V9: Cancerogenesis

www.healthline.com specifies:

“A **neoplasm** is an abnormal growth of cells, also known as a **tumor**.

Neoplastic diseases are conditions that cause tumor growth — both benign and malignant.

Benign tumors are noncancerous growths. They usually grow slowly and can’t spread to other tissues.

Malignant tumors are cancerous and can grow slowly or quickly.

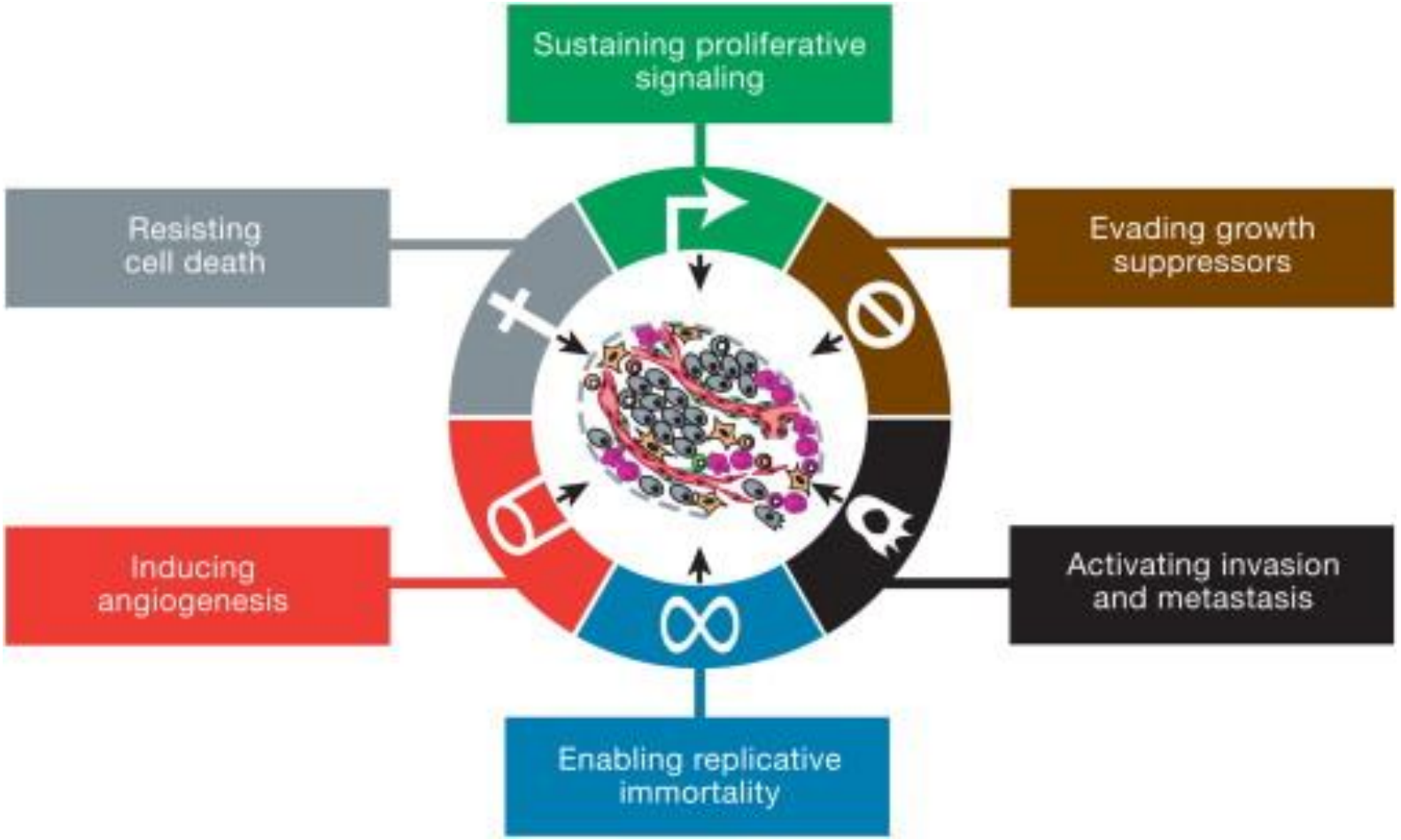
Malignant tumors carry the risk of **metastasis**, or spreading to multiple tissues and organs.”

https://blog.dana-farber.org/ explains

“Cancer is a disease in which cells, almost anywhere in the body, begin to divide uncontrollably. A **tumor** is when this uncontrolled growth occurs in solid tissue such as an organ, muscle, or bone. Tumors may spread to surrounding tissues through the blood and lymph systems.”

Hallmarks of cancers

In 2000, Hanahan & Weinberg proposed that the six listed hallmarks of cancer are characteristic common properties of the diverse tumors (neoplastic diseases).



Hanahan & Weinberg,
Cell **144**, 646–674 (2011)

Mutations sustain proliferative signalling: Raf, ras, Pi3K

High-throughput DNA sequencing analyses of cancer cell genomes revealed somatic mutations in certain human tumors that predict constitutive activation of signaling circuits that are usually triggered by activated growth factor receptors.

~40% of human melanomas contain activating mutations affecting the structure of the **B-Raf protein**.

These lead to constitutive signaling through the Raf to mitogen-activated protein (MAP)-kinase pathway.

Similarly, mutations in the catalytic subunit of **phosphoinositide 3-kinase (PI3-kinase**) isoforms are detected in several tumor types, which serve to hyperactivate the PI3-kinase signaling circuitry, including its key **Akt/PKB** signal transducer.

Hanahan & Weinberg,
Cell **144**, 646–674 (2011)

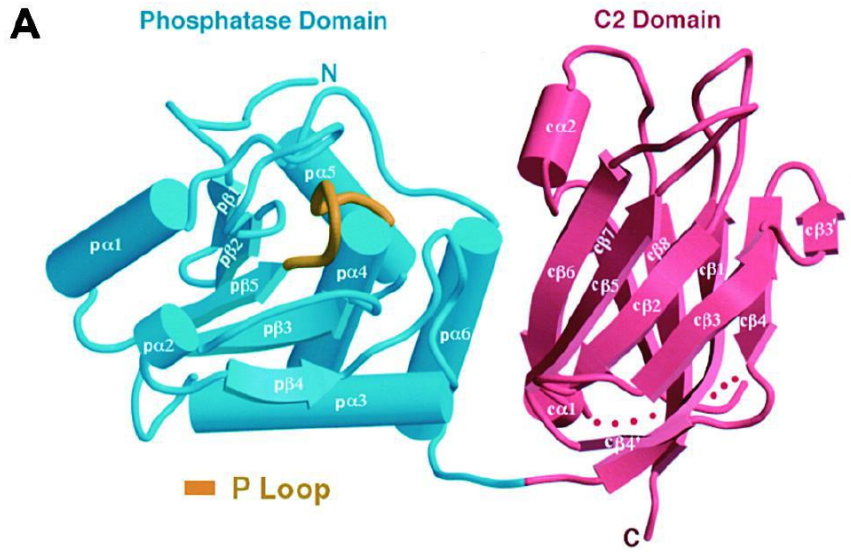
Attenuate proliferative signalling: PTEN

Negative-feedback mechanisms operate at multiple nodes within the proliferative signaling circuitry.

