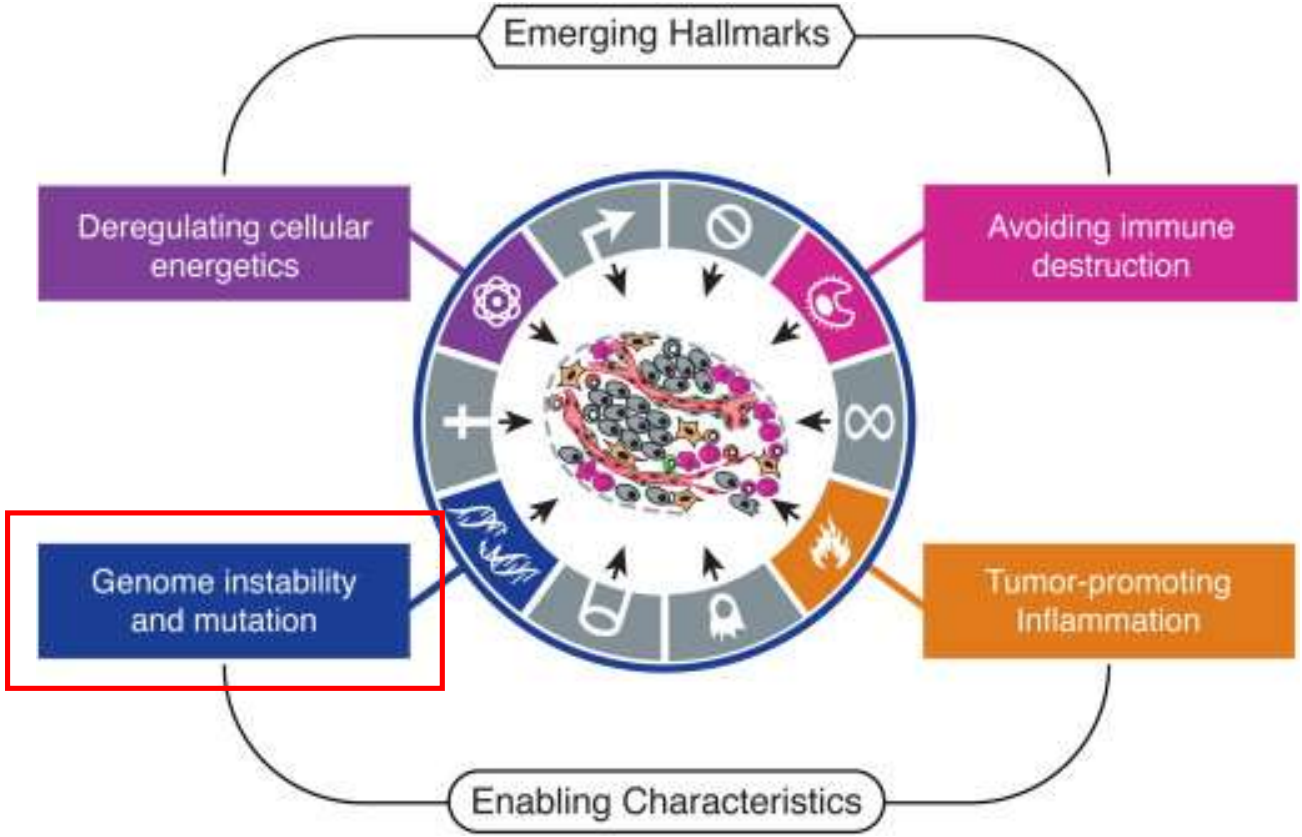
Tumors must acquire additional hallmarks capabilities



- Deregulating cellular energetics – modification or reprograming of cellular metabolism in order to effectively support neoplastic proliferation.
- Avoiding immune destruction - allows cancer cells to evade immunological destruction mediated by T and B lymphocytes, macrophages, and natural killer cells.
- Genomic instability and mutation - endow cancer cells with genetic alterations that drive tumor progression.
- Tumor-promoting inflammation - inadvertent support of multiple hallmark capabilities due to inflammatory responses of innate immune cells.

Cell, Volume 144, Issue 5, 4 March 2011, Pages 646-674

Tumors must acquire additional four hallmarks capabilities



Cell, Volume 144, Issue 5, 4 March 2011, Pages 646-674

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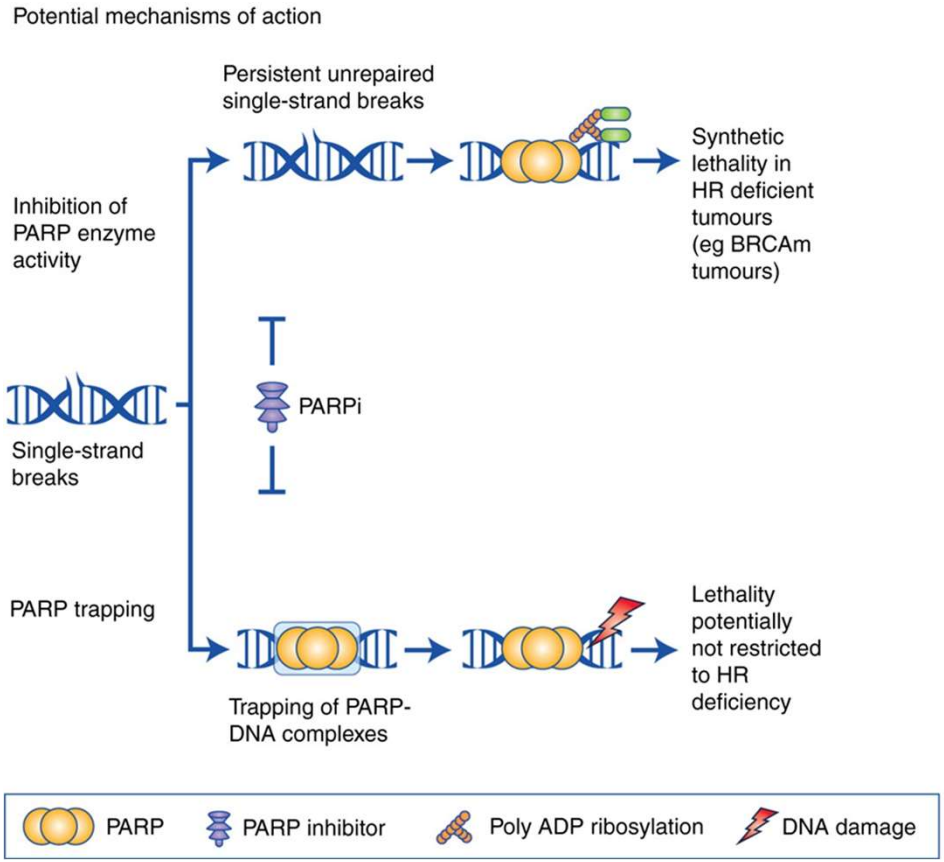
Genome instability and mutation

- Genomic instability and mutation - endow cancer cells with genetic alterations that drive tumor progression.

- This may be acquired through:
  - Clonal selection – through nonmutational changes affecting the regulation of gene expression.
  - Epigenetic mechanism - DNA methylation and histone modifications.

- Alterations in DNA maintenance machinery due to defects in proteins involved in:
  - Detecting DNA damage and activating the repair machinery.
  - Directly repairing damaged DNA.
  - Inactivating or intercepting mutagenic molecules before they have damaged the DNA.

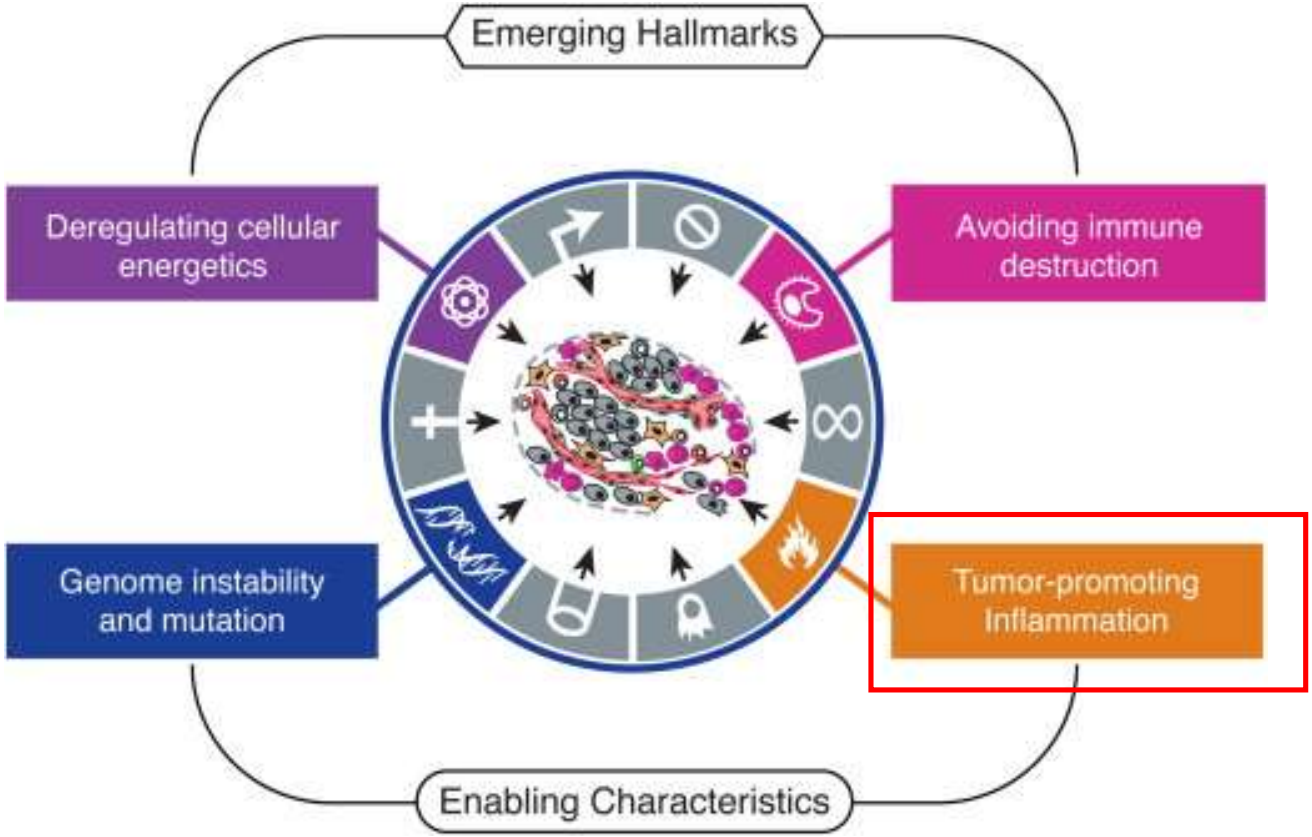
Genome instability and mutation



- Inactivating or intercepting mutagenic molecules before they have damaged the DNA.
- Inhibition of PARP enzyme activity or catalytic inhibition interferes with the repair of single-strand breaks, leading to stalled DNA replication forks that requires HR repair. In HR-deficient tumors, such as those with BRCA mutations, PARP inhibition results in synthetic lethality.
- PARP trapping refers to trapping of PARP proteins on DNA, which also leads to replication fork damage, but because this pathway utilizes additional repair mechanisms, it is not restricted to tumors with HR deficiency.

British Journal of Cancer volume 119, pages141–152 (2018)

Tumors must acquire additional four hallmarks capabilities



Cell, Volume 144, Issue 5, 4 March 2011, Pages 646-674

MUNI SCI

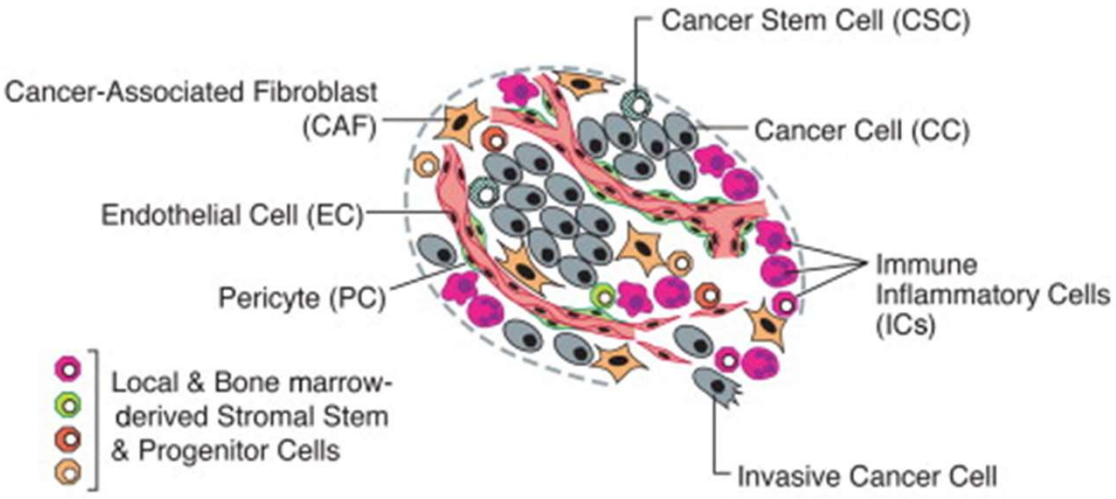
Tumor promoting inflammation

- Every neoplastic lesion contains immune cells present at densities ranging from subtle infiltrations detectable only with cell type-specific antibodies to gross inflammations.

