Stem Cells, Cancer & Therapy
BT632

Dr. Bithiah Grace Jaganathan

Course Syllabus

Cancer types, oncogenes and tumor suppressor genes; Cancer origin, progression and relapse; Cancer stem cells; Cancer and normal stem cells: common and shared pathways; Immuno-oncology and cancer microenvironment; Cancer therapy: Chemotherapy, radiation, cell and integrative therapy; Cancer multidrug resistance; Pharmacogenomics and Precision medicine.

About the course

Cancer is a dysregulation of normal cells

Healthy tissue is maintained by a stem cell population

Aberrant regulation of stem cells leads to

- Cancer
- Degenerative diseases

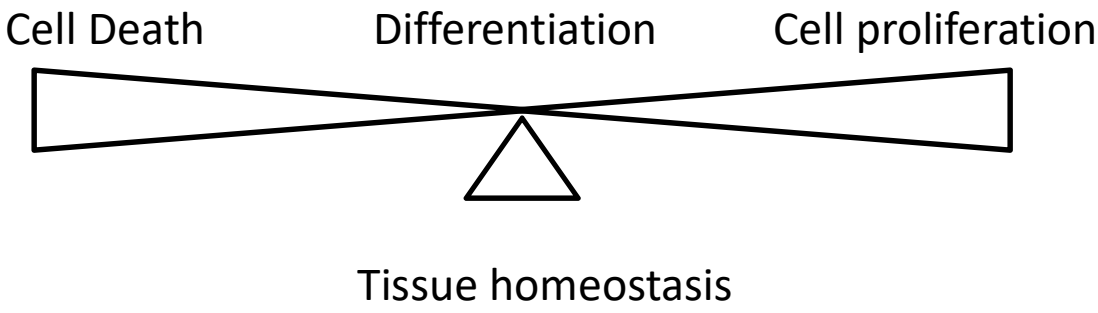
Understanding normal stem cells will point out the dysregulation in cancer cells

Is cancer a stem cell disease?

Cancer shows clonal heterogeneity

Epigenetics and microenvironment contribute to cancer development

Loss of homeostasis



Cancer is caused by the accumulation of genetic and epigenetic mutations in genes that normally play a role in the regulation of cell proliferation thus leading to uncontrolled cell growth

Cancer is generated through

- Mutations
  - Dominant gain of function of oncogenes
  - Recessive loss of function of tumor suppressor genes

Genetic changes

- Mutations
- Gene amplifications
- Loss of heterozygosity (LOH)
- Translocations

Oncogenes and Tumor Suppressor genes

I. Growth factors
II. Growth factor receptors
III. Signal-transduction proteins
IV. Transcription factors
V. Pro- or anti-apoptotic proteins
VI. Cell cycle control proteins

Proto-Oncogenes

Tumor suppressor genes

VII. DNA repair proteins

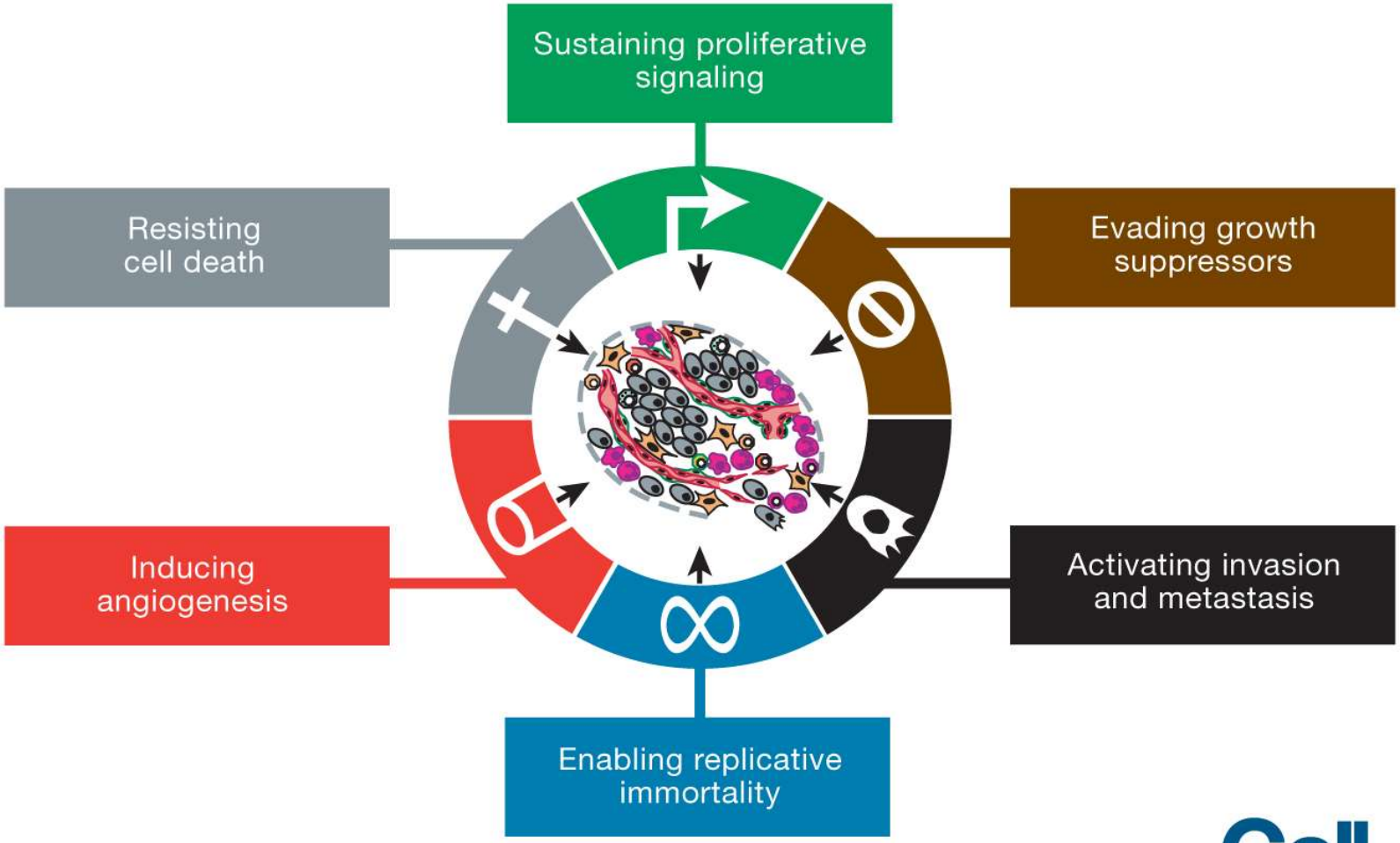
Increases mutation in proto-oncogenes / tumor suppressor genes