A prominent example involves the **PTEN phosphatase**, which counteracts PI3-kinase by degrading its product, phosphatidylinositol (3,4,5) trisphosphate (PIP_3).

Loss-of-function mutations in PTEN amplify **PI3K signaling** and promote tumorigenesis in a variety of experimental models of cancer.

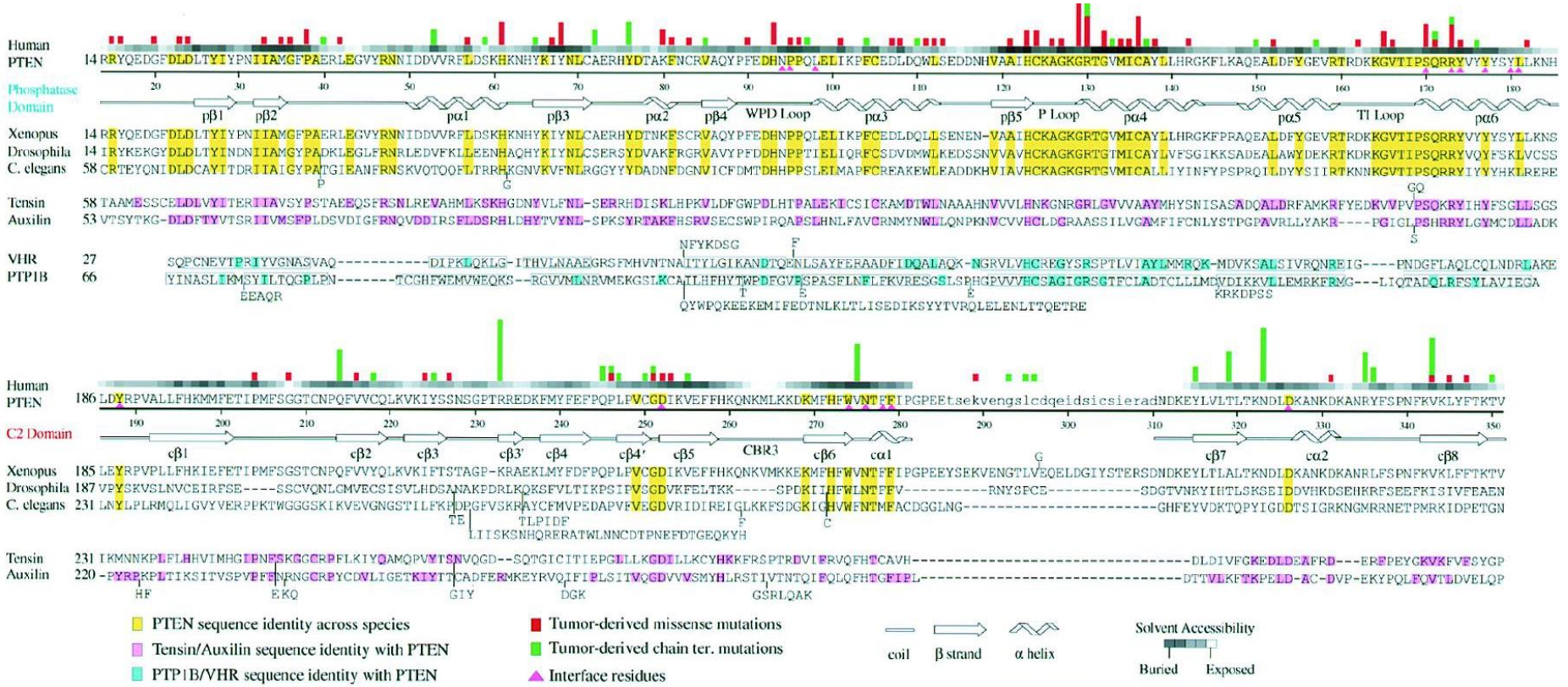
In human tumors, PTEN expression is often lost by promoter methylation.



Hanahan & Weinberg,
Cell **144**, 646–674 (2011)

Lee, …. Nikola Pavletich,
Cell **99**, 323-334 (1999)

Attenuate proliferative signalling: PTEN



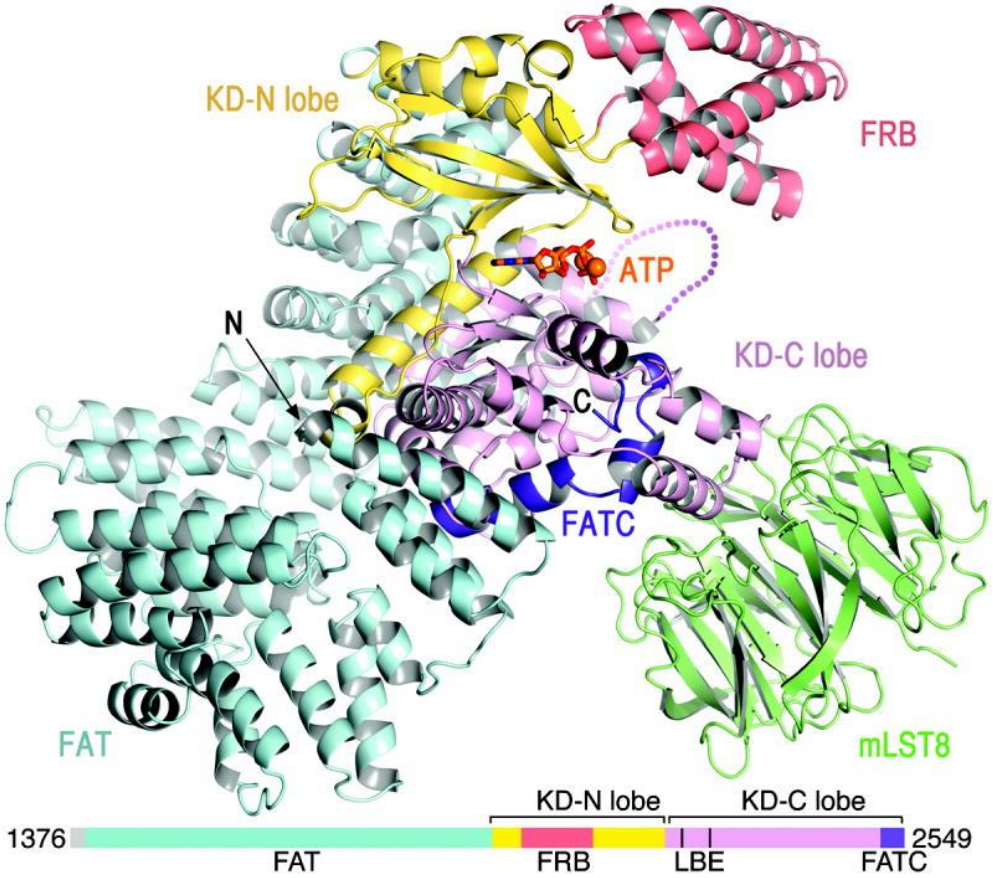
Conservation of PTEN and histogram of 93 **missense (red bars)** and **80 chain termination (green bars)** tumor-derived mutations known in 1999.