- Inflammation contributes to multiple hallmark capabilities by supplying bioactive molecules to the tumor microenvironment, including:
  - Growth factors that sustain proliferative signaling.
  - Survival factors that limit cell death.
  - Proangiogenic factors.
  - Extracellular matrix-modifying enzymes that facilitate angiogenesis, invasion, and metastasis.
  - Signals that lead to activation of EMT.
  - Various factors facilitating other hallmarks programs.

Tumor promoting inflammation

- Most solid tumors are assembled of distinct cell types.
- Both the parenchyma and stroma of tumors contain distinct cell types and subtypes that collectively enable tumor growth and progression.
- Also, the immune inflammatory cells present in tumors can include both tumor-promoting as well as tumor-killing subclasses.



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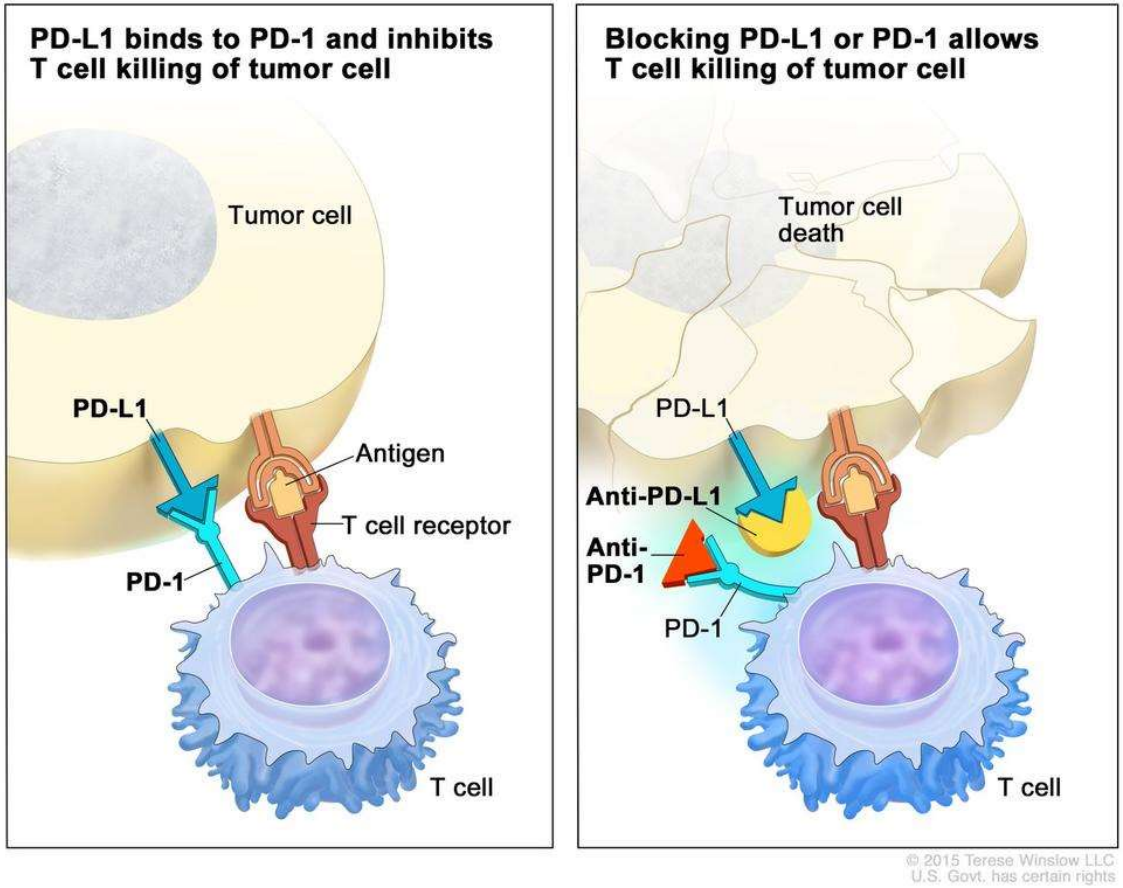
Tumor promoting inflammation

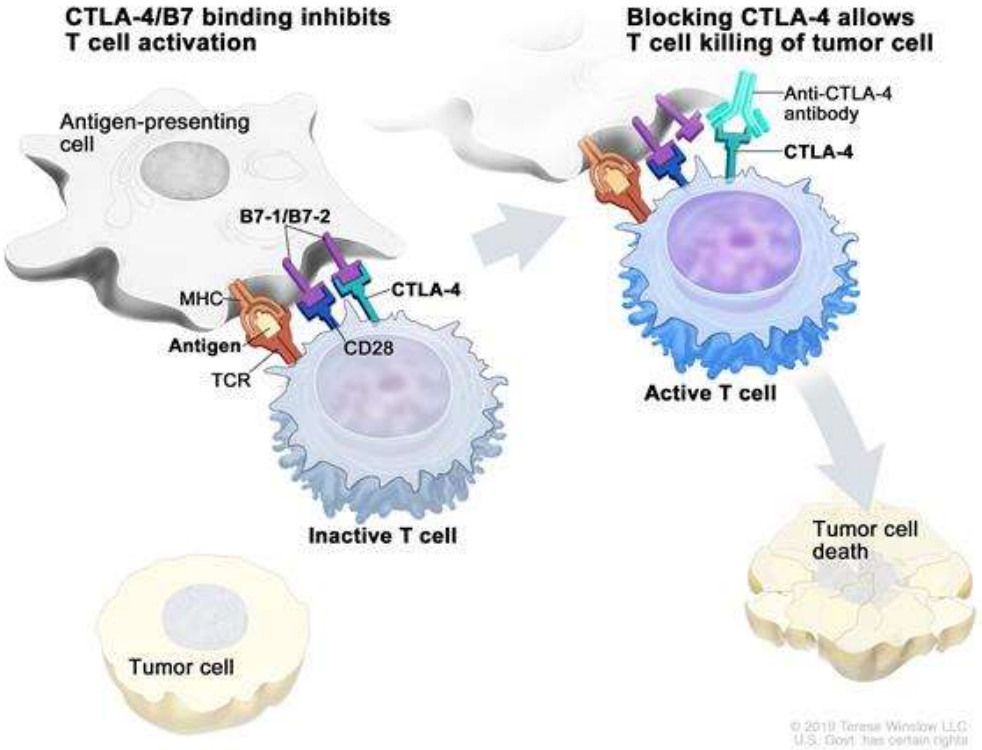


- Checkpoint proteins, such as PD-L1 on tumor cells and PD-1 on T cells, help keep immune responses in check.
- The binding of PD-L1 to PD-1 keeps T cells from killing tumor cells in the body.
- Blocking the binding of PD-L1 to PD-1 with an immune checkpoint inhibitor (anti-PD-L1 or anti-PD-1) allows the T cells to kill tumor cells.

https://www.cancer.gov/about-cancer/treatment/types/immunotherapy/checkpoint-inhibitors

Tumor promoting inflammation

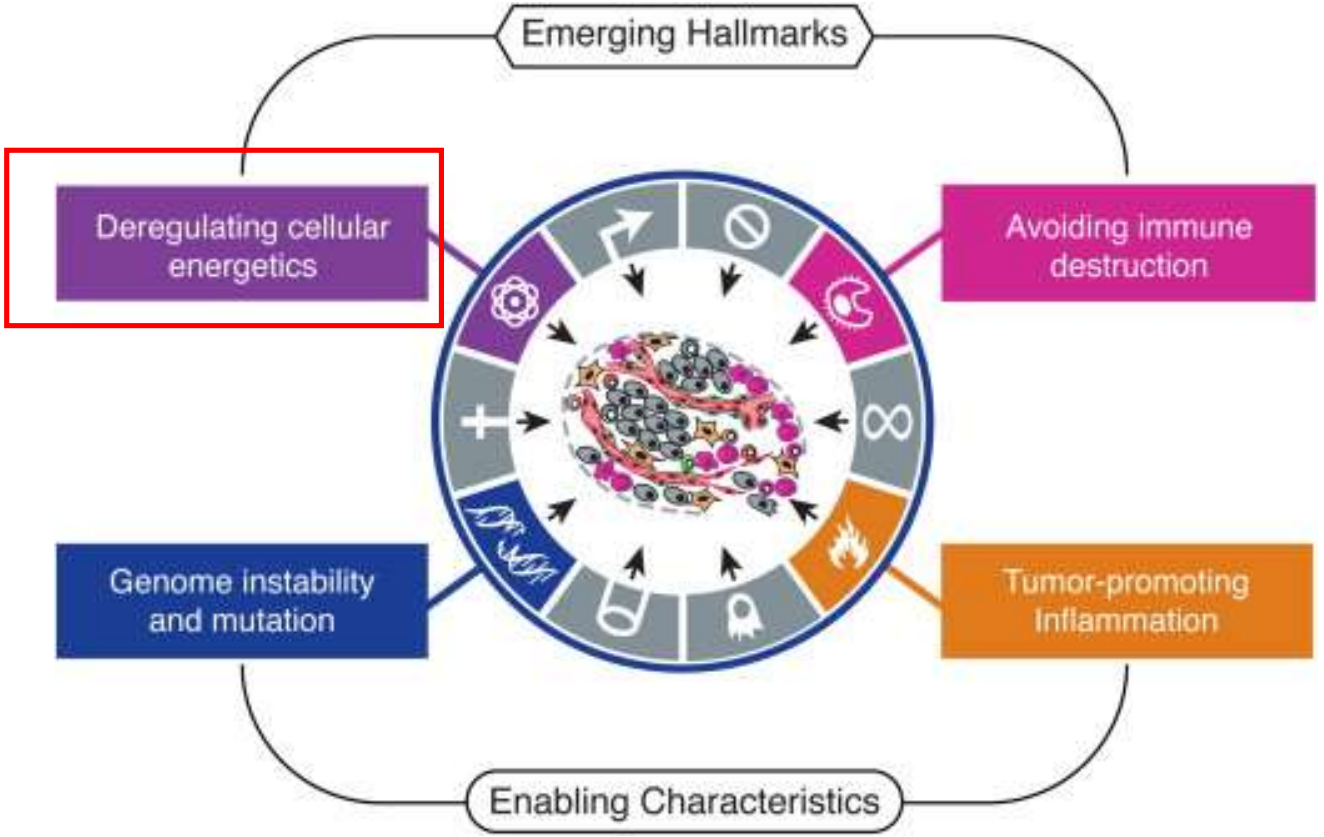
- T Cells have CTLA-4 that works as a regulating switch for T cell activity.
- By blocking the CTLA-4 pathway that T cell activation can be restored.
- Ipilimumab is a drug blocking the CTLA-4 protein. When this protein is blocked the T-cell is activated and the tumor cell is killed.



https://www.biologycorner.com/2020/05/27/jim-allison-breakthrough/

MUNI SCI

Tumors must acquire additional four hallmarks capabilities



Cell, Volume 144, Issue 5, 4 March 2011, Pages 646-674

MUNI SCI

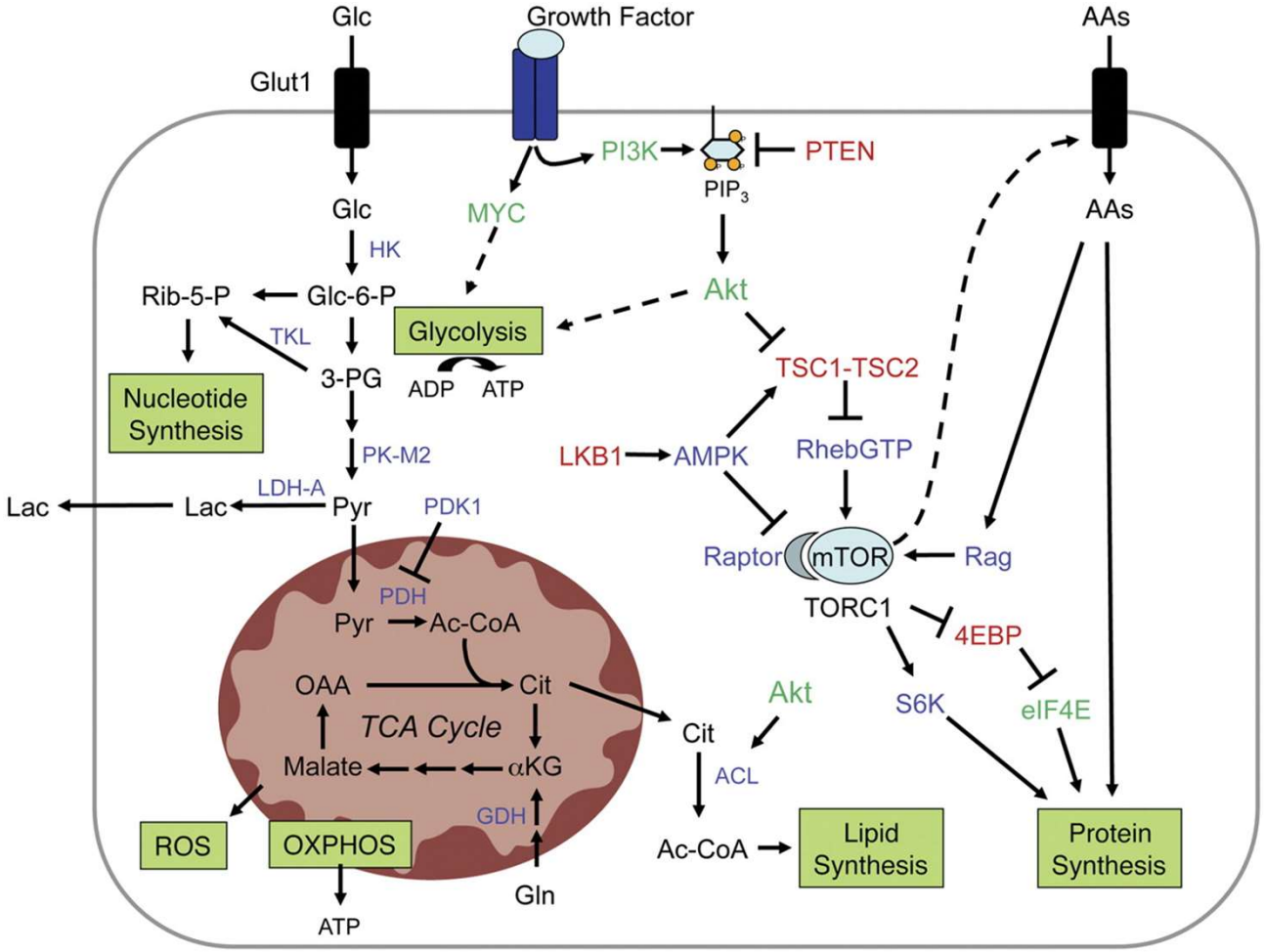
Deregulating cellular energetics

- Reprogramming energy metabolism.
- The Warburg Effect: Cancer cells reprogram their glucose metabolism, by limiting their energy production largely to glycolysis.
- The cells upregulate glucose transporters, notably GLUT1, which substantially increases glucose import into the cytoplasm.
- Growth factor-independent activation of the PI3K/Akt and c-MYC pathways facilitates increased rates of glucose uptake and glycolysis.
- The hypoxia response system acts pleiotropically to upregulate glucose transporters and multiple enzymes of the glycolytic pathway.