Gain of function mutation in proto-oncogenes and loss of function mutation in tumor suppressor genes

Tumorigenesis

- Multi-step process
- Progressive transformation of normal cells
- Age-dependent incidence

Hallmarks of cancer



Hanahan and Weinberg, 2011

Cell PRESS

Sustained proliferative signaling

- Dysregulation of growth factor signaling
- Spatially & temporally regulated paracrine signaling
- Sequestration in ECM and pericellular space

Growth factor signaling in cancer

- Cancer autocrine signaling
- Paracrine signaling to microenvironment
- Receptor overexpression
- Ligand independent signaling
- Constitutively active signaling
- Disruption of negative feedback signaling
- Higher but not excessive proliferative signaling

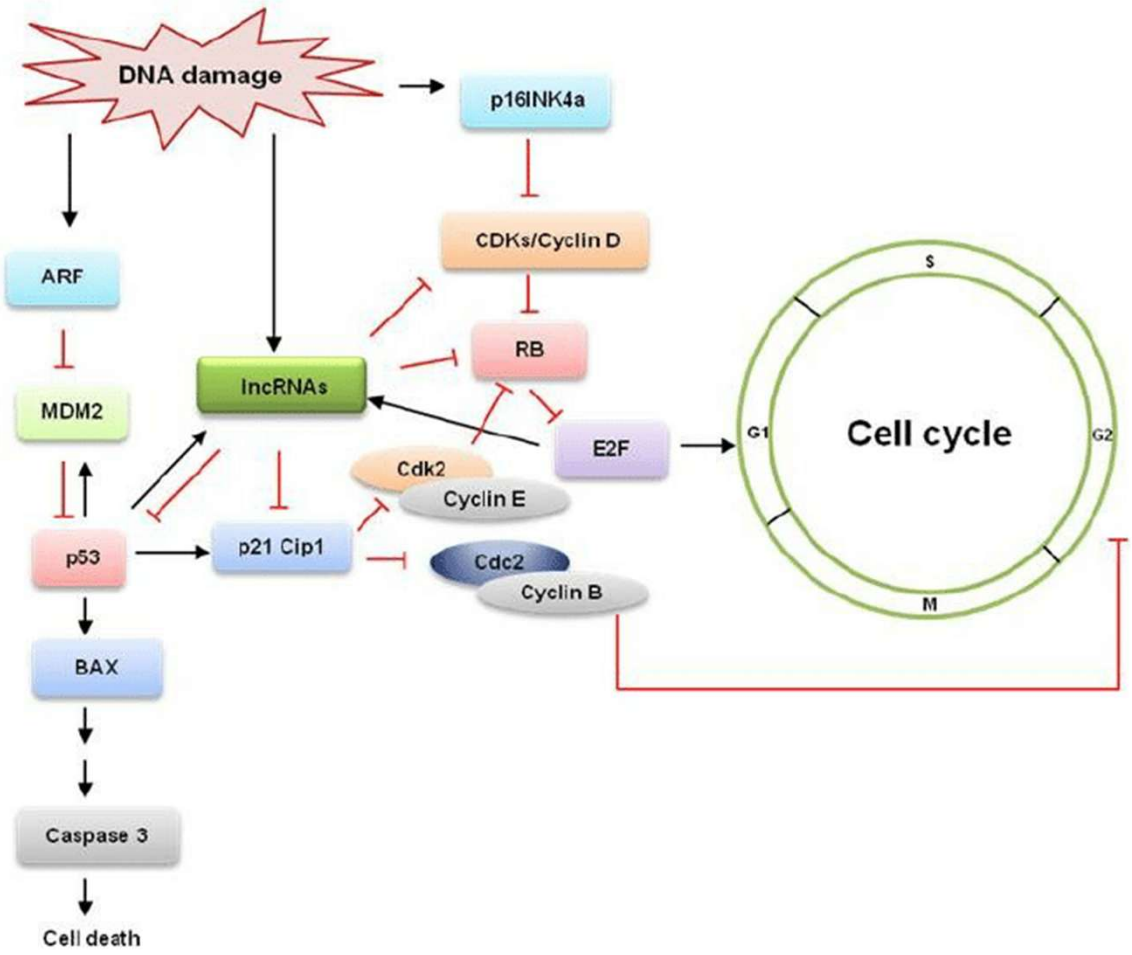
Sustained proliferative signaling

- Somatic mutations – activate downstream signaling
  - B-Raf, PI3K, AKT
- Disruption of negative feedback
  - PI3K, PTEN
  - mTOR inhibitors
- Excessive proliferative signaling
  - Inactivation of senescence/apoptosis genes

Evading growth suppressors

- Mutation in tumor suppressors RB and TP53
- RB transduces extracellular signals
- TP53 transduces intracellular signals
- RB and TP53 show functional redundancy
- RB null mice are normal with pituitary tumors at later life
- TP53 null mice are normal with leukemia and sarcoma at later life

Evading growth suppressors



Cancer progression

- Hyperplasia-Excessive normal cells
- Metaplasia-replacement of normal cells
- Dysplasia-cytologically abnormal
- Neoplasm-abnormal growth

Evasion of contact inhibition

- NF2 couples E-cad with EGFR to strengthen cell-cell adhesion
- LKB1 overrules Myc signaling in organized epithelial cells
- Anti-proliferative TGF-b signal promotes EMT

- Are these gene oncogenes or TS?

Resisting cell death

- Apoptosis evasion (extrinsic and intrinsic)
  - TP53 loss
  - Bcl-2, Bcl-XL overexpression
  - Bax, Bak, Bim, Puma downregulation
- Autophagy (dual function)
  - through Beclin-1
  - Beclin-1 null mouse had increased susceptibility to cancer
  - Nutrient starvation shrunk cancer cells to a reversible dormancy through autophagy
- Necrosis
  - Promotes inflammation
  - Helps in angiogenesis, cancer proliferation, invasion
  - Releases proliferation inducing IL1a

Replicative immortality

- Telomere elongation (TTAGGG)
- Telomerase activation/alternate telomere copying
- Telomere shortening-limit and enhance tumorigenesis
- Telomere shortening with TP53 inactivation leads to BFB cycle-delayed telomerase activation
- TERT provides resistance to apoptosis/DNA repair, etc

Angiogenesis

- Switching on of angiogenic switch
- VEGF-A and FGF upregulated
- Abnormal, leaky, enlarged, hemorrhagic vessels
- Early dripping of angiogenic switch during dysplasia
- Controlled by circulating angiogenic inhibitors (TSP-1)-inhibition leads to impaired wound healing and tissue remodeling