Lee, …. Nikola Pavletich, *Cell* **99**, 323-334 (1999)

Attenuate proliferative signalling: mTOR

Another example involves the **mTOR kinase**, a coordinator of cell growth and metabolism that lies both upstream and downstream of the PI3K pathway.

In the circuitry of some cancer cells, mTOR activation results, via negative feedback, in the inhibition of PI3K signaling.



KD: kinase domain

Yang, … , Nikola Pavletích,
Nature **497**, 217–223 (2013)

Corruption of the TGF-beta pathway

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4717672/ writes

„The TGF-β family is key to specifying the body plan during metazoan development. Members of this family, including nodal, activins, inhibins, bone morphogenetic proteins (BMPs) and growth differentiation factors (GDFs), specify the anterior/posterior and dorsal/ventral axes, endoderm, mesoderm and ectoderm, left–right asymmetry and details of individual organs. TGF-β1, TGF-β2 and TGF-β3 are important in development, wound healing, immune responses and tumour-cell growth and inhibition."

TGF-β (transforming growth factor beta) has also antiproliferative effects that need to be evaded by cancer cells.

In many late-stage tumors, TGF-β signaling is redirected away from suppressing cell proliferation. Instead, one finds that TGF-β activates a cellular program, termed the

epithelial-to-mesenchymal transition (EMT), that confers on cancer cells traits associated with high-grade malignancy.

Hanahan & Weinberg,
Cell **144**, 646–674 (2011)

Evading Growth Suppressors

Dozens of tumor suppressors that limit cell growth and proliferation in various ways have been discovered through their characteristic inactivation in one or another form of animal or human cancer.

Many of these genes have been validated as bona fide tumor suppressors through gain- or loss-of-function experiments in mice.

The two prototypical tumor suppressors encode the RB (retinoblastoma-associated) and TP53 proteins.

They operate as central control nodes within two key complementary cellular regulatory circuits that govern the **decisions of cells** to **proliferate** or, alternatively, **activate senescence** and **apoptotic programs**.

Hanahan & Weinberg,
Cell **144**, 646–674 (2011)

Evading Growth Suppressors: RB (see lecture V6)

The RB protein integrates signals from diverse extracellular and intracellular sources and, in response, decides whether or not a cell should proceed through its growth-and-division cycle.

Cancer cells with defects in RB pathway function are thus missing the services of a critical gatekeeper of cell-cycle progression.

Absence of RB permits persistent cell proliferation.

Hanahan & Weinberg,
Cell **144**, 646–674 (2011)

“Guardian of the cell”: TP53

TP53 receives inputs from **stress** and **abnormality sensors** that function within the cell's intracellular operating systems:

if the degree of damage to the genome is excessive, or if the levels of nucleotide pools, growth-promoting signals, glucose, or oxygenation are suboptimal,

TP53 can call a **halt** to further **cell-cycle progression** until these conditions have been normalized.

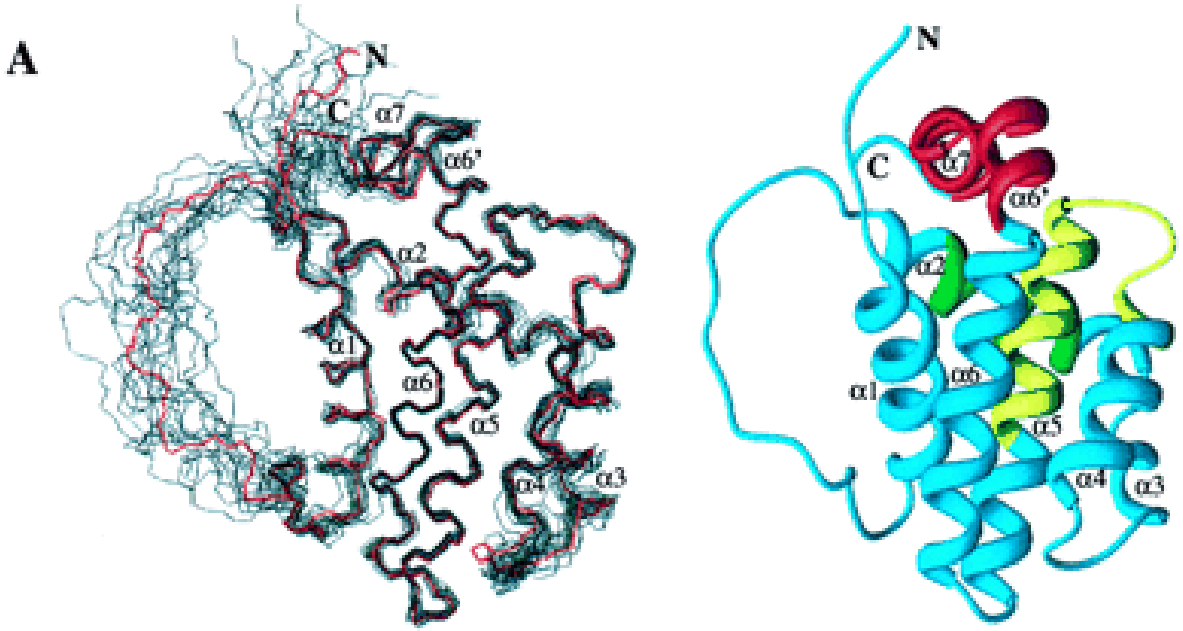
Alternatively, in the face of alarm signals indicating overwhelming or irreparable damage to such cellular subsystems, TP53 can trigger **apoptosis**.