Genes & Dev. 2009. 23: 537-548

Deregulating cellular energetics

- Glycolysis is the process in which glucose is broken down to produce energy.
- It produces two molecules of pyruvate, ATP, NADH and water.
- The process takes place in the cytoplasm of a cell and does not require oxygen.



Genes & Dev. 2009. 23: 537-548

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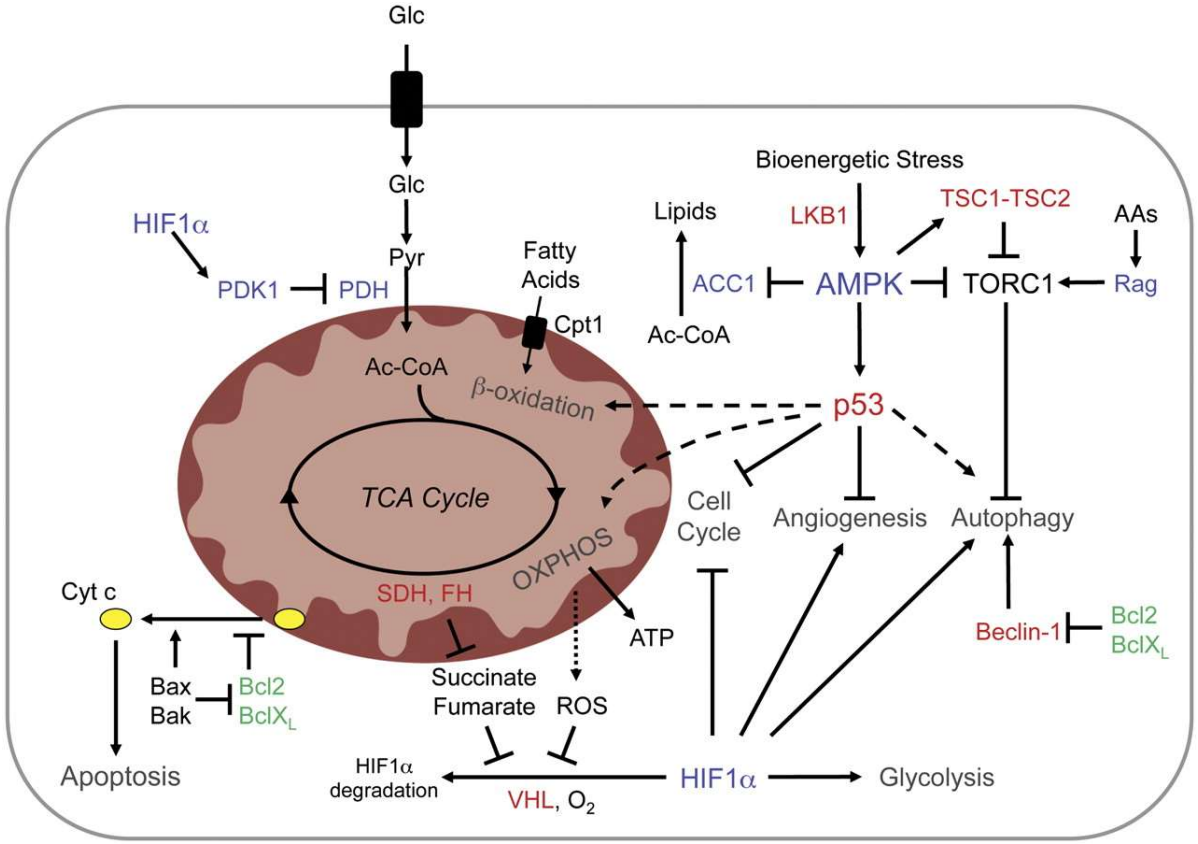
Deregulating cellular energetics

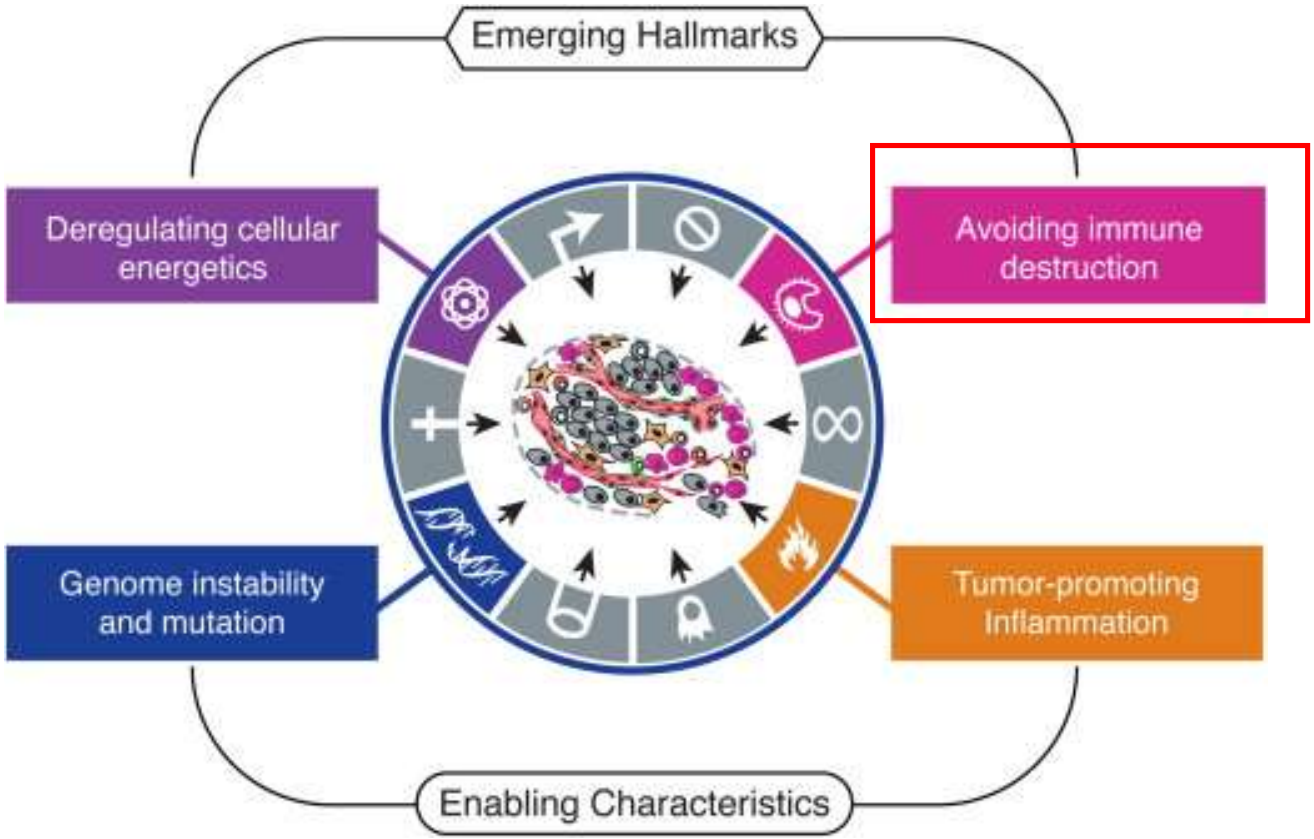


- Intracellular sensors of energy, nutrients, and oxygen promote metabolic adaptation to stress during tumorigenesis.
- Major physiological strategies of metabolic adaptation include:
  - Cell cycle inhibition.
  - Inhibition of biosynthetic pathways (lipid, protein synthesis).
  - Increases in bioenergetic pathways (β-oxidation, glycolysis, and OXPHOS).
  - Induction of autophagy.
- Oncogenes are displayed in green and tumor suppressors in red.

Genes & Dev. 2009. 23: 537-548

MUNI SCI

Tumors must acquire additional four hallmarks capabilities



Cell, Volume 144, Issue 5, 4 March 2011, Pages 646-674

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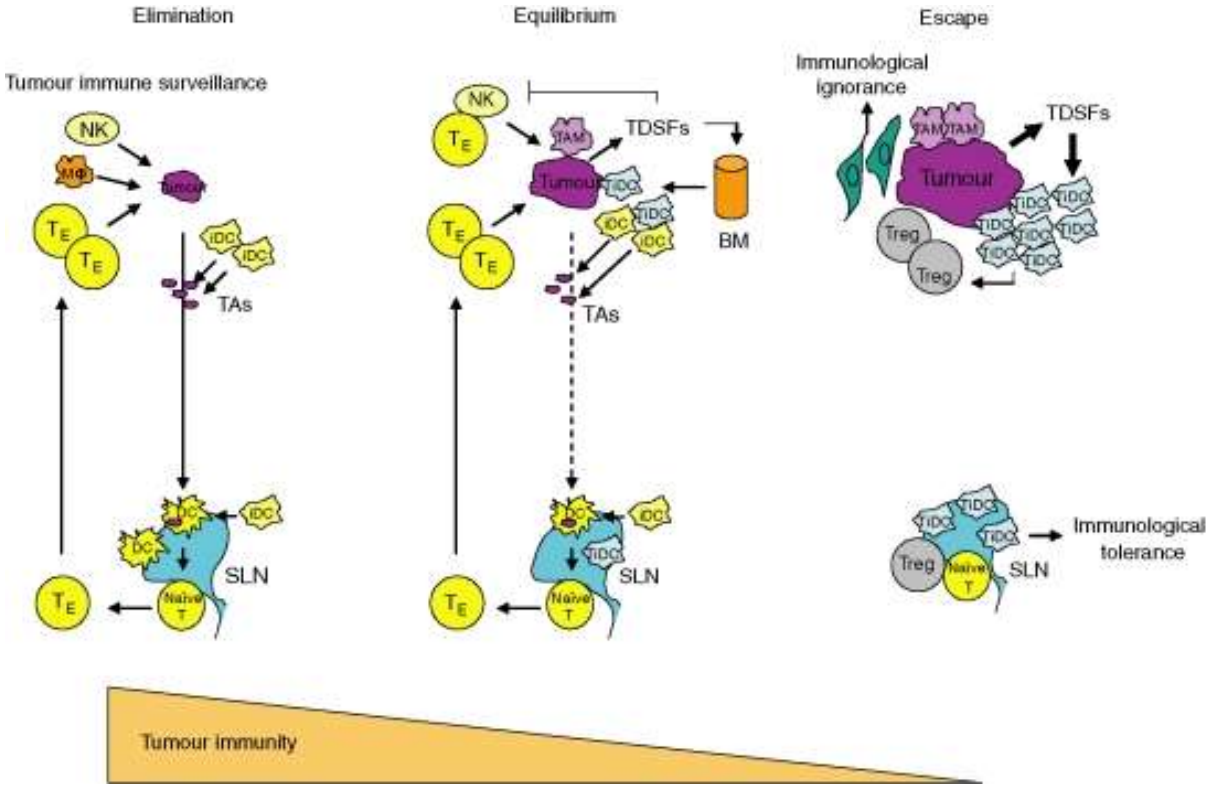
Evading immune destruction

- Cells and tissues are under constant surveillance by the immune system and immune surveillance is responsible for recognizing and eliminating the vast majority of incipient cancer cells and thus nascent tumors.
- Tumors somehow managed to avoid detection by the various arms of the immune system or have been able to limit the extent of immunological killing, thereby evading eradication.
- Immune system operates as a significant barrier to tumor formation and progression, at least in some forms of non-virus-induced cancer.
- Mice lacking NK and T cells were more susceptible to cancer development.
- Patients with higher CTLs and NK cells have a better prognosis.

Evading immune destruction

- Nascent transformed cells are directly eradicated by innate and adaptive immune responses.
- During tumor growth, tumor cells are required for angiogenesis and stromal remodeling, which produce tumor cell variants that have low immunogenicity and are resistant to immune attack.
- Tumor progression leads to the release of tumor-derived soluble factors that are involved in several mechanisms of immune evasion in the escape phase.