Angiogenesis

- Stromal deserts or densely vascularized
- Ras, Myc upregulate angiogenic factors
- Immune inflammatory cells promote angiogenesis
- Invade tumors and assemble at tumor periphery

Invasion and Metastasis

- ECM modification
- Downregulation of E-cad-required for cell-cell attachment and quiescence
- Upregulation of N-cadherin
- EMT-invade, resist apoptosis and disseminate
- Snail, Slug, Twist, Zeb1/2
- EMT-transient state
- Tumor stroma secrete-CCL5/RANTES
- Macrophages secrete-metalloproteinases
  - TAMs secrete EGF – cancer secrete CSF-1

Invasion and Metastasis

- Anoikis resistance during circulation
- Tethering, rolling and transmigration
- Reverse EMT or MET at secondary site
- Mechanostate of tumor induces EMT
- Collective invasion – non metastatic
- Amoeboid migration
- Inflammatory cells in invasion

Invasion and Metastasis

- Dissemination and seeding is ineffective
- Dormant Micrometastasis
  - Lack of angiogenesis
  - Inhospitable microenvironment
- Macrometastasis after the removal of primary tumor –breast/melanoma
- Seeding other metastatic sites and primary site

Genomic Instability

- Mutations-provide selective advantages
- Increased mutation rate
- Accumulation of mutation-spatial and temporal
- Loss of DNA repair genes-caretake genes
- Mutation profile of cancers

Tumor promoting inflammation

- Infiltrated by innate and adaptive immune cells
- Growth factors, survival factors, proangiogenic factors
- ROS of immune cells cause genome instability in cancer cells

Reprogramming Energy Metabolism

- Glycolysis
- Glucose+2 NAD^++2 ADP+2Pi→2 Pyruvate+2 NAD H+2 ATP+2 H_2O+2 H^+
- **In Normal Cells (Aerobic Conditions)**
- Mitochondrial Entry**:**
  Under aerobic conditions, pyruvate is transported into the mitochondria.
- Conversion to Acetyl-CoA:
  - **Enzyme:** Pyruvate dehydrogenase complex
  - **Reaction:**
    Pyruvate+NAD^++CoA→Acetyl-CoA+CO2+NADH

Reprogramming Energy Metabolism

- **In Aerobic Glycolysis (Warburg Effect)**
- **Conversion to Lactate:**
  In many cancer cells, even in the presence of sufficient oxygen, pyruvate is preferentially reduced to lactate. This is catalyzed by lactate dehydrogenase in the cytosol.
- **Reaction:**
  $\text{Pyruvate} + \text{NADH} + \text{H}^+ \rightarrow \text{Lactate} + \text{NAD}^+$
- Modifying the tumor microenvironment (acidification due to lactate production).

Reprogramming Energy Metabolism

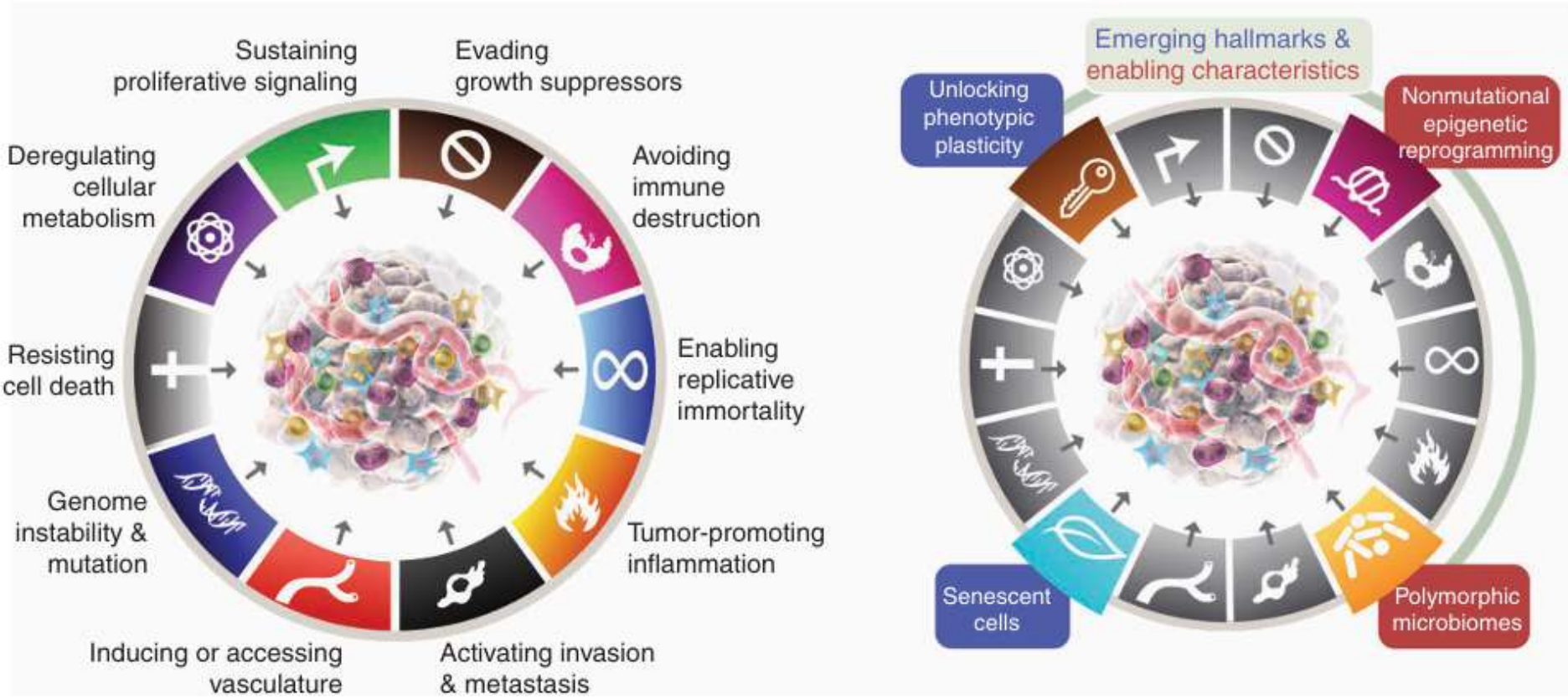
- **ATP Yield**
  - **Normal Aerobic Metabolism:**
    Glucose oxidation via glycolysis, citric acid cycle, and oxidative phosphorylation yields up to 38 ATP per glucose molecule.
  - **Aerobic Glycolysis (Cancer):**
    Primarily yields 2 ATP per glucose via glycolysis, but the rapid rate and production of biosynthetic intermediates support cell proliferation.