The various effects of activated TP53 are complex and highly context dependent, varying by cell type as well as by the severity and persistence of conditions of cell stress and genomic damage.

Hanahan & Weinberg,
Cell **144**, 646–674 (2011)

Resisting Cell Death: Bcl-2

The “apoptotic trigger” that conveys signals between the regulators and effectors is controlled by counterbalancing pro- and antiapoptotic members of the **Bcl-2** family of regulatory proteins.



Backbone (N, Cα, C') superposition of 15 low-energy NMR-derived structures and Ribbons depiction of the average minimized structure for Bcl-2.

Petros, …, Stephen Fesik, *PNAS* **98**, 3012 (2001)

Resisting Cell Death: Bcl-2

Bcl-2, along with its close relatives Bcl-x_L, Bcl-w, Mcl-1, A1 are **inhibitors of apoptosis**.

They act by binding to and thereby suppressing two proapoptotic triggering proteins (Bax and Bak) that are embedded in the mitochondrial outer membrane.

When relieved of inhibition by their antiapoptotic relatives, Bax and Bak disrupt the integrity of the outer mitochondrial membrane, causing the release of proapoptotic signaling proteins including cytochrome *c*.

The released cytochrome *c* activates, in turn, a cascade of **caspases** that act via their proteolytic activities to induce the multiple cellular changes associated with the apoptotic program.

Hanahan & Weinberg,
Cell **144**, 646–674 (2011)

Enabling Replicative Immortability: Telomeres

Telomeres protecting the ends of chromosomes are centrally involved in the capability for unlimited proliferation.

The telomeres, composed of multiple tandem hexanucleotide repeats, shorten progressively in nonimmortalized cells propagated in culture, eventually losing the ability to protect the ends of chromosomal DNAs from end-to-end fusions.

Such fusions generate unstable dicentric chromosomes whose resolution results in a scrambling of karyotype that threatens cell viability.

Accordingly, the length of telomeric DNA in a cell dictates how many successive cell generations its progeny can pass through before telomeres are largely eroded and have consequently lost their protective functions, triggering entrance into crisis.

Hanahan & Weinberg,
Cell **144**, 646–674 (2011)

Enabling Replicative Immortability: Telomerase

Telomerase, the specialized DNA polymerase that adds telomere repeat segments to the ends of telomeric DNA, is almost absent in nonimmortalized cells but expressed at functionally significant levels in the vast majority (~90%) of spontaneously immortalized cells, including human cancer cells.

By extending telomeric DNA, telomerase is able to counter the progressive telomere erosion that would otherwise occur in its absence.

The presence of telomerase activity, either in spontaneously immortalized cells or in the context of cells engineered to express the enzyme, is correlated with a resistance to induction of both senescence and crisis/apoptosis.

Conversely, suppression of telomerase activity leads to telomere shortening and to activation of one or the other of these proliferative barriers.

Hanahan & Weinberg,
Cell **144**, 646–674 (2011)

Inducing Angiogenesis: VEGF-A

The VEGF-A gene (Vascular Endothelial Growth Factor A) encodes ligands that are involved in orchestrating new blood vessel growth during embryonic and postnatal development, and then in homeostatic survival of endothelial cells, as well as in physiological and pathological situations in the adult.

VEGF signaling via three receptor tyrosine kinases (VEGFR-1–3) is regulated at multiple levels. VEGF gene expression can by upregulated both by hypoxia and by oncogene signaling.

Additionally, VEGF ligands can be sequestered in the extracellular matrix in latent forms that are subject to release and activation by extracellular matrix-degrading proteases (e.g., MMP-9).

In addition, other proangiogenic signals, such as members of the fibroblast growth factor (FGF) family, have been implicated in sustaining tumor angiogenesis when their expression is chronically upregulated.

Hanahan & Weinberg,
Cell **144**, 646–674 (2011)

Activating Invasion and metastasis: ECM, E-cadherin

When carcinomas arising from epithelial tissues progress to higher pathological grades of malignancy (reflected in local invasion and distant metastasis) the associated cancer cells typically developed alterations in their shape as well as in their **attachment** to other cells and to the **extracellular matrix (ECM).**

In particular, carcinoma cells loose E-cadherin, a key cell-to-cell adhesion molecule.

E-cadherin forms adhering junctions with adjacent epithelial cells. Thereby, E-cadherin helps to assemble epithelial cell sheets and maintain the quiescence of the cells within these sheets.

Increased expression of E-cadherin prevents invasion and metastasis, whereas reduced expression potentiates these phenotypes.

E-cadherin is frequently downregulated and occasionally inactivated by mutations in human carcinomas. This strongly supports its role as a key suppressor of this hallmark capability.

Hanahan & Weinberg,
Cell **144**, 646–674 (2011)