- iDC, immature dendritic cell; TAs, tumour antigens; SLN, sentinel lymph node; TAM, tumour-associated macrophage; TDSFs, tumour-derived soluble factors; Tregs, regulatory T cells; BM, bone marrow.

mice with combined immunodeficiencies in both T cells and NK cells were even more susceptible to cancer development

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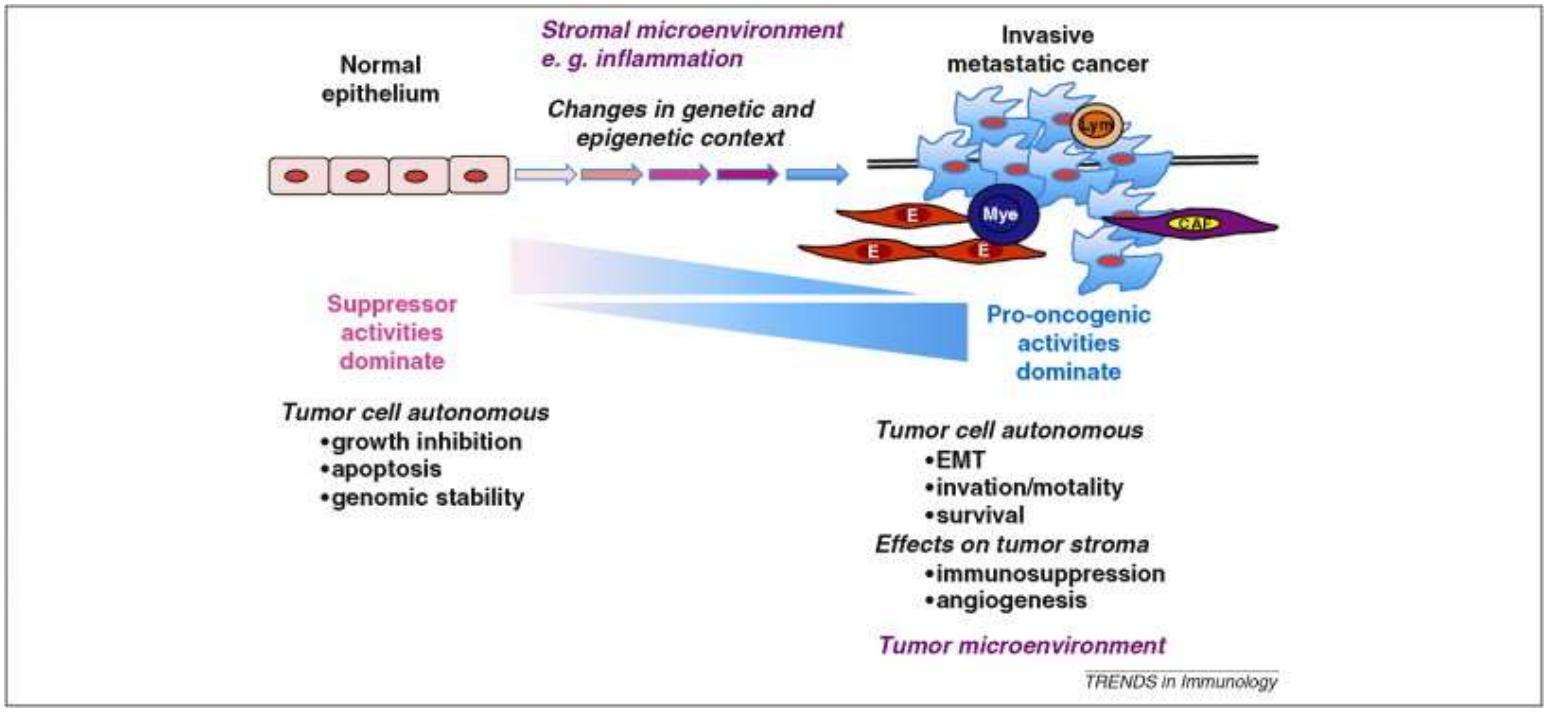
Evading immune destruction

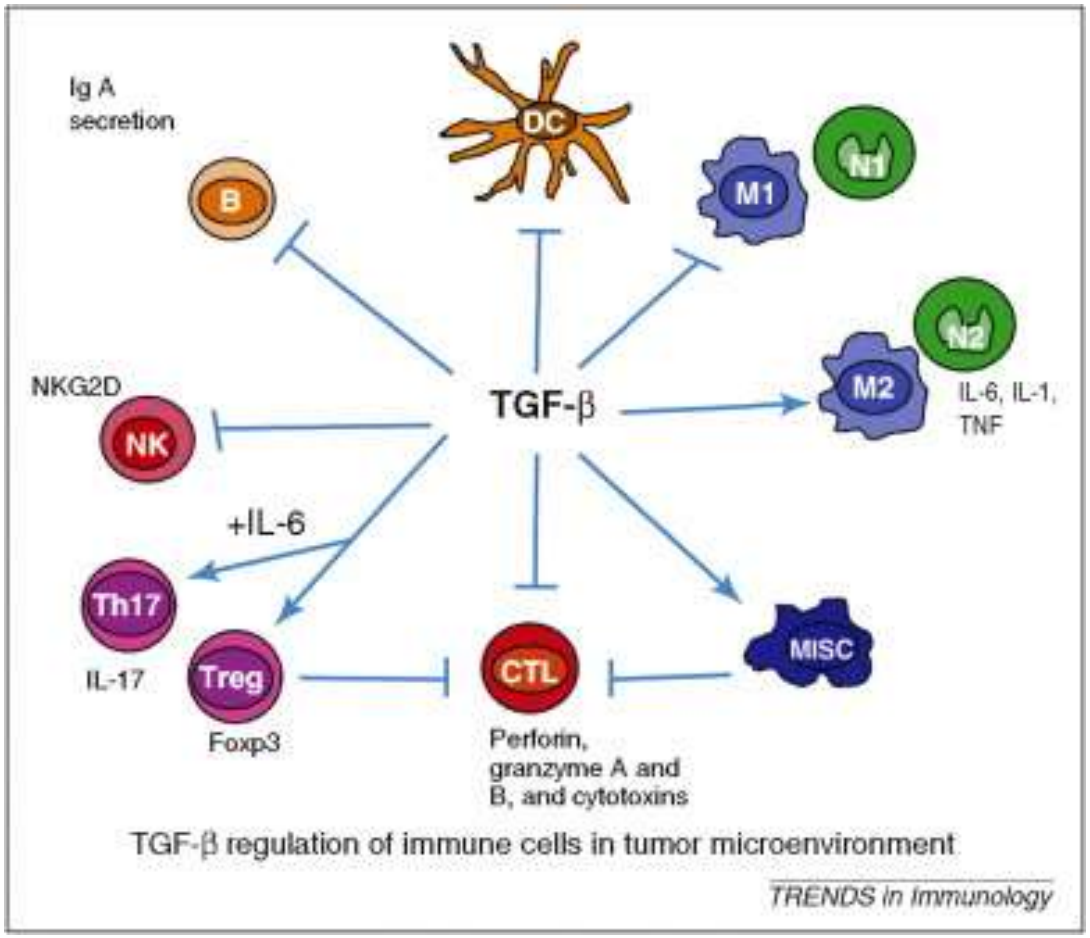
- Progression to metastatic disease is generally accompanied by decreased or altered TGF-β responsiveness and increased expression or activation of the TGF-β ligand.



Trends in Immunology, Volume 31, Issue 6, June 2010, Pages 220-227

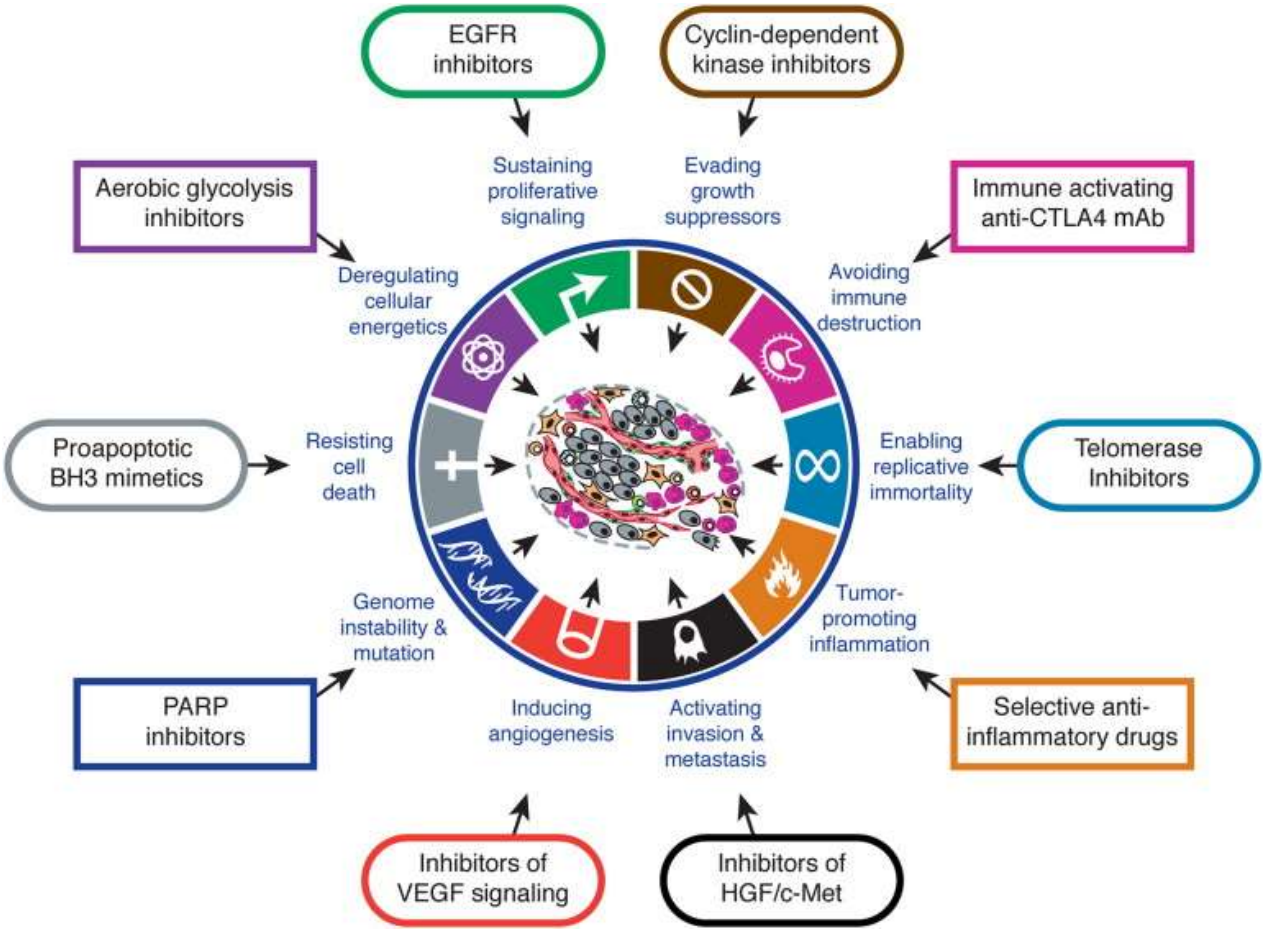
Evading immune destruction

- TGF-β affects multiple components of the immune system.
- TGF-β inhibits the function of natural killer (NK) and CD8+ CTL (cytotoxic T lymphocytes), by blocking production of perforin, granzymes and cytotoxins.
- TGF-β induces Treg and Th17 cell differentiation and inhibits B-cell proliferation and dendritic function.
- TGF-β, inhibits macrophage and neutrophil development, but promotes type II macrophages and neutrophils, and mediates the immune suppression function of immune suppressor cells - MISCs.



Trends in Immunology, Volume 31, Issue 6, June 2010, Pages 220-227.