Reprogramming Energy Metabolism

- GLUT1 increase
- Activated by Ras, Myc and mutated p53
- Hypoxia in tumor-HIF1a-increase glycolysis
- Two populations-lactate producing and lactate consuming with citric acid cycle

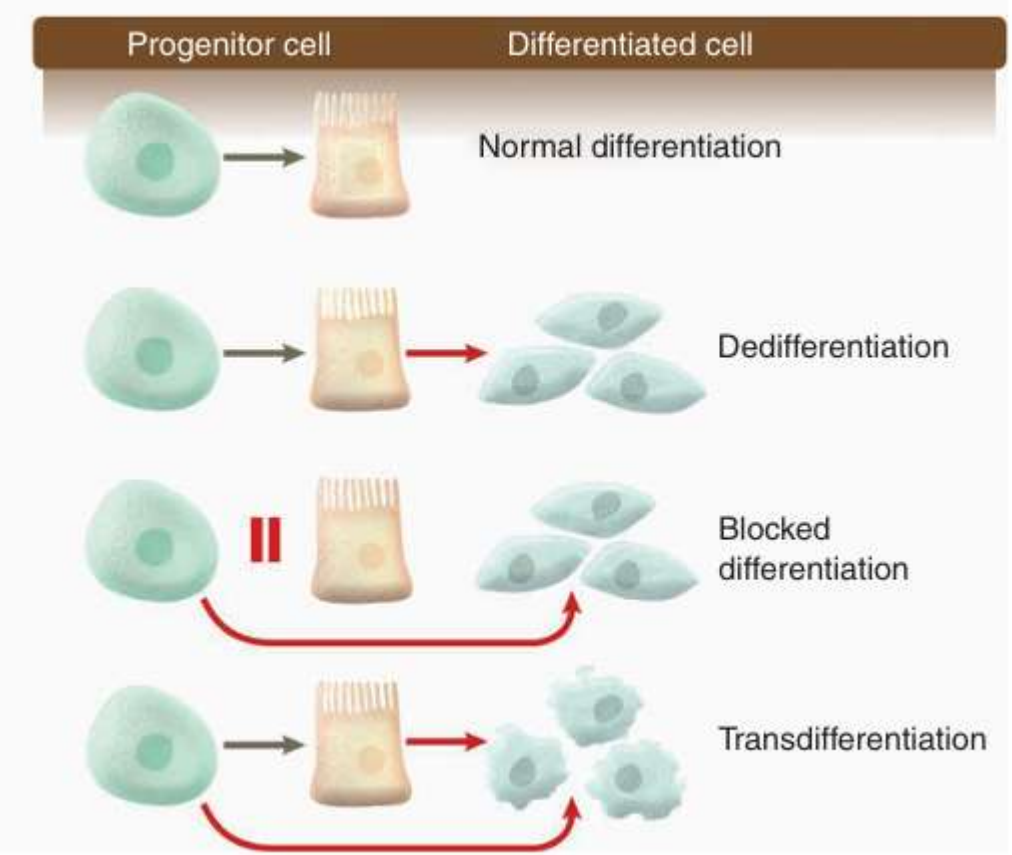
Evading immune destruction

- Increase in cancer in immunosuppressed conditions
- High infiltration of immune cells in tumor- better prognosis


Sustaining proliferative signaling
Evading growth suppressors
Avoiding immune destruction
Enabling replicative immortality
Tumor-promoting inflammation
Activating invasion & metastasis
Inducing or accessing vasculature
Genome instability & mutation
Resisting cell death
Deregulating cellular metabolism
Emerging hallmarks & enabling characteristics
Unlocking phenotypic plasticity
Nonmutational epigenetic reprogramming
Senescent cells
Polymorphic microbiomes

Phenotypic plasticity

- Differentiation is anti-proliferative
- Differentiated cells - Dedifferentiation into progenitor cells
- Block in differentiation of progenitor cells
- Transdifferentiation into a different lineage



Dedifferentiation

- HOXA5 and SMAD4 – expressed in colonic epithelial cells
  - Lost in colon carcinomas
  - Forced expression, suppresses stem cell phenotype, impairs invasion and metastasis
- SMAD4 suppresses Wnt driven hyper proliferation
- MITF – differentiation marker is downregulated
- BRAFmutated -originate from mature melanocytes

Dedifferentiation

- Pancreatic cancer – similar to embryonic islet precursors
- Upregulation of miRNA required for progenitor state rather than sustained proliferation or resistance to cell death – initiate the carcinogenesis

Blocked differentiation

- PML-RARa in APML – blocks myeloid differentiation
  - treatment with retinoic acid – induces differentiation
- AML1-ETO in AML – HDAC inhibitor induces differentiation
- SOX10 blocks the differentiation of progenitors into melanocytes in BRAF driven melanocytes

Blocked differentiation

- Circumvented differentiation – partially or undifferentiated progenitor exit cell cycle – quiescent-proliferate again

Transdifferentiation

- Metaplasia
- Barrett's esophagus – stratified squamous epithelium converted to simple columnar epithelium
- pancreatic ductal adenocarcinoma (PDAC) - cells of origin, the pancreatic acinar cell - transdifferentiate into a ductal cell phenotype during the initiation of neoplastic development.

Transdifferentiation

- Two TFs—PTF1a and MIST1— govern, via their expression in the context of self-sustaining, “feed-forward” regulatory loops
- PTF1a impairs KRAS-induced transdifferentiation and proliferation, - force the redifferentiation of already neoplastic cells into a quiescent acinar cell phenotype
- suppression of PTF1a expression elicits acinar-to-ductal metaplasia, namely transdifferentiation, and thereby sensitizes the duct-like cells to oncogenic KRAS transformation, accelerating subsequent development of invasive PDAC