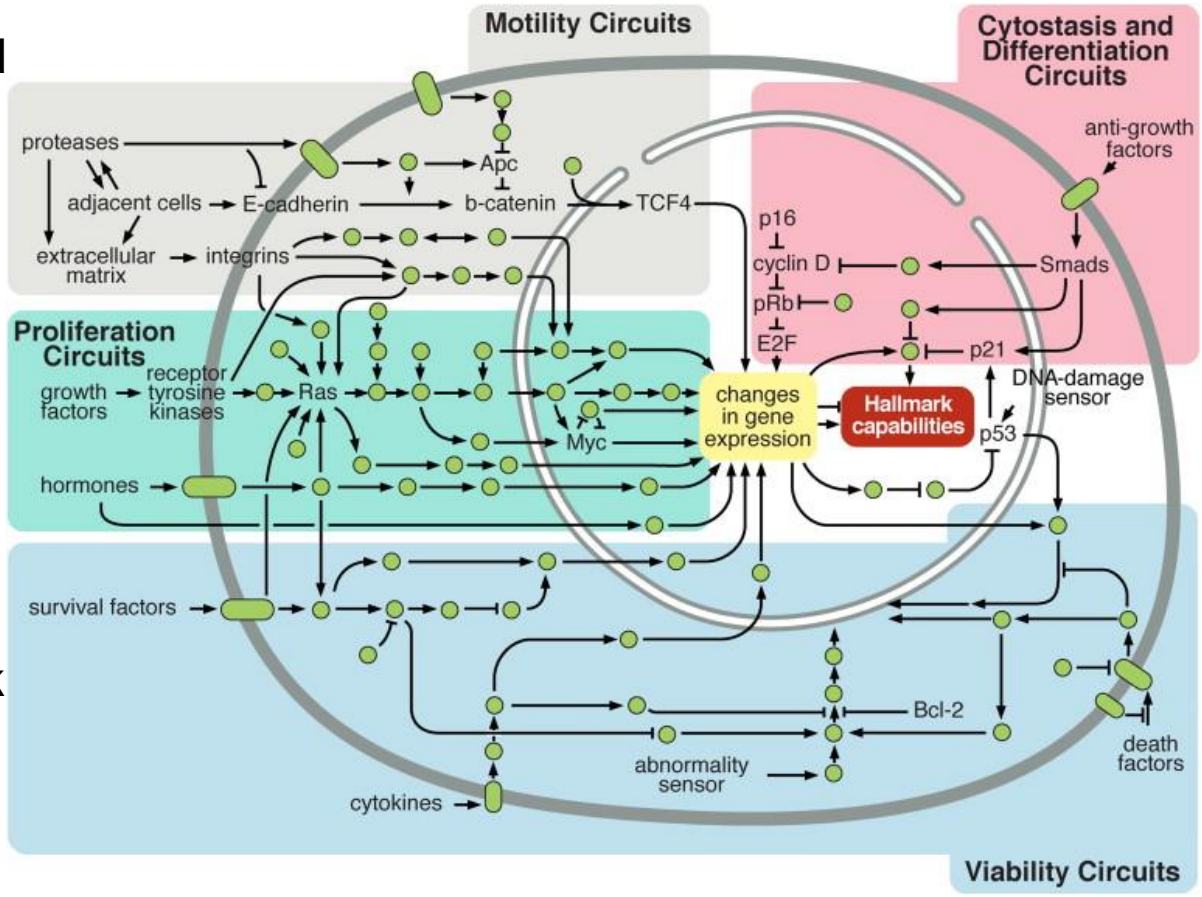
Intracellular Signaling Networks

An elaborate integrated circuit operates within normal cells and is reprogrammed to regulate hallmark capabilities within cancer cells.

Separate subcircuits, depicted here in differently colored fields, are specialized to orchestrate the various capabilities.

This depiction is simplistic. There is considerable **crosstalk** between such subcircuits.

Also, each of these subcircuits is connected with signals originating from other cells in the **tumor microenvironment**.

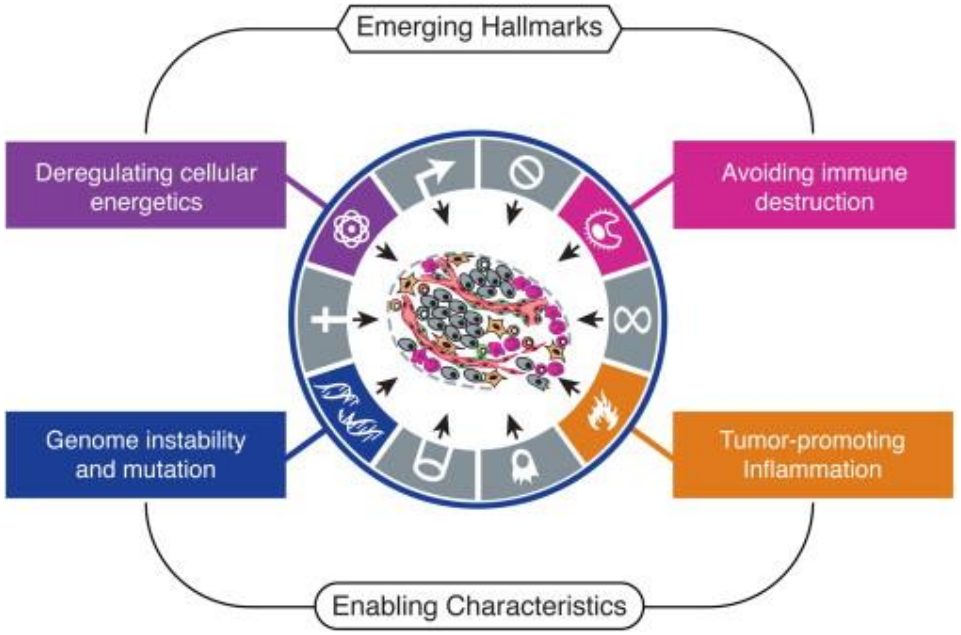


Hanahan & Weinberg, *Cell* **144**, 646–674 (2011)

Emerging Hallmarks

Additional hallmarks of cancers.

(1) In tumors, the **cellular metabolism** is reprogrammed so that neoplastic proliferation is most effectively supported.
(2) Cancer cells **evade immunological destruction** by T and B lymphocytes, macrophages, and natural killer cells.



(3) **Genomic instability** and thus **mutability** endow cancer cells with genetic alterations that drive tumor progression.
(4) **Inflammation** by innate immune cells designed to fight infections and heal wounds can instead result in their inadvertent support of multiple hallmark capabilities. This manifests the tumor-promoting consequences of inflammatory responses.

Hanahan & Weinberg,
Cell **144**, 646–674 (2011)

Driver and passenger mutations

Genomewide sequencing has shown that every tumor harbors thousands of genetic (and epigenetic) alterations that are not present in the patient's germline.

Only a very small fraction of these alterations are in "**driver genes**".
When driver genes are mutated, this endows the tumor cell with a **growth advantage** over surrounding cells.