Therapeutic Targeting of the Hallmarks of Cancer



Cell, Volume 144, Issue 5, 4 March 2011, Pages 646-674

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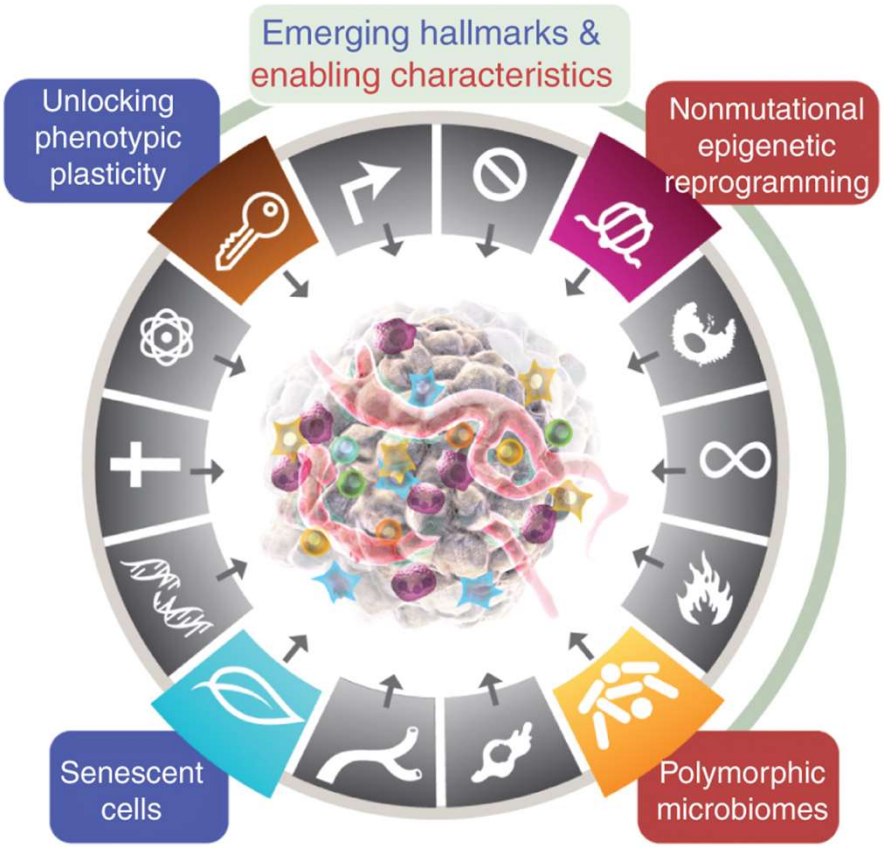
- The Hallmarks of Cancer: New Dimensions

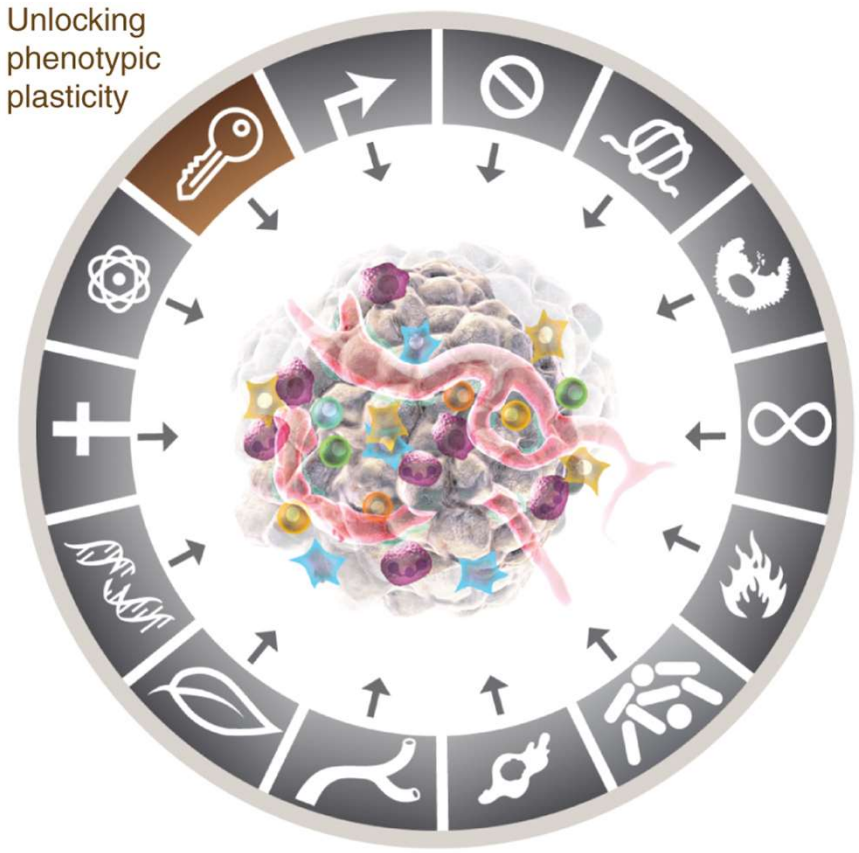
Hallmarks of cancer: New Dimensions

- In 2022 additional emerging hallmarks and enabling characteristics of cancer were proposed:
- Unlocking phenotypic plasticity.
- Nonmutational epigenetic reprogramming.
- Polymorphic microbiomes.
- Senescent cells.



Cancer Discov (2022) 12 (1): 31–46.

Tumors acquire additional four hallmarks capabilities



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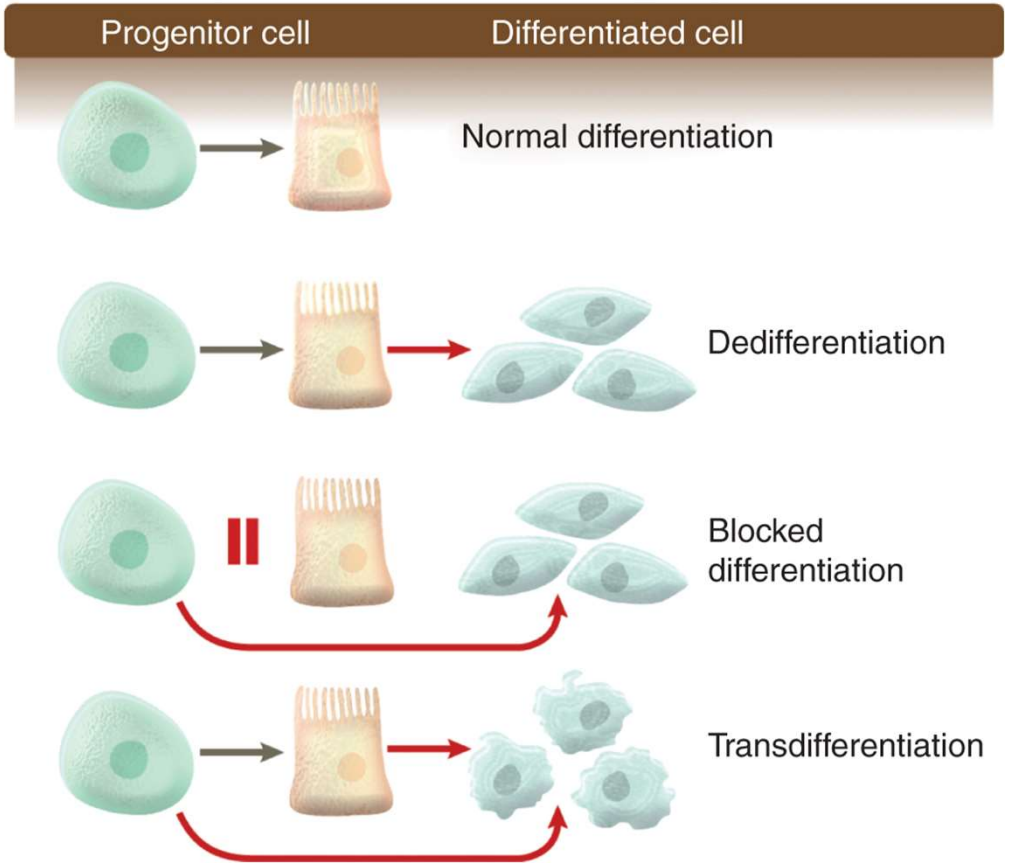
Phenotypic plasticity

- Phenotypic plasticity enables various disruptions of cellular differentiation, including:

- Dedifferentiation from mature to progenitor states.

- Blocked (terminal) differentiation from progenitor cell states.

- Transdifferentiation into different cell lineages.



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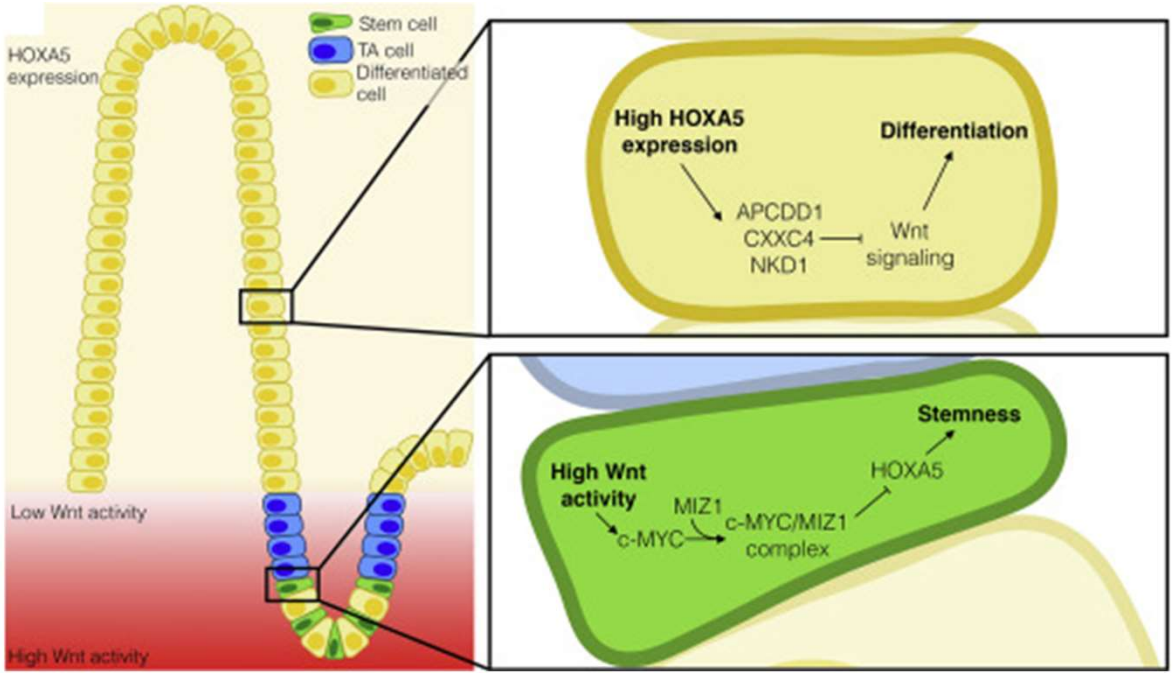
Phenotypic plasticity

- For example the developmental transcription factors HOXA5 are highly expressed in differentiating colonic epithelial cells, and typically lost in advanced colon carcinomas, which characteristically express markers of stem and progenitor cells.



Cancer Cell, Volume 28, Issue 6, 14 December 2015, Pages 683-685

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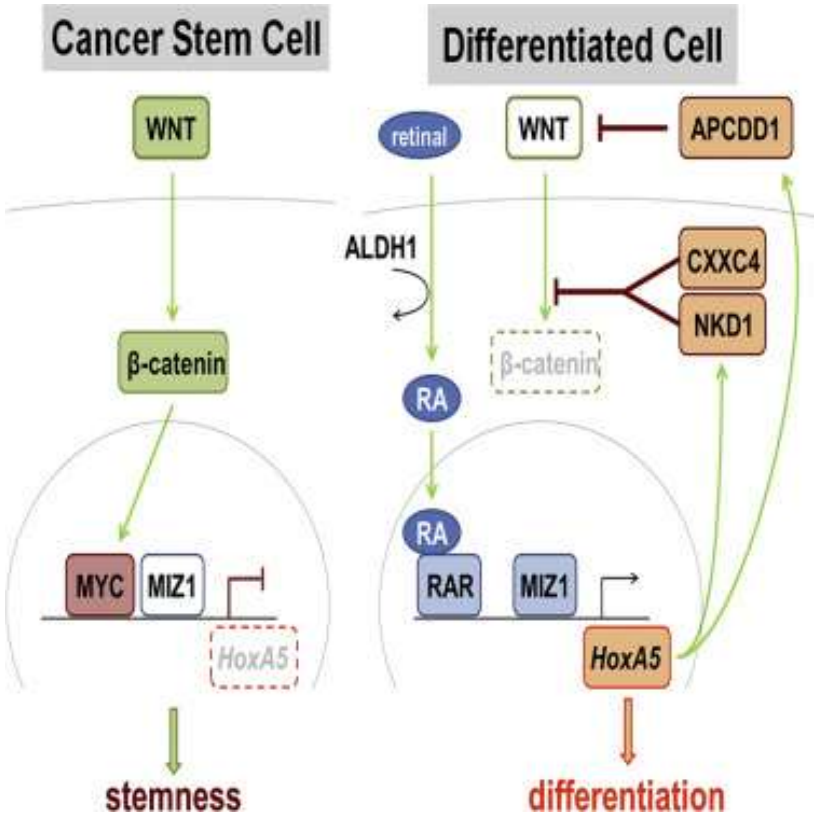
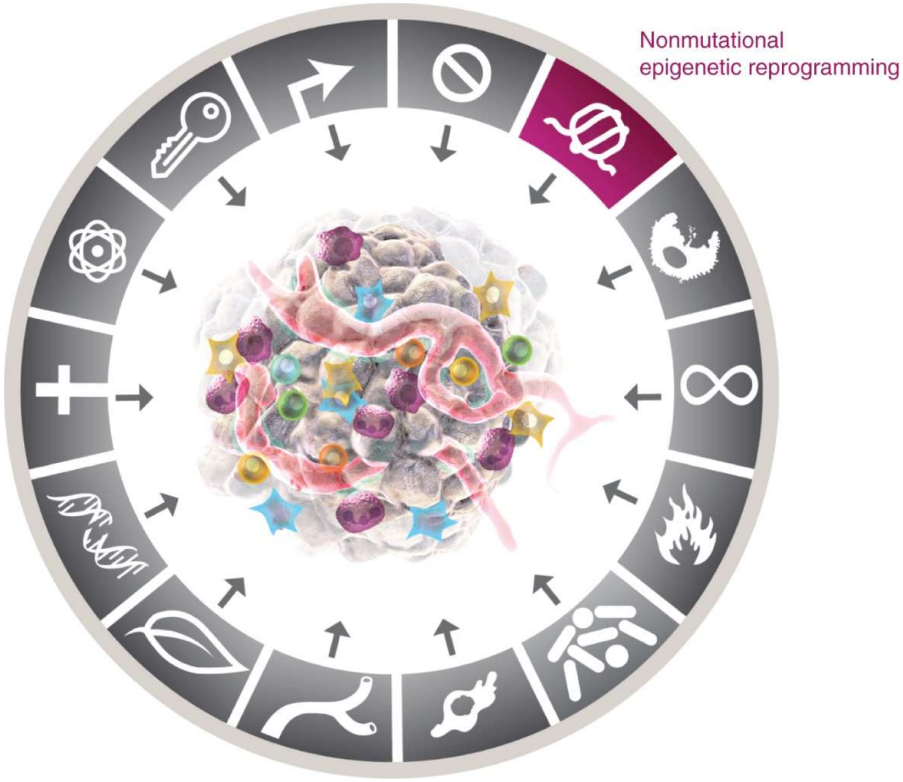
Phenotypic plasticity



- In colon cancer, HOXA5 is downregulated, and its re-expression induces loss of the cancer stem cell phenotype, preventing tumor progression and metastasis.
- Tumor regression by HOXA5 induction can be triggered by retinoids, which represent tangible means to treat colon cancer by eliminating cancer stem cells.