Nonmutational Epigenetic Reprogramming

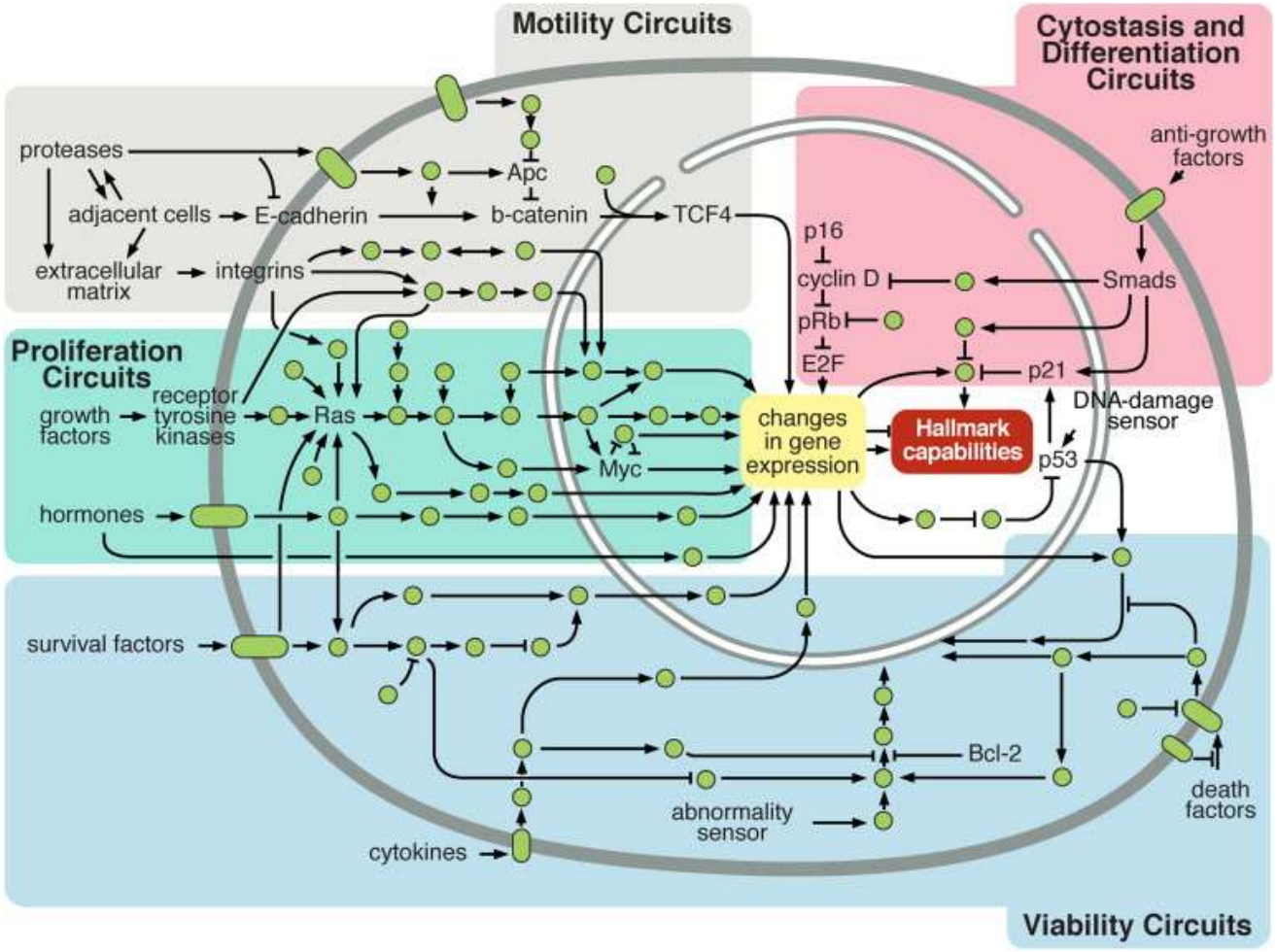
- Genomic instability
- Microenvironment mediated –stromal and immune cells mediated
- Leads to transcriptional heterogeneity