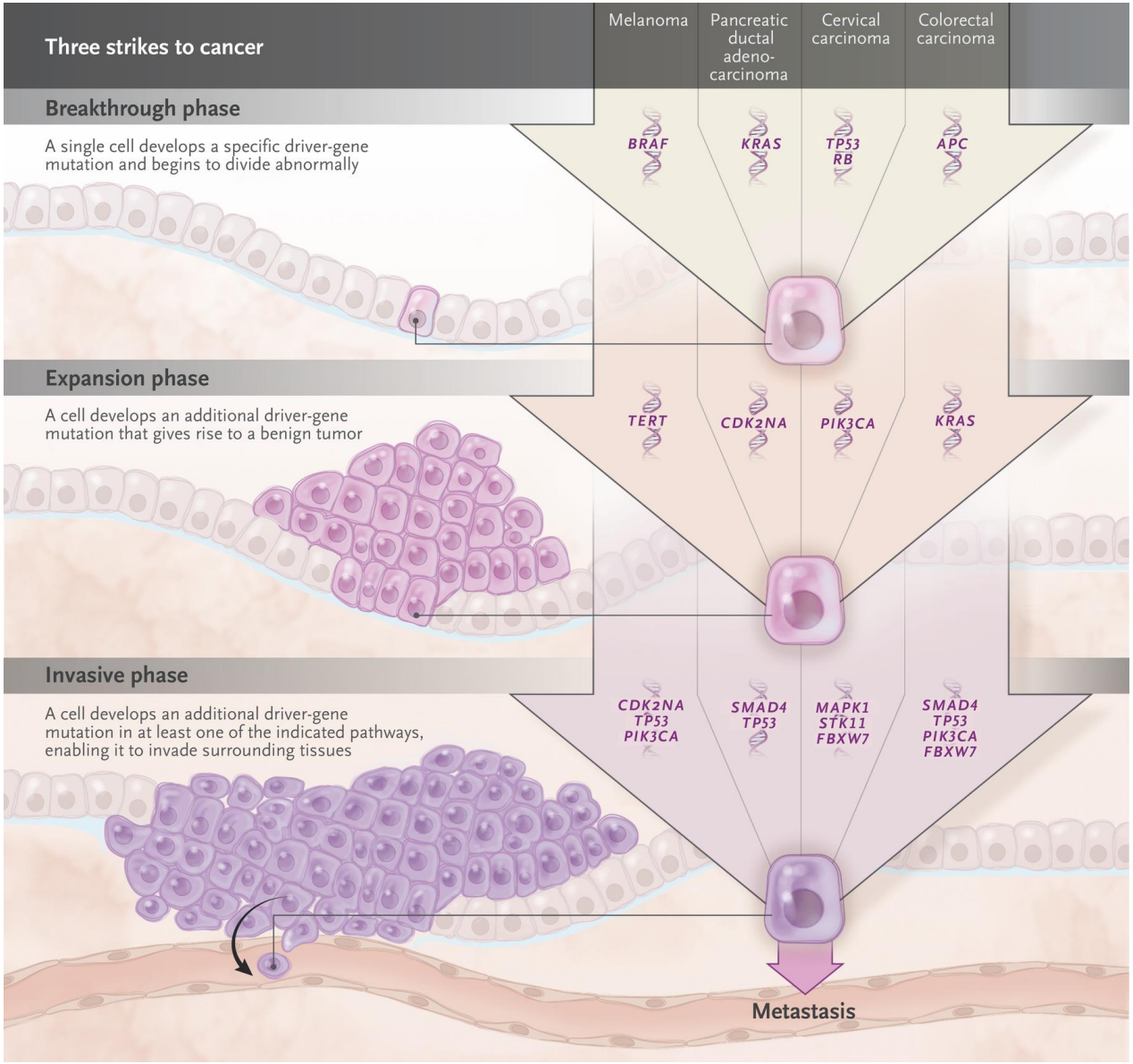
The remaining alterations are "**passenger mutations**," found in tumor cells only because they occurred coincidentally during the long march toward tumorigenesis.

Only about 200 of the 20,000 genes in the human genome have been shown to act as driver genes for common cancers.

Driver genes appear to function through a limited number of pathways that regulate cells' growth and fate.

Vogelstein & Kinzler,
NEJM **373**, 1895-1898 (2015)

Three Strikes and You're Out



Bert Vogelstein from Johns Hopkins is one of the most influential cancer researchers.

Tumors evolve in 3 phases shown in the figure.

Vogelstein & Kinzler, *NEJM* **373**, 1895-1898 (2015)

Driver and passenger mutations

Driver-gene mutations should be viewed at a pathway level rather than at an individual-gene level. E.g., colorectal cancers are initiated by mutations in genes in the adenomatous polyposis coli (APC) pathway. This pathway includes *APC, CTNNB1, SOX9, TCF7L1, TCF7L2,* and *AMER1*.

The order in which driver-gene mutations occur is also important. E.g., RAS-pathway mutations are the initiating events for pancreatic ductal adenocarcinomas and melanomas but occur later in colorectal tumorigenesis.

Genome-sequencing data **exclude the possibility of spontaneous tumors**: a normal adult cell cannot suddenly transform into a cancer cell.

Every time a cancer (or normal) cell divides, a few new mutations occur. Cancers continuously evolve, always generating more passenger mutations and occasionally another driver-gene mutation that increases growth.

Vogelstein & Kinzler,
NEJM **373**, 1895-1898 (2015)

The human cancer transcriptome

Analysis of transcriptomic data for ca 8000 patients suffering from 17 major cancer types showed that:

- a large fraction of cancer protein-coding genes are differentially expressed and, in many cases, have an impact on overall patient survival.

- shorter patient survival was generally associated with up-regulation of genes involved in mitosis and cell growth and down-regulation of genes involved in cellular differentiation.

Uhlen et al, *Science* **357**, eaan2507 (2017)

The human cancer transcriptome

On the one hand, 41% of the protein-coding genes were expressed in all analyzed cancers.

On the other hand, 46% of the protein-coding genes displayed tumor type-restricted expression.

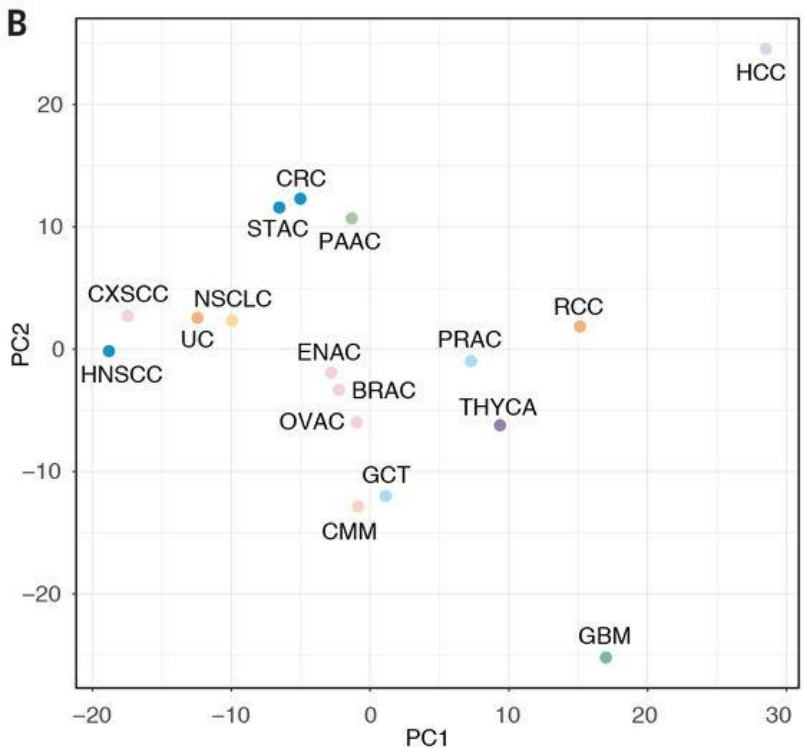
13% of the protein-coding genes were not detected in any tumor types investigated.