Cancer Cell, Volume 28, Issue 6, 14 December 2015, Pages 815-829

Tumors acquire additional four hallmarks capabilities



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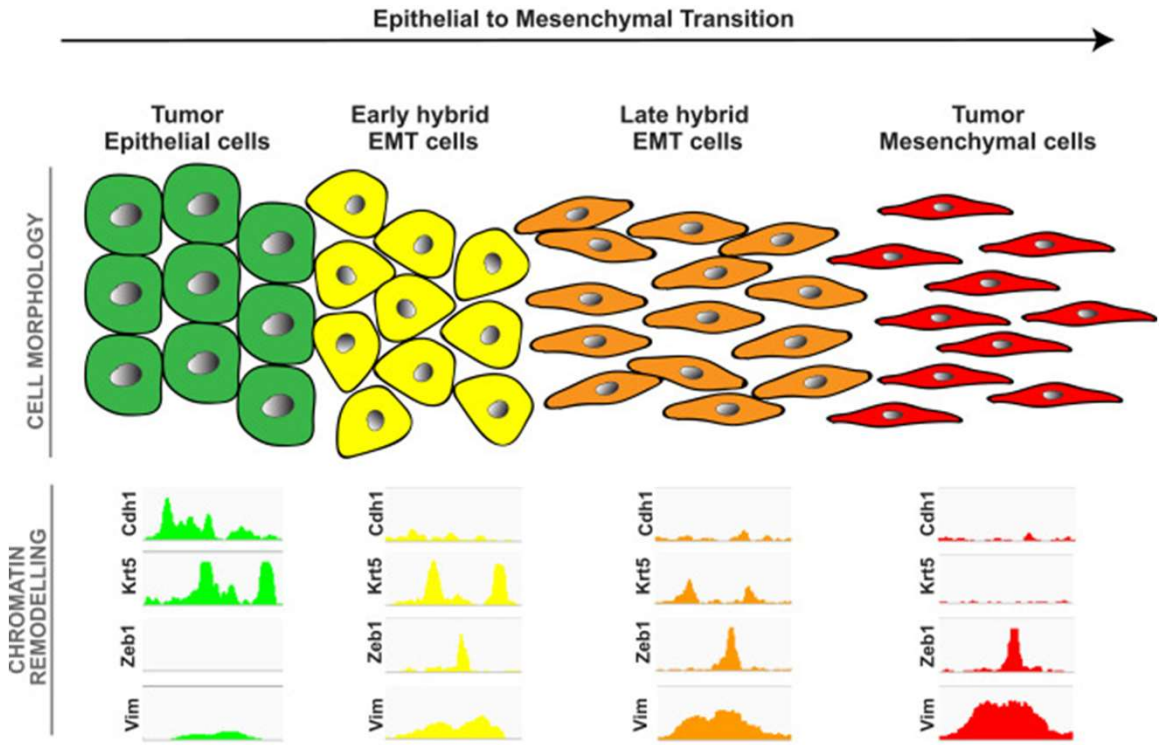
Nonmutational epigenetic reprogramming

- Nonmutational epigenetic regulation of gene expression is of course well established as the central mechanism mediating embryonic development, differentiation, and organogenesis.

- Analogous epigenetic alterations can contribute to the acquisition of hallmark capabilities during tumor development and malignant progression.



Cell Stem Cell, Volume 24, Issue 1, 3 January 2019, Pages 65-78

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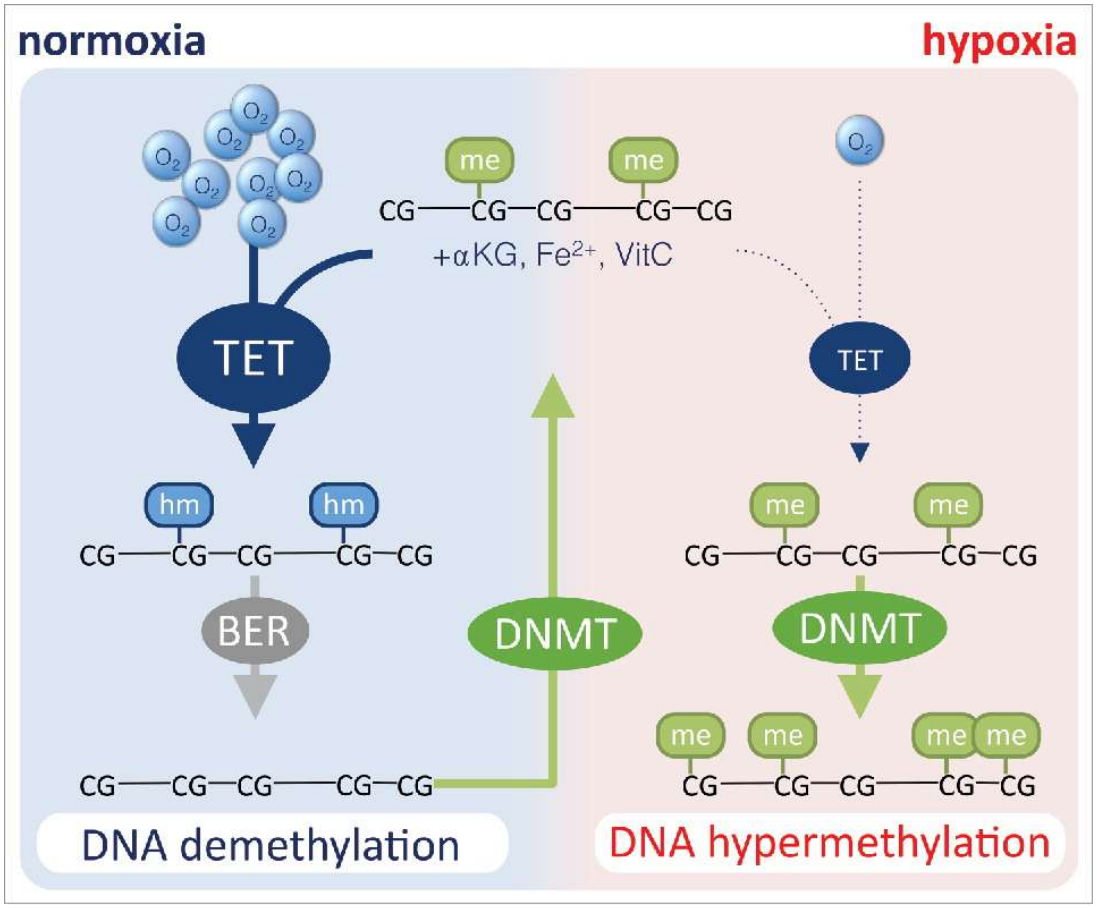
Nonmutational epigenetic reprogramming



- One common characteristic of tumors (or regions within tumors) is hypoxia, consequent to insufficient vascularization.
- Hypoxia, for example, reduces the activity of the TET demethylases, resulting in substantive changes in the methylome, in particular hypermethylation.
- Under hypoxic conditions (right) TET activity is compromised, leading to accumulation of methyl groups and causing DNA hypermethylation.

Mol Cell Oncol 2016;3:e1240549.

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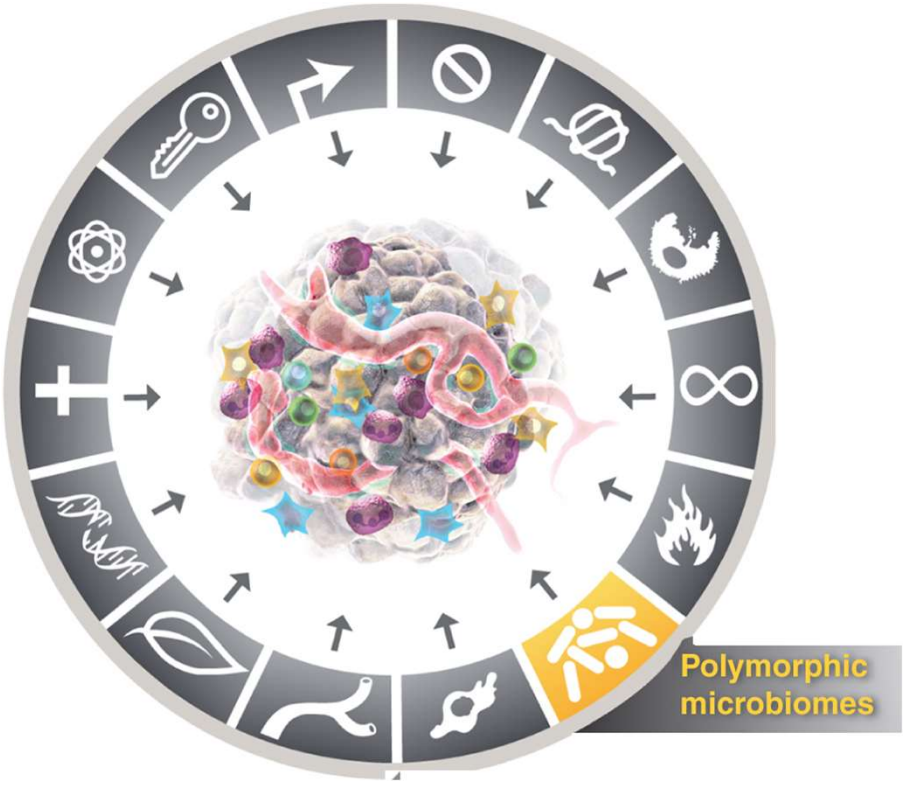
Tumors acquire additional four hallmarks capabilities



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Polymorphic microbiomes

- Microbiota, that symbiotically associate with the barrier tissues of the body exposed to the external environment - the epidermis and the internal mucosa, in particular the gastrointestinal tract, as well as the lung, the breast, and the urogenital system.

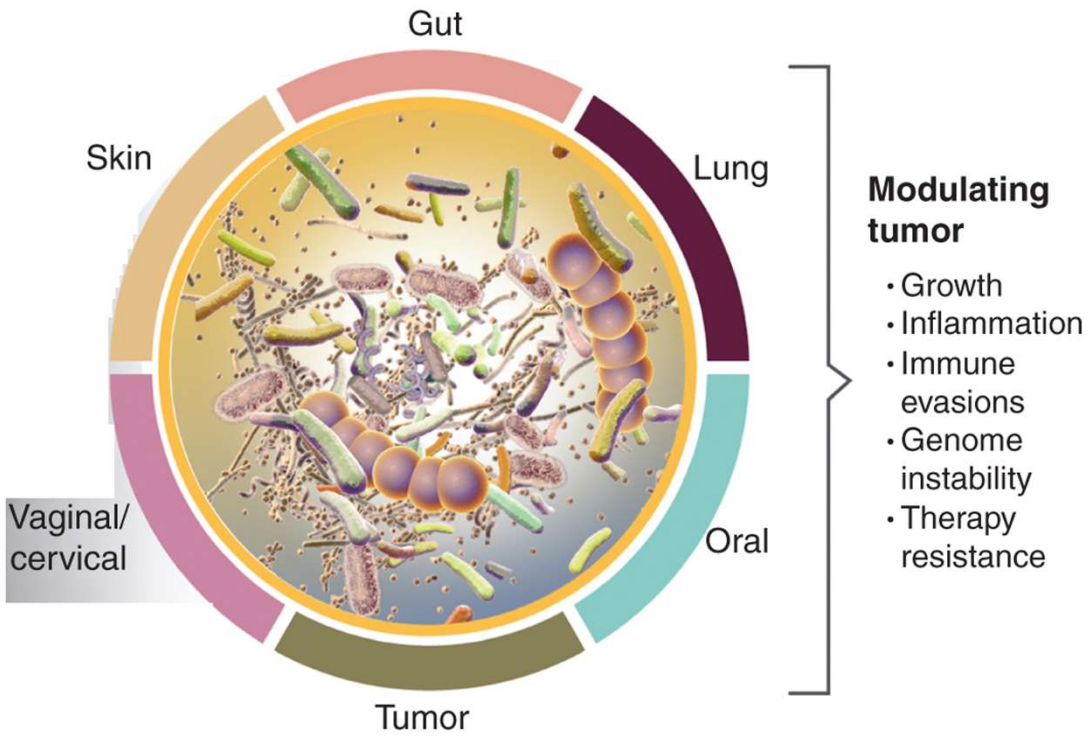
- For cancer, the evidence is increasingly compelling that polymorphic variability in the microbiomes between individuals in a population can have a profound impact on cancer phenotypes.