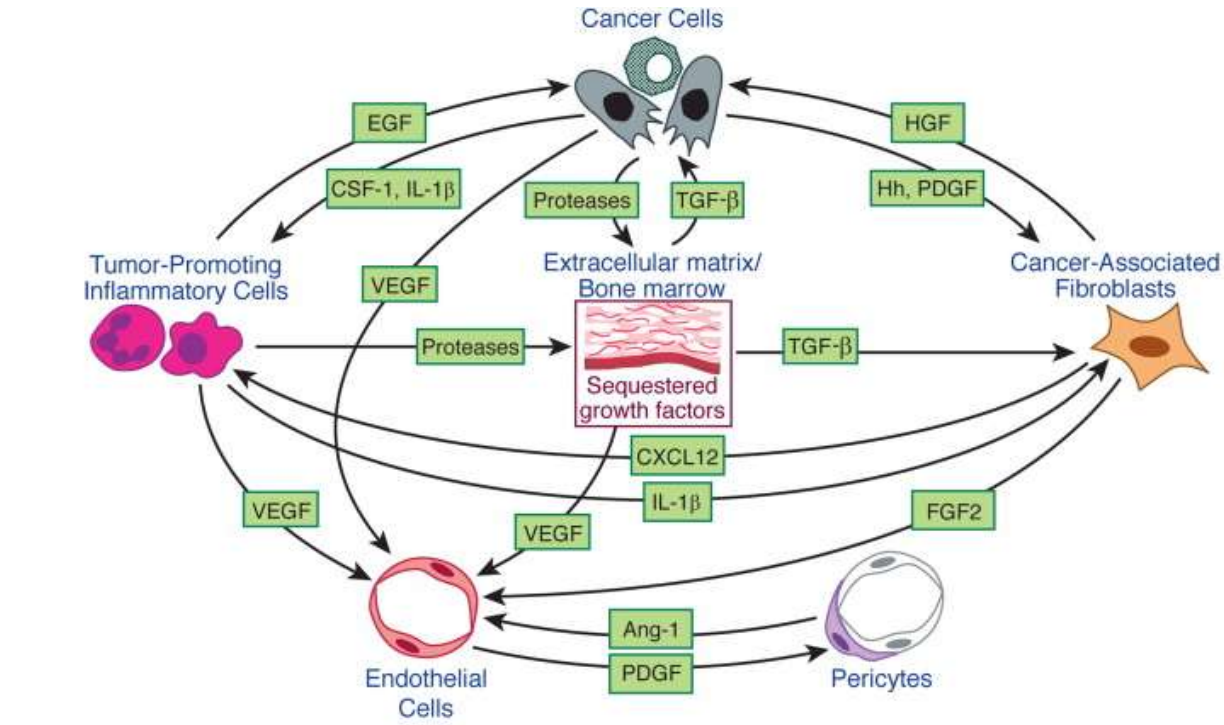
Polymorphic mirobiomes

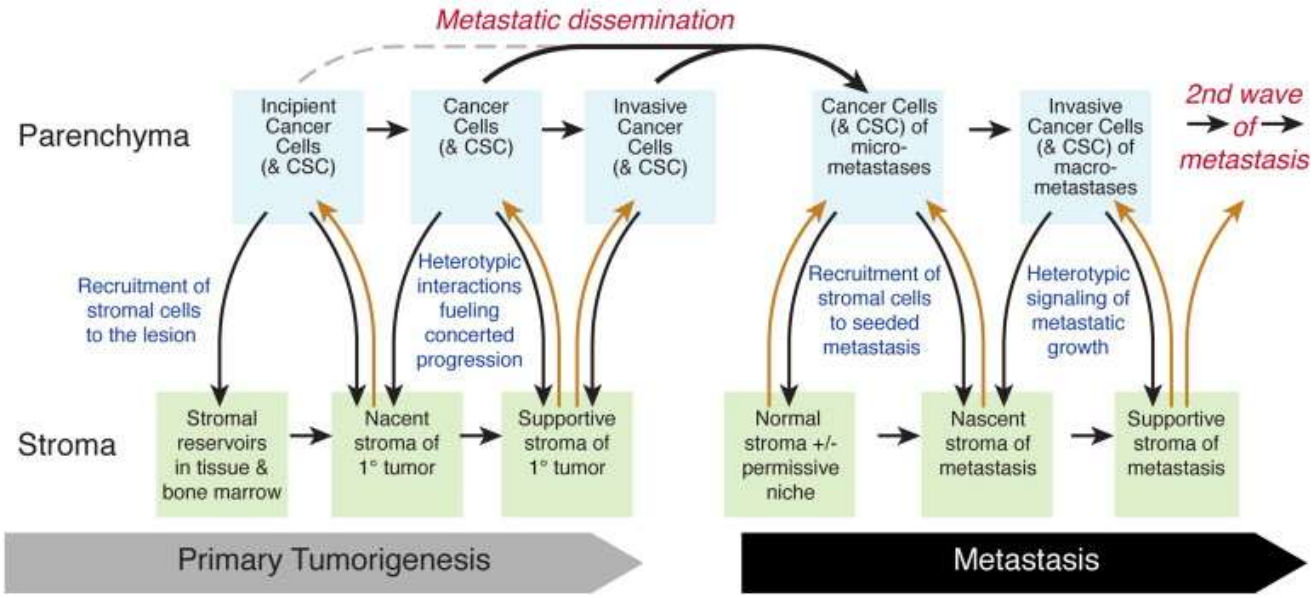
- Microbiota changes with aging and cancer
- Gut microbiomes in altering the metabolism
- Immunomodulatory factors by microbes
- Intratumoral microbiota

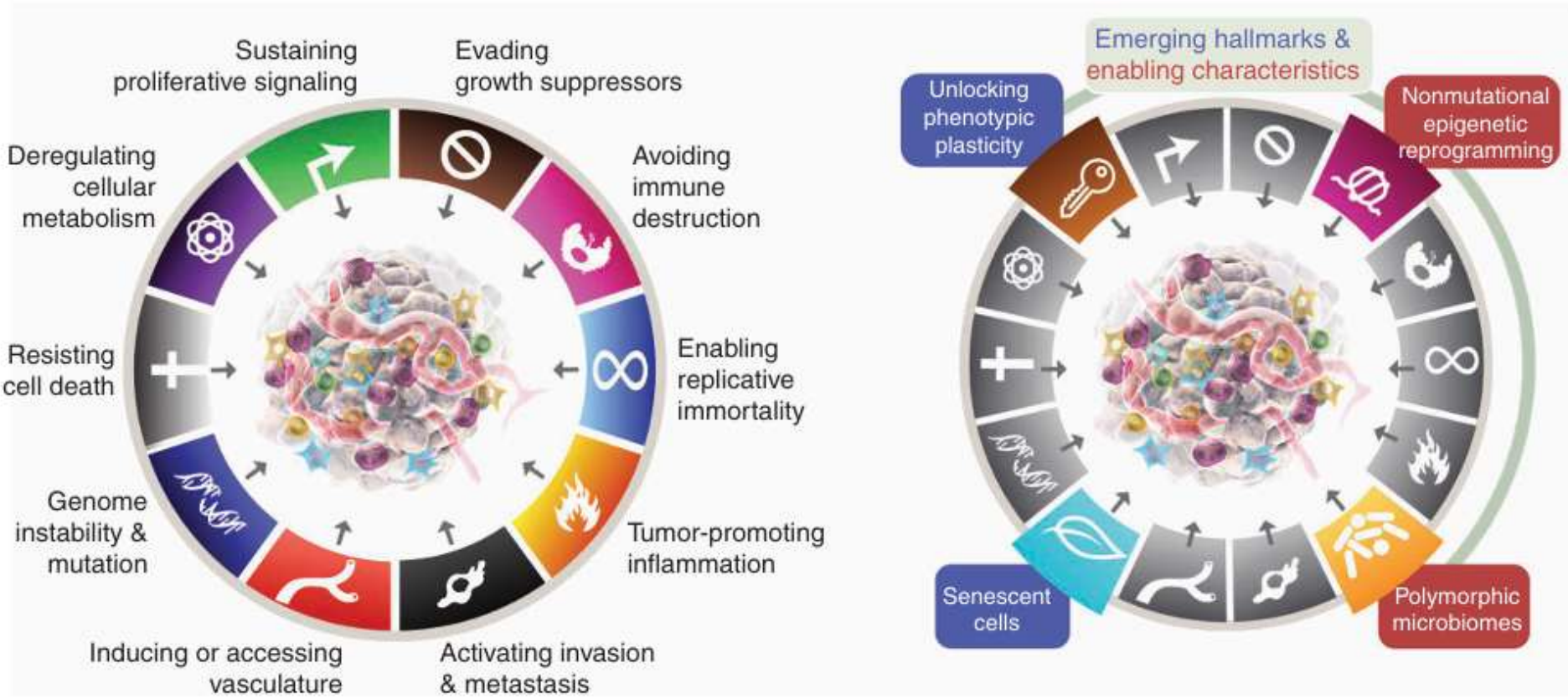
Senescent Cells

- Irreversible cell cycle arrest
- Senescence-associated secretory phenotype
- Reversible senescence


Motility Circuits
Cytostasis and Differentiation Circuits
Proliferation Circuits
Viability Circuits
proteases
adjacent cells
E-cadherin
Apc
b-catenin
TCF4
extracellular matrix
integrins
anti-growth factors
p16
cyclin D
Smads
pRb
E2F
p21
growth factors
receptor tyrosine kinases
Ras
Myc
changes in gene expression
Hallmark capabilities
DNA-damage sensor
p53
hormones
survival factors
Bcl-2
abnormality sensor
death factors
cytokines


Cancer Cells
EGF
HGF
CSF-1, IL-1β
Proteases
TGF-β
Hh, PDGF
Tumor-Promoting Inflammatory Cells
VEGF
Extracellular matrix/ Bone marrow
Cancer-Associated Fibroblasts
Proteases
Sequestered growth factors
TGF-β
CXCL12
IL-1β
VEGF
VEGF
FGF2
Ang-1
PDGF
Endothelial Cells
Pericytes