The majority of the genes (*n* = 5772) detected in all samples were shared between cancers and normal tissues,
whereas 2401 additional genes were expressed in all cancers analyzed, but with more restricted expression in the normal tissues.

These **“housekeeping” genes** in tumors are enriched in biological functions related to DNA replication and the regulation of apoptosis and mitosis.

Uhlen et al, *Science* **357**, eaan2507 (2017)

PCA of the human cancer transcriptome



Cancer types that share a similar tissue type of origin or similar morphological features and phenotypic expression patterns are grouped together.

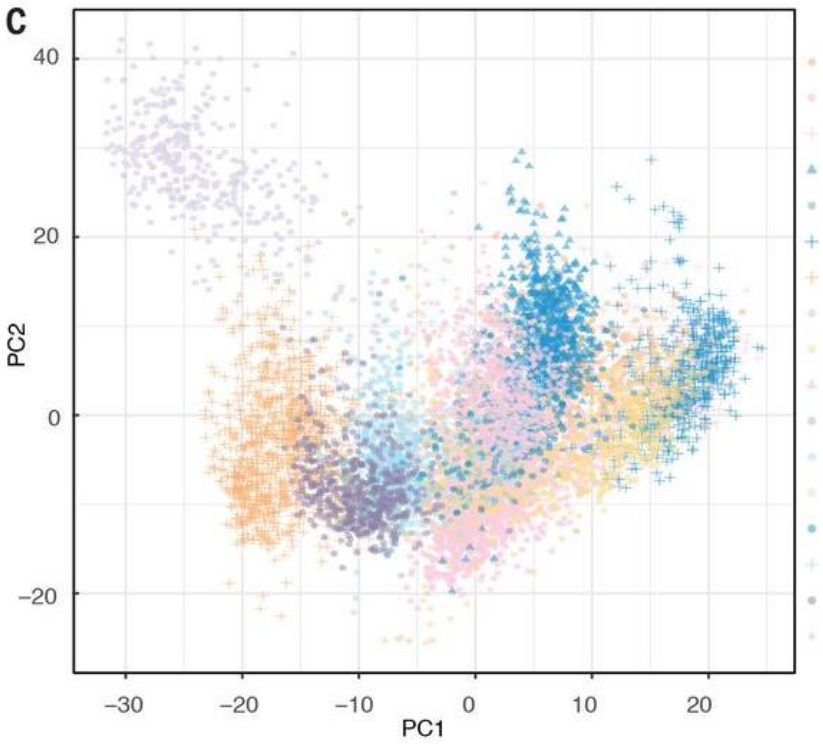
E.g., cancers with a dominating squamous cell carcinoma phenotype, such as cervical (CXSCC) or head and neck cancer (HNSCC), clustered together close to the related urothelial cell carcinoma and non–small cell lung cancer (NSCLC), which also contains a large fraction of squamous cell carcinoma.

Adenocarcinomas that originate from the gastrointestinal tract, including pancreatic cancer (PAAC), cluster separately from the cluster containing the 3 adenocarcinomas representing female cancer (i.e., breast BRAC, endometrial ENAC, and ovarian cancer OVAC).

Glioma (brain GBM) and hepatocellular (liver HCC) carcinoma were the most divergent tumor types.

Uhlen et al, *Science* **357**, eaan2507 (2017)

The human cancer transcriptome

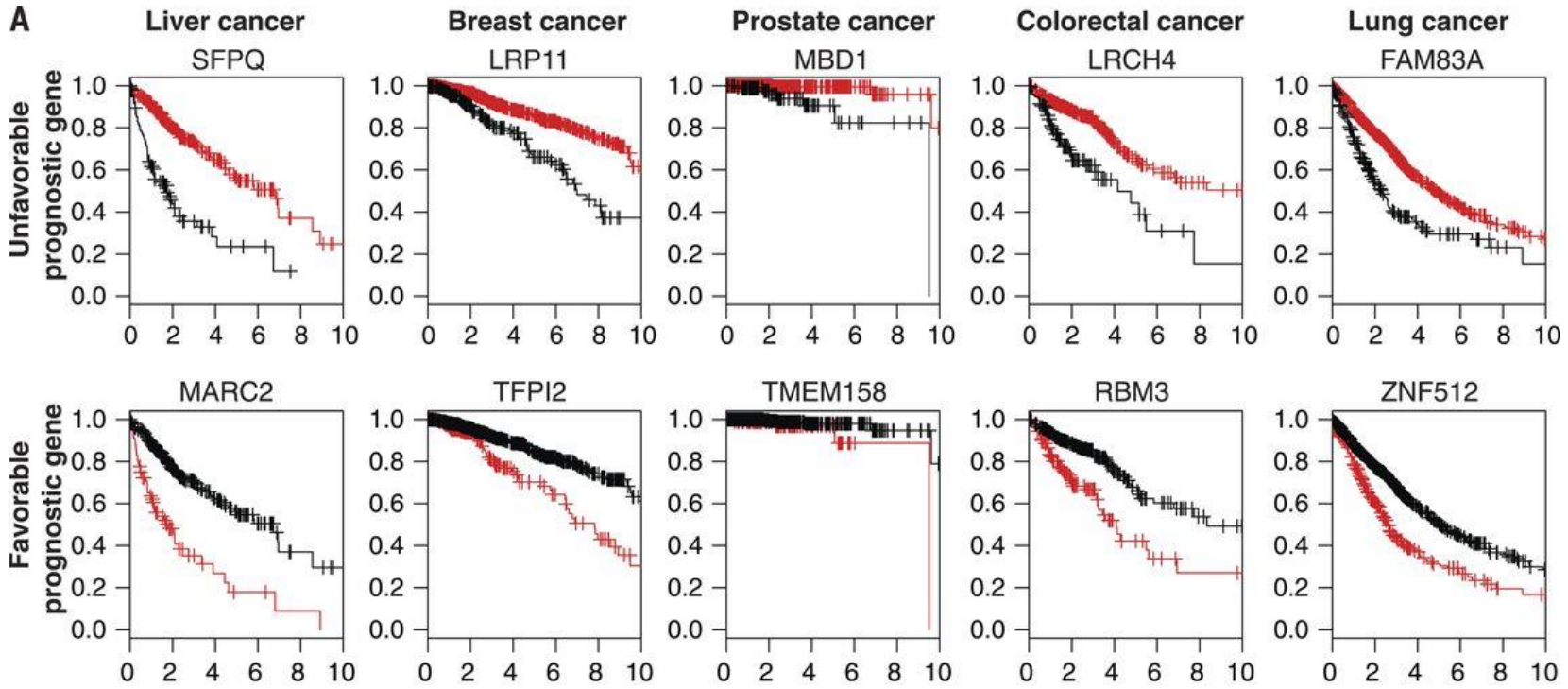


Same analysis for individual tumors

-> there is a large overlap in expression among individuals patients of different cancer types.