- Microbiotic organisms have protective or deleterious effects on cancer development, malignant progression, and response to therapy.

Polymorphic microbiomes

- Polymorphic microbiomes in one individual versus another, can diversely influence—by either inducing or inhibiting—many of the hallmark capabilities.

- Tumor microbiome, are implicated in modulating the acquisition - both positively and negatively - of the illustrated hallmark capabilities in certain tumor types.



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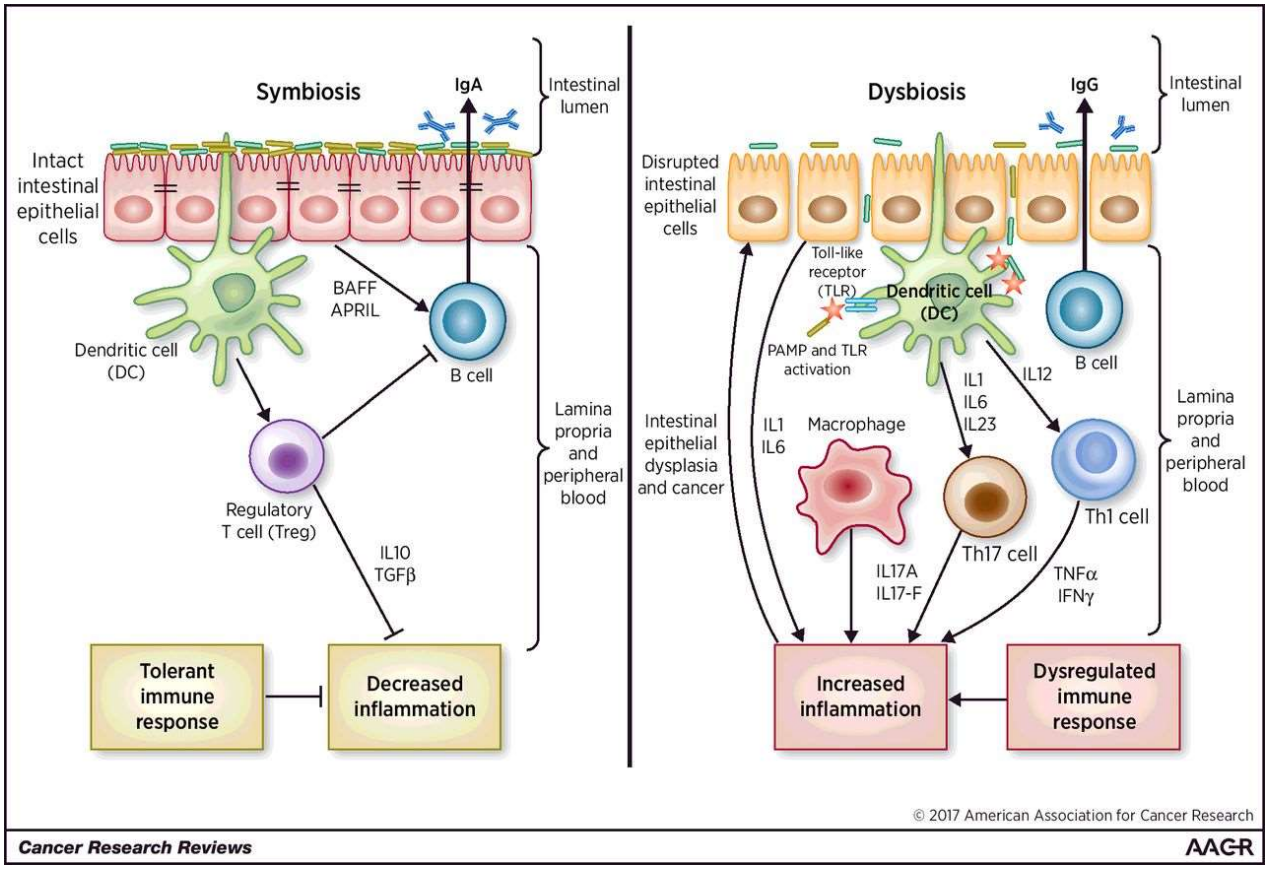
Polymorphic microbiomes



- Dysbiosis is an immunocompromised state characterized by pathobiont colonization that leads to hyperinflammation, dysplasia, and tumorigenesis.
- Pathobiont overgrowth leads to the loss of barrier integrity and a breach in the intestinal epithelial cell barrier.
- The secretion of IL1 and IL6 from intestinal epithelial cells fuels a Th1 and Th17 response by DCs and macrophages and leads to higher levels of commensal-specific IgG by B cells.

Cancer Res (2017) 77 (8): 1783–1812.

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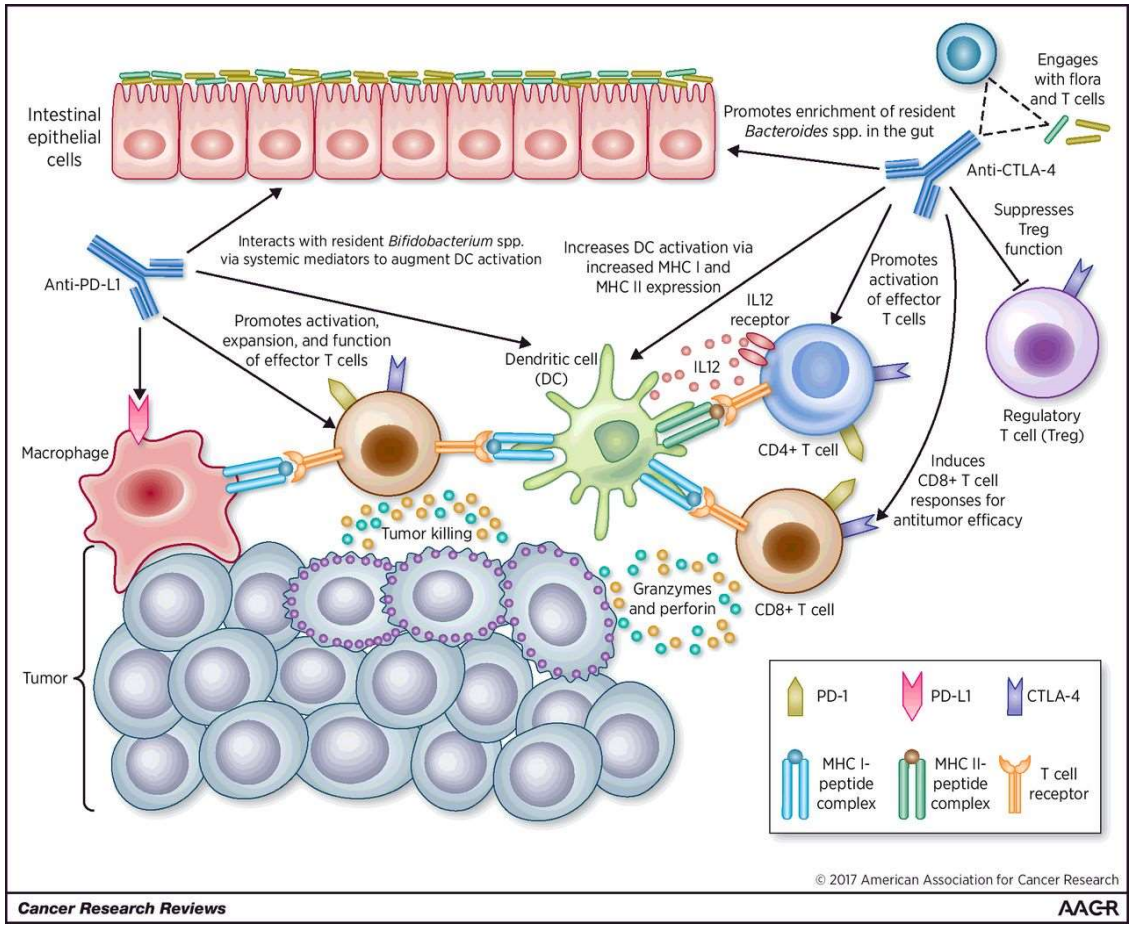
Polymorphic microbiomes

- Both anti-CTLA-4 and anti-PD-L1 therapies rely on gut microbiota for efficacy in immune activation.
- Anti-PD-L1 therapy has been shown to rely on the preexistence of sufficient *Bifidobacterium* species, which are also thought to augment responses via PD-L1 binding on APCs, such as DCs and macrophages.
- Similarly, anti-CTLA-4 indirectly alters the intestinal flora and enriches the Bacteroides species, possibly by promoting deterioration of the intestinal epithelial cell barrier via activation of local lymphocytes.



Cancer Res (2017) 77 (8): 1783–1812.

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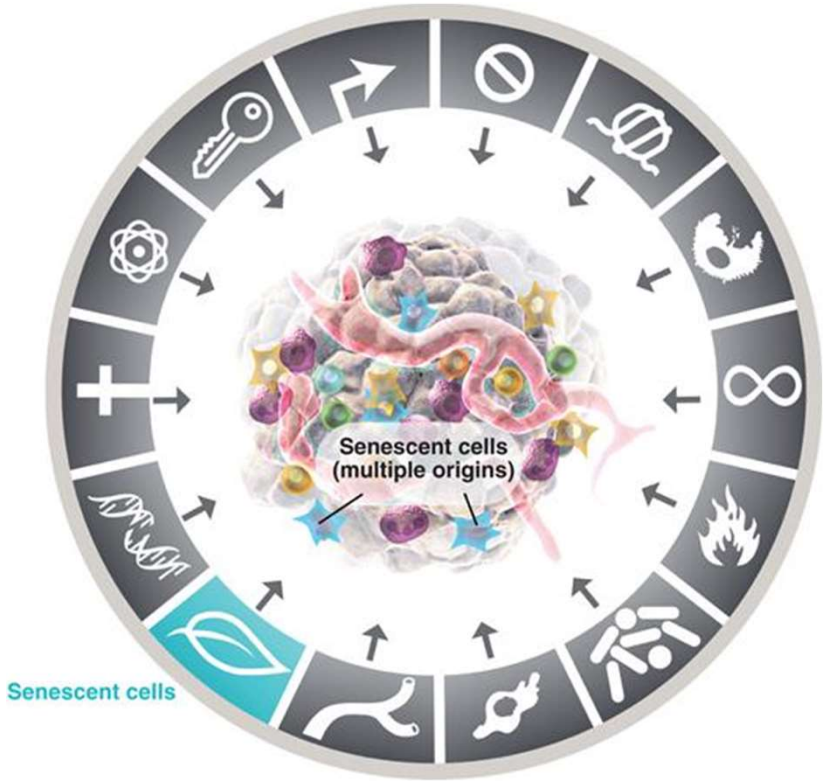
Tumors acquire additional four hallmarks capabilities



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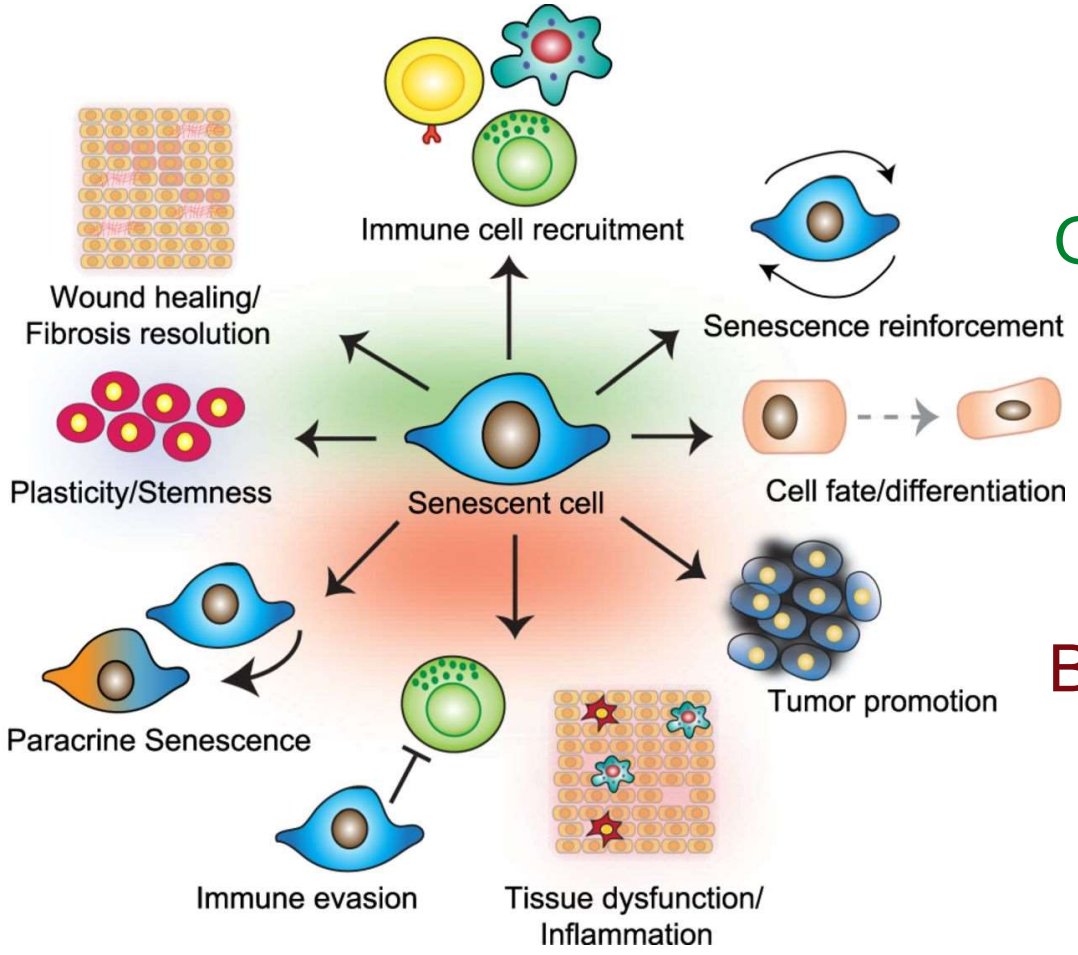
Senescent cells

- Cellular senescence is a common outcome of various anticancer interventions.
- Senescence-associated secretory phenotypes (SASPs) have pro-tumorigenic functions.
- Evidence exists of increased cellular senescence in patients treated for various types of cancer.
- Accumulation and persistence of therapy-induced senescent cells can promote tissue dysfunction and the early onset of various age-related symptoms in treated cancer patients.