Metastatic dissemination
Parenchyma
Incipient Cancer Cells (& CSC)
Cancer Cells (& CSC)
Invasive Cancer Cells (& CSC)
Cancer Cells (& CSC) of micro-metastases
Invasive Cancer Cells (& CSC) of macro-metastases
2nd wave of metastasis
Recruitment of stromal cells to the lesion
Heterotypic interactions fueling concerted progression
Recruitment of stromal cells to seeded metastasis
Heterotypic signaling of metastatic growth
Stroma
Stromal reservoirs in tissue & bone marrow
Nacent stroma of 1° tumor
Supportive stroma of 1° tumor
Normal stroma +/- permissive niche
Nascent stroma of metastasis
Supportive stroma of metastasis
Primary Tumorigenesis
Metastasis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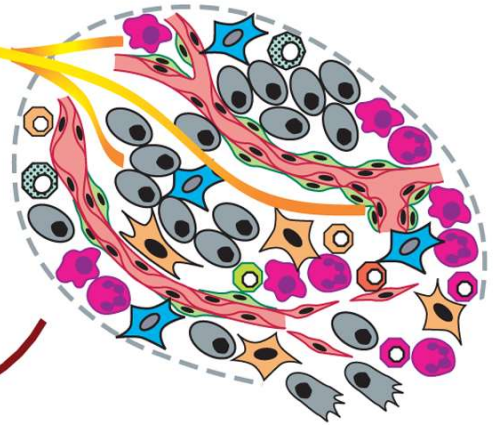
Resisting cell death
Deregulating cellular metabolism
Emerging hallmarks & enabling characteristics
Unlocking phenotypic plasticity
Nonmutational epigenetic reprogramming
Senescent cells
Polymorphic microbiomes

Neural interactions facilitate cancer hallmarks

- Sustaining proliferative signaling
- Resisting cell death
- Activating invasion & metastasis
- Tumor-promoting inflammation

Innervation by multiple neuronal subtypes



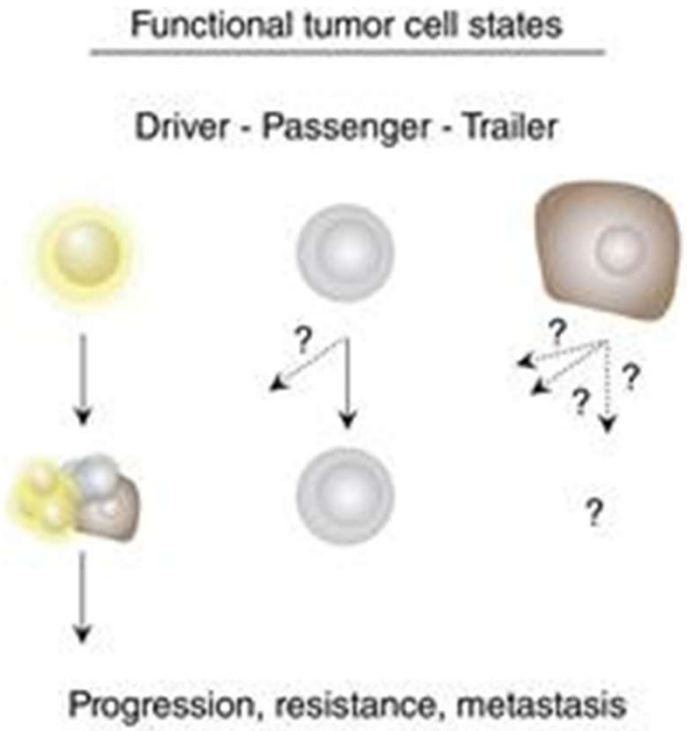
Cancer-induced neural remodeling

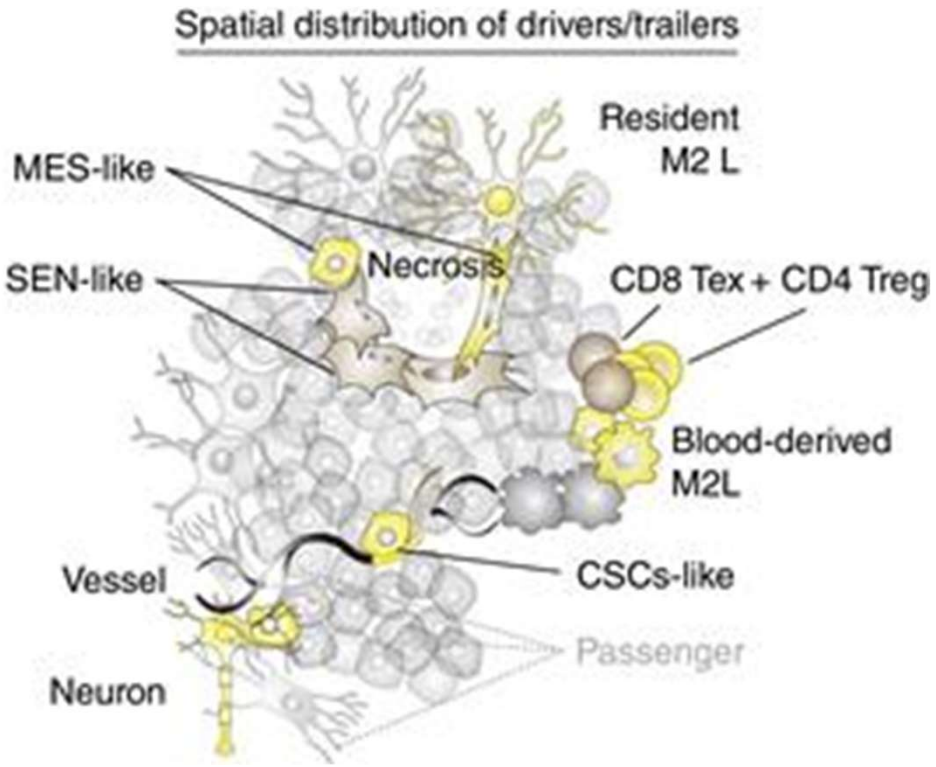
- Encouraging nerve ingrowth
- Increasing neuronal excitability
- Reinforcing nerve-cancer interactions