Uhlen et al, *Science* **357**, eaan2507 (2017)

Clinical outcome



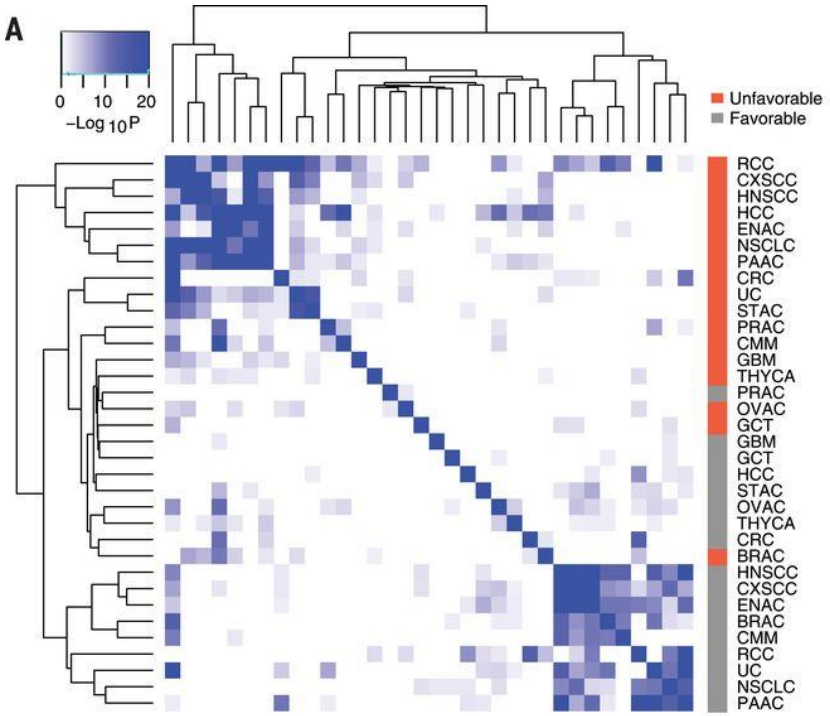
Black: high expression of this gene, red: low expression.

(Top) 5 genes with unfavorable effect on survival.

(Bottom) 5 genes with unfavorable effect on survival.

Uhlen et al, *Science* **357**, eaan2507 (2017)

Overlap of prognostic genes among cancer types



For most cancers, little correlation was observed, suggesting a relatively limited number of common prognostic genes.

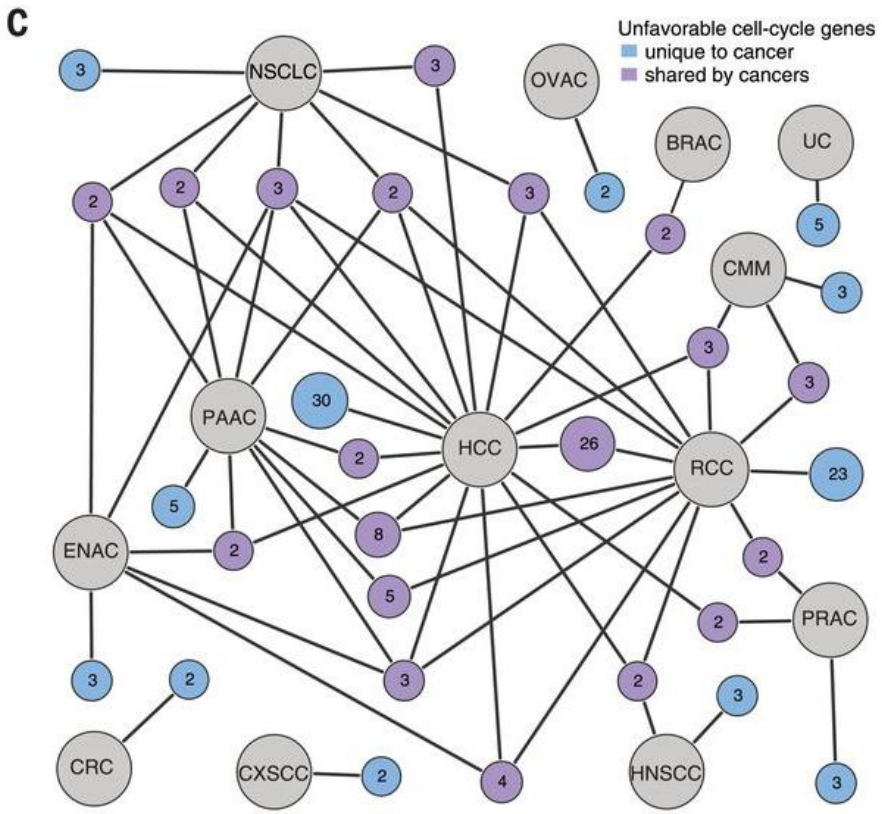
In contrast, a significant overlap of favorable prognostic genes was observed for other cancers (e.g., renal, breast, lung, and pancreatic cancers).

Similarly, unfavorable prognostic genes for some cancers, including renal, liver, lung, and pancreatic cancer, clustered together.

No prognostic genes were shared among more than 7 of the cancer types

Uhlen et al, *Science* **357**, eaan2507 (2017)

Functional annotation of prognostic genes



GO analysis of most significant prognostic genes:
many of the common unfavorable genes are related to cell proliferation, including mitosis, cell cycle regulation, and nucleic acid metabolism.

In contrast, few GO functions were significantly overrepresented by the common favorable genes;
the most enriched GO functions were positive regulation of cell activation, regulation of immune cell activation, and cell-cell adhesion.

Uhlen et al, *Science* **357**, eaan2507 (2017)

Paper #9

Structural basis for cancer immunotherapy by the first-in-class checkpoint inhibitor ipilimumab

Udupi A. Ramagopal, Weifeng Liu, Sarah C. Garrett-Thomson, Jeffrey B. Bonanno, Qingrong Yan, Mohan Srinivasan, Susan C. Wong, Alasdair Bell, Shilpa Mankikar, Vangipuram S. Rangan, Shrikant Deshpande, Alan J. Korman, and Steven C. Almo

PNAS 114, E4223-E4232 *(2017)*

Paper presentation Jan 19, 2021