Good

Bad

Trends in Cancer, Volume 6, Issue 10, October 2020, Pages 838-857
Genes & Dev. 2020. 34: 1565-1576

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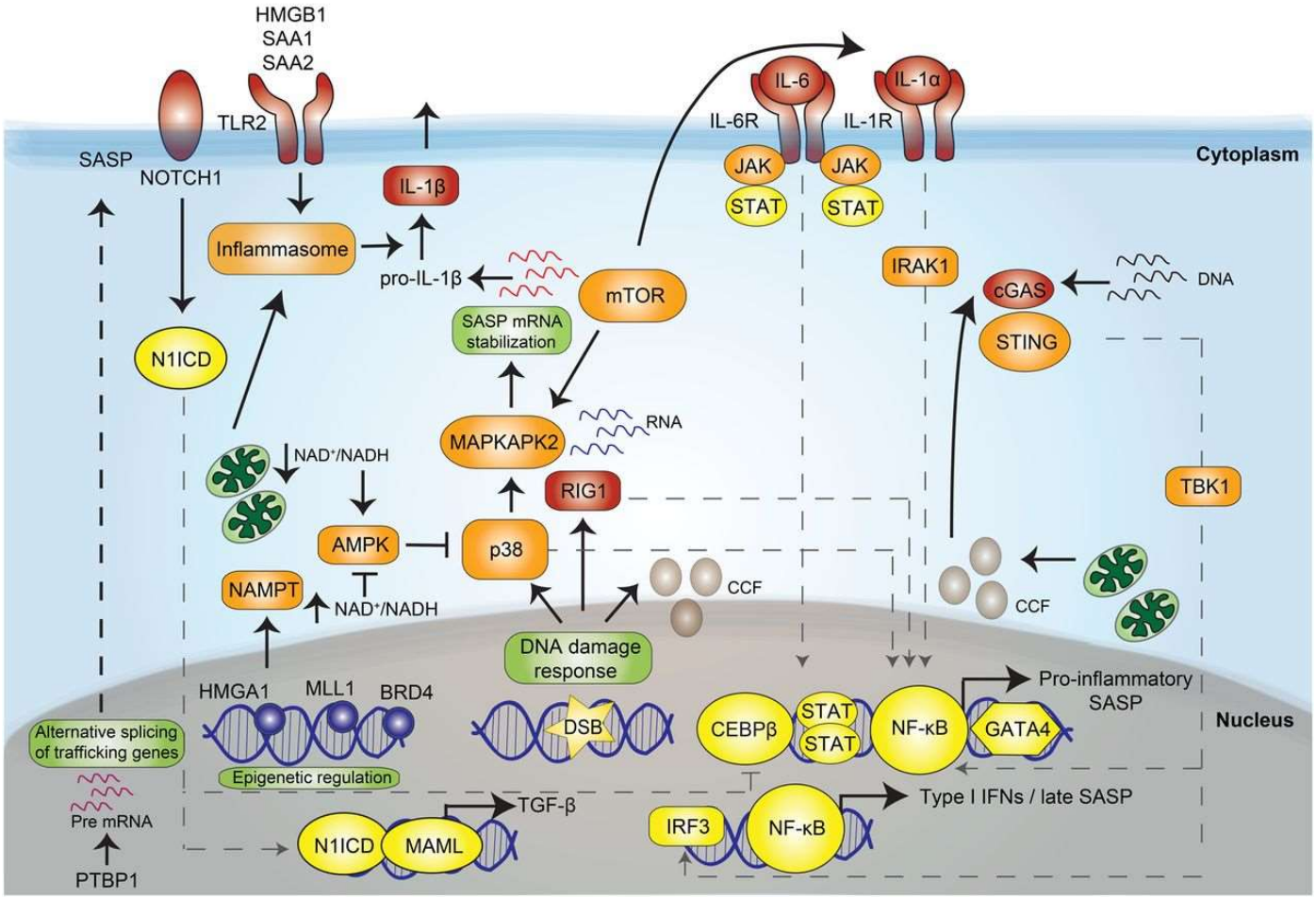
Senescent cells



- Regulation of the senescence-associated secretory phenotype (SASP).
- Transcription factors - NF-κB, C/EBPβ, STAT, MAML (yellow).
- Intracellular signaling components – mTOR, TBK1, MAPK, JAK (orange).
- Sensors and receptors and ligands – IL-6, IL-1, TLR2, NOTCH1 (red).

Genes & Dev. 2020. 34: 1565-1576

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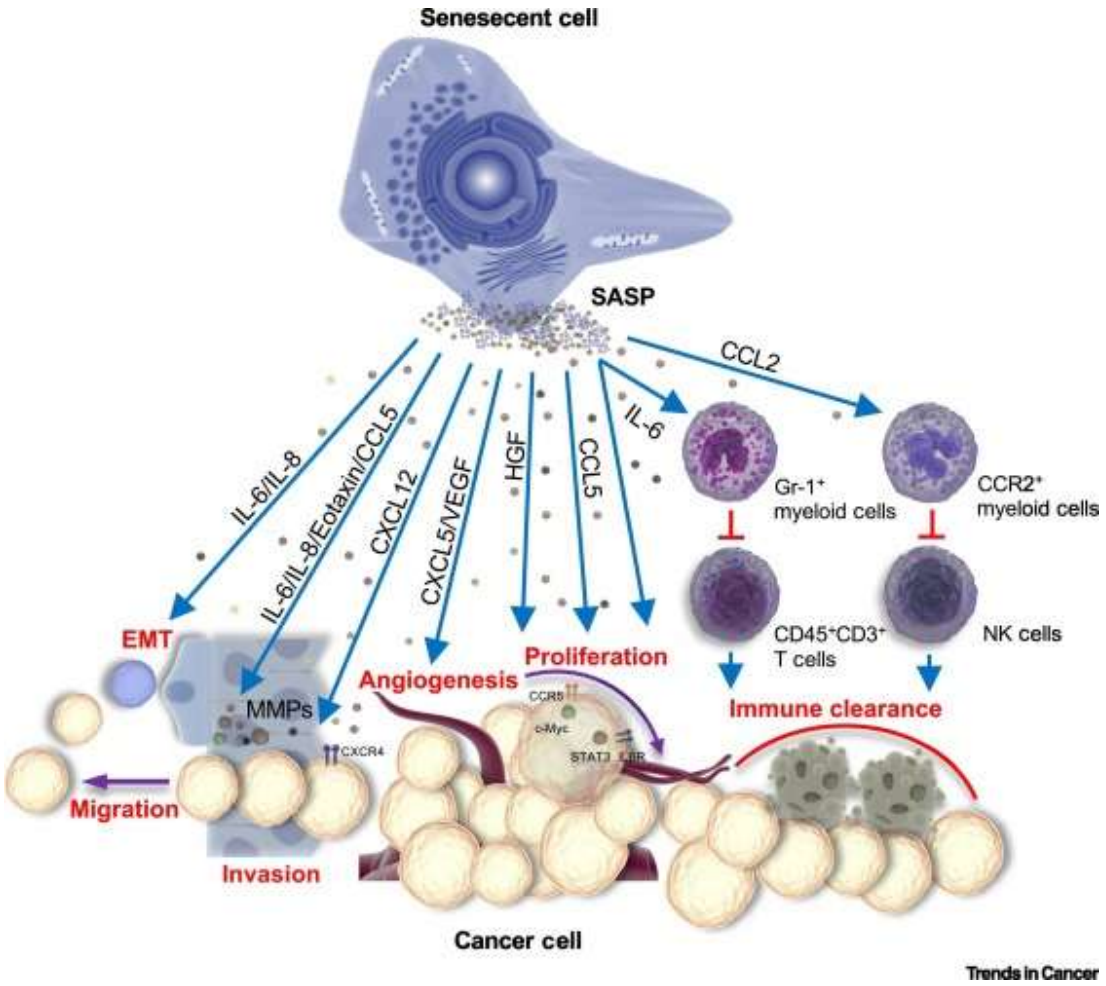
Senescent cells



- Senescence-associated secretory phenotype (SASP) factors are engaged in senescent cells through the activation of the NF-κB, C/EBPβ, and p38MAPK pathways.

- SASP factors contribute to various aspects of cancer progression:
- IL-6 and IL-8 promote EMT.
- IL-6–IL-6R cancer cell proliferation.
- CXCL5 and VEGF promote angiogenesis.
- CXCL12–CXCR4 cancer cell invasion and migration.
- IL-6 suppresses the CD45+CD3+ T cell-mediated immune clearance of cancer cells.
- CCL2 suppresses the natural killer (NK).

Trends in Cancer, Volume 6, Issue 10, October 2020, Pages 838-857

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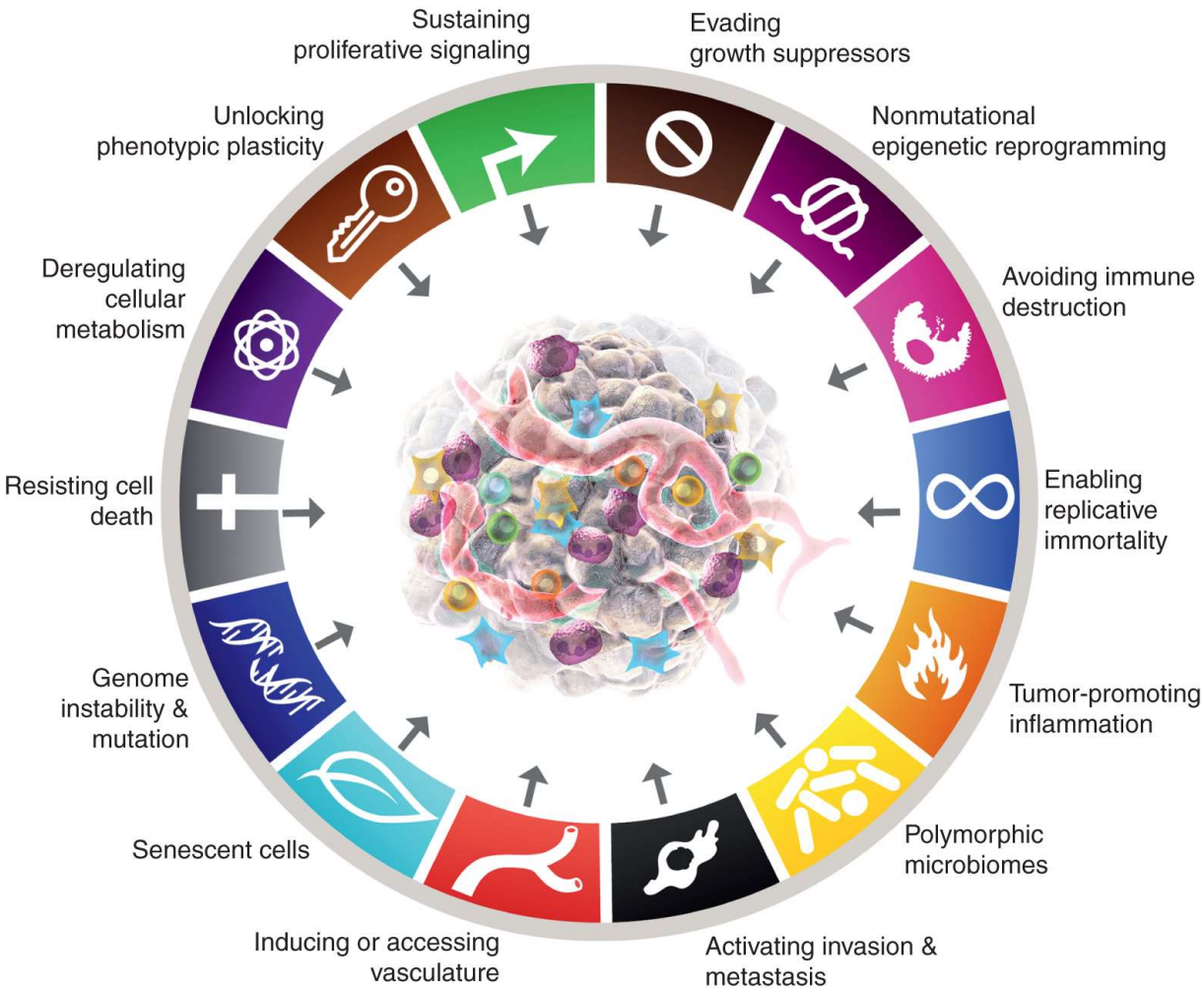
Hallmarks of Cancer



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THANK YOU FOR YOUR ATTENTION

"We're looking for somebody in medical research."

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