Cell Types of the Tumor Microenvironment

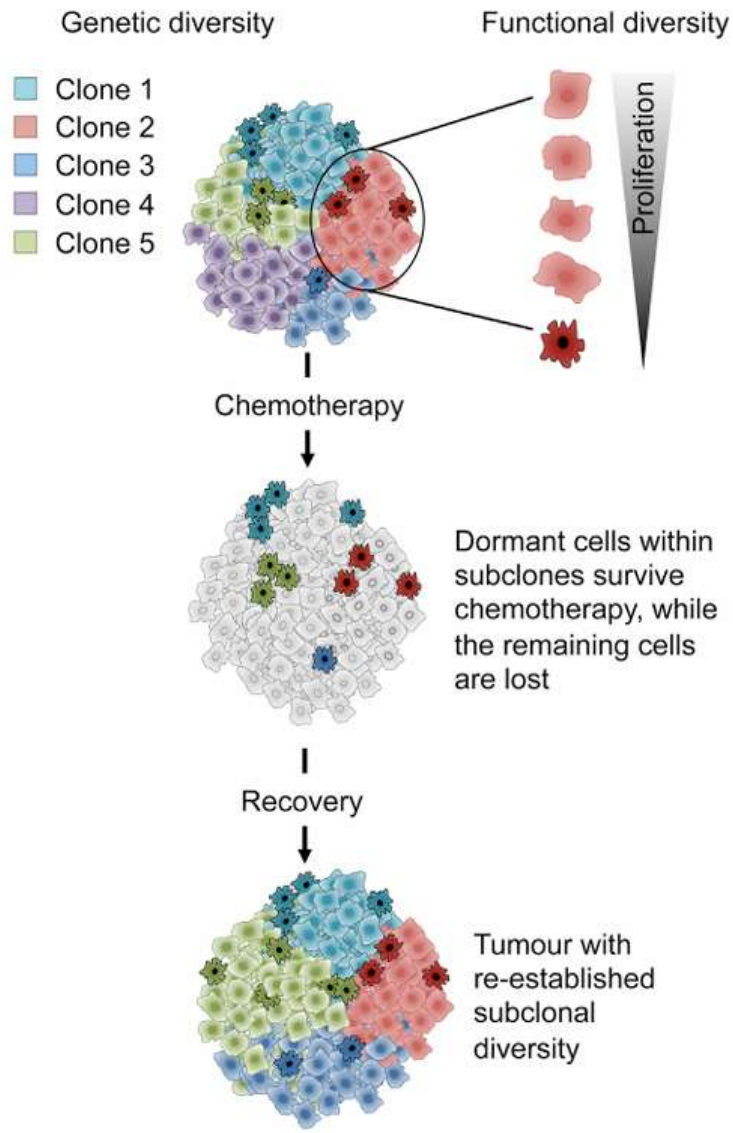
- Cancer Cells
- Invasive Cancer Cells
- Cancer Stem Cells
- Cancer-associated Fibroblasts
- Endothelial Cells
- Pericytes
- Tumor-promoting immune cells
- Stromal Progenitor Cells
- Senescent Cells (various origins)
- Neurons (and their projections)

Cancer heterogeneity

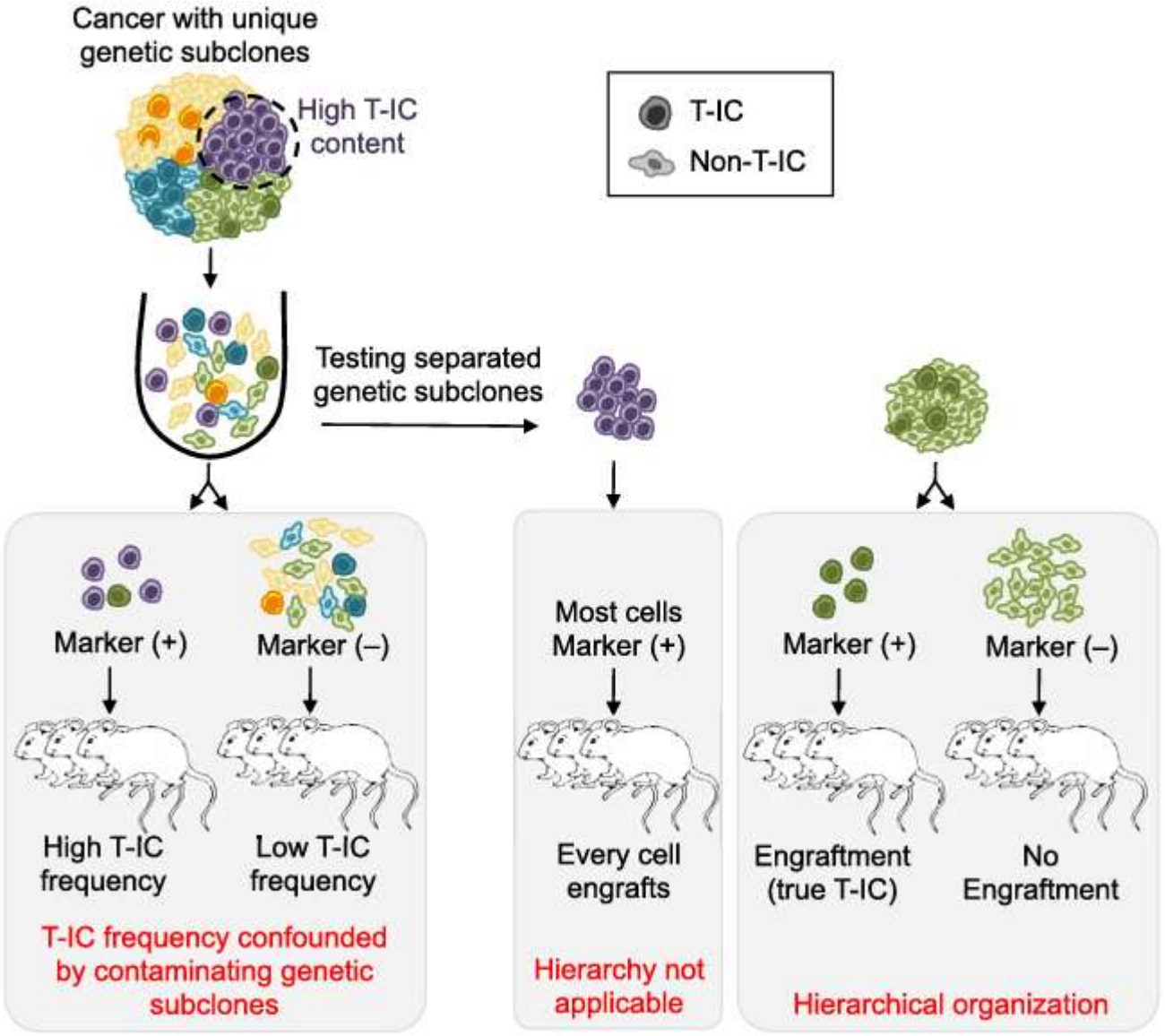




Cancer heterogeneity



TIC-multiple markers



Genetic subclones