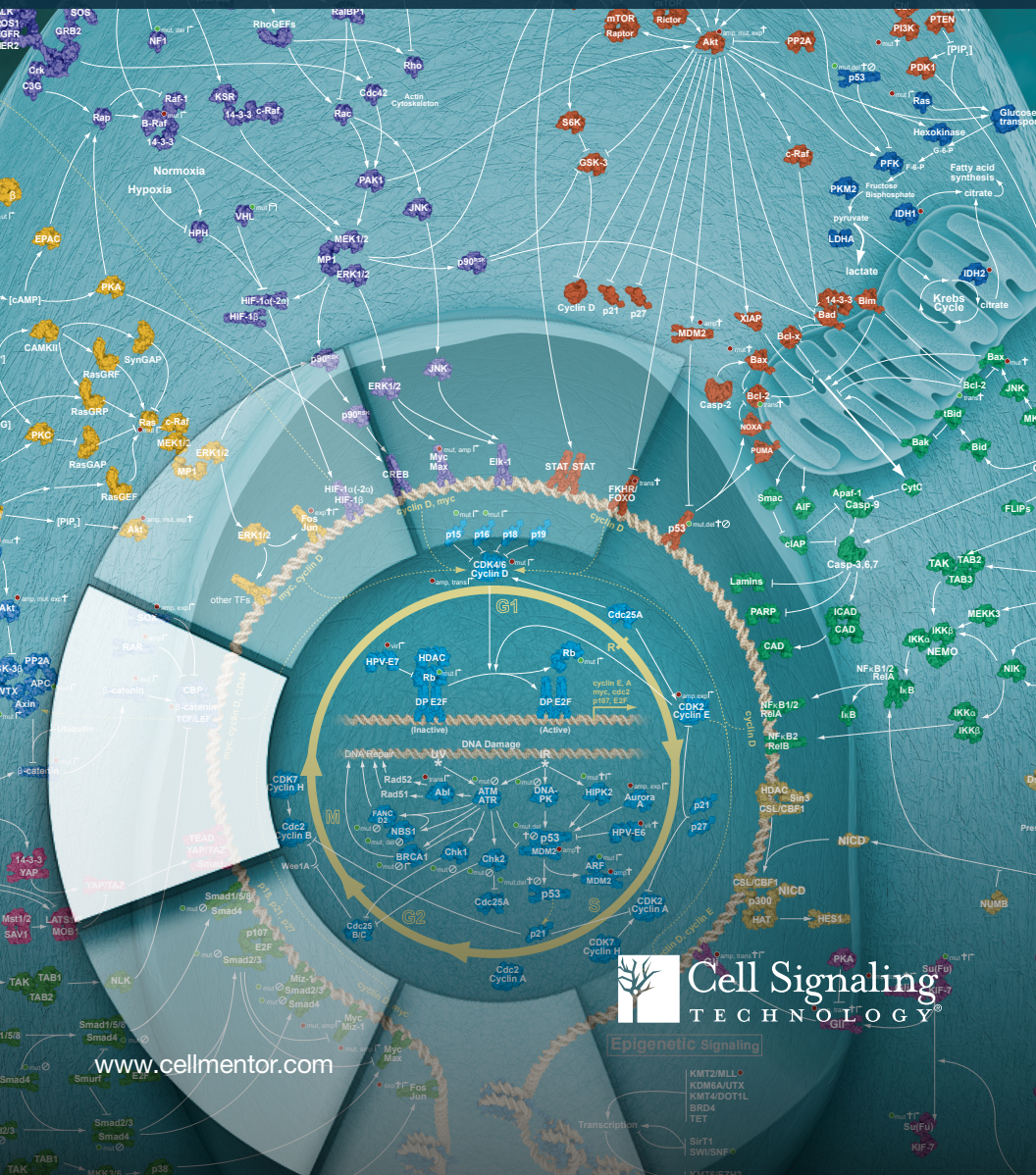
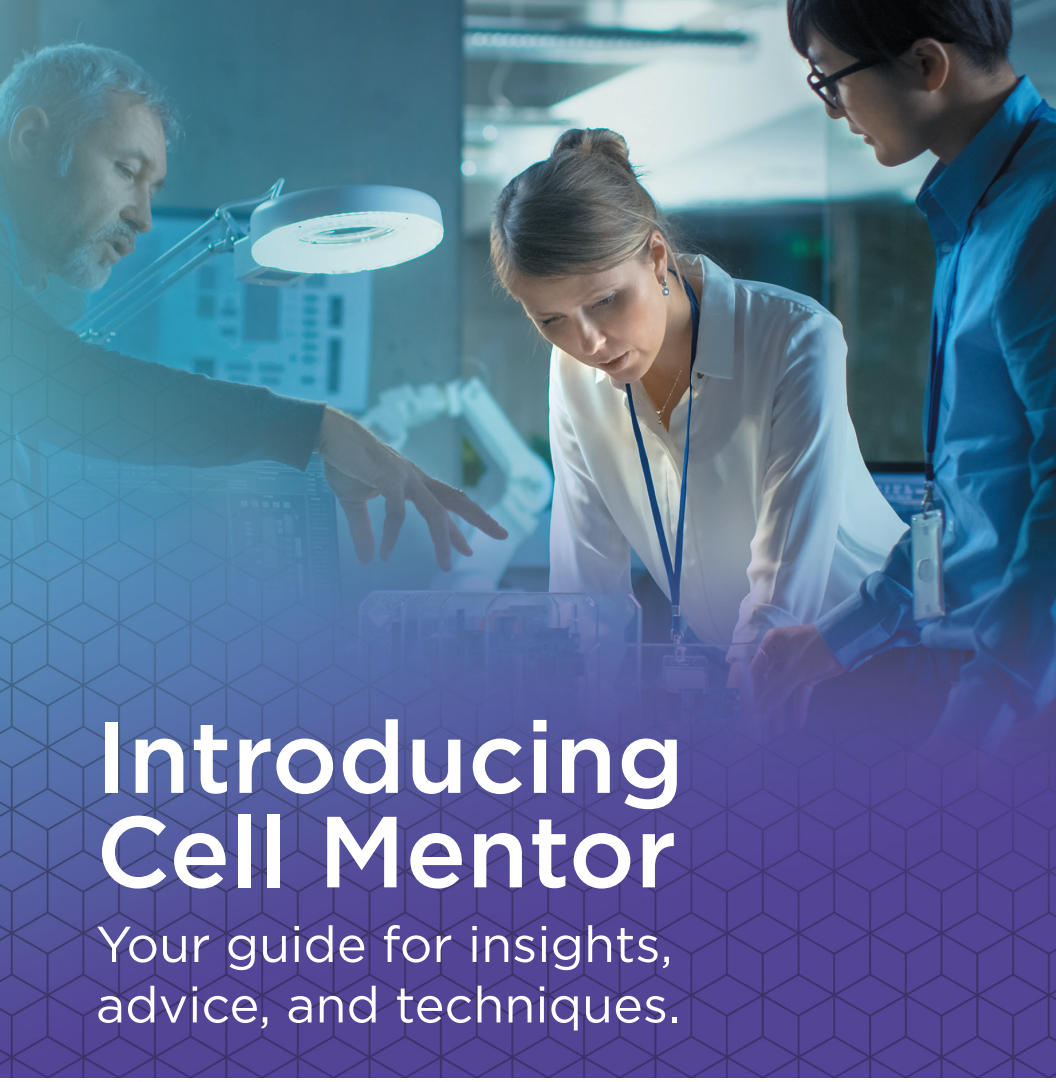
Cell MENTOR Handbooks

The Researcher's Guide to the Hallmarks of Cancer Research Targets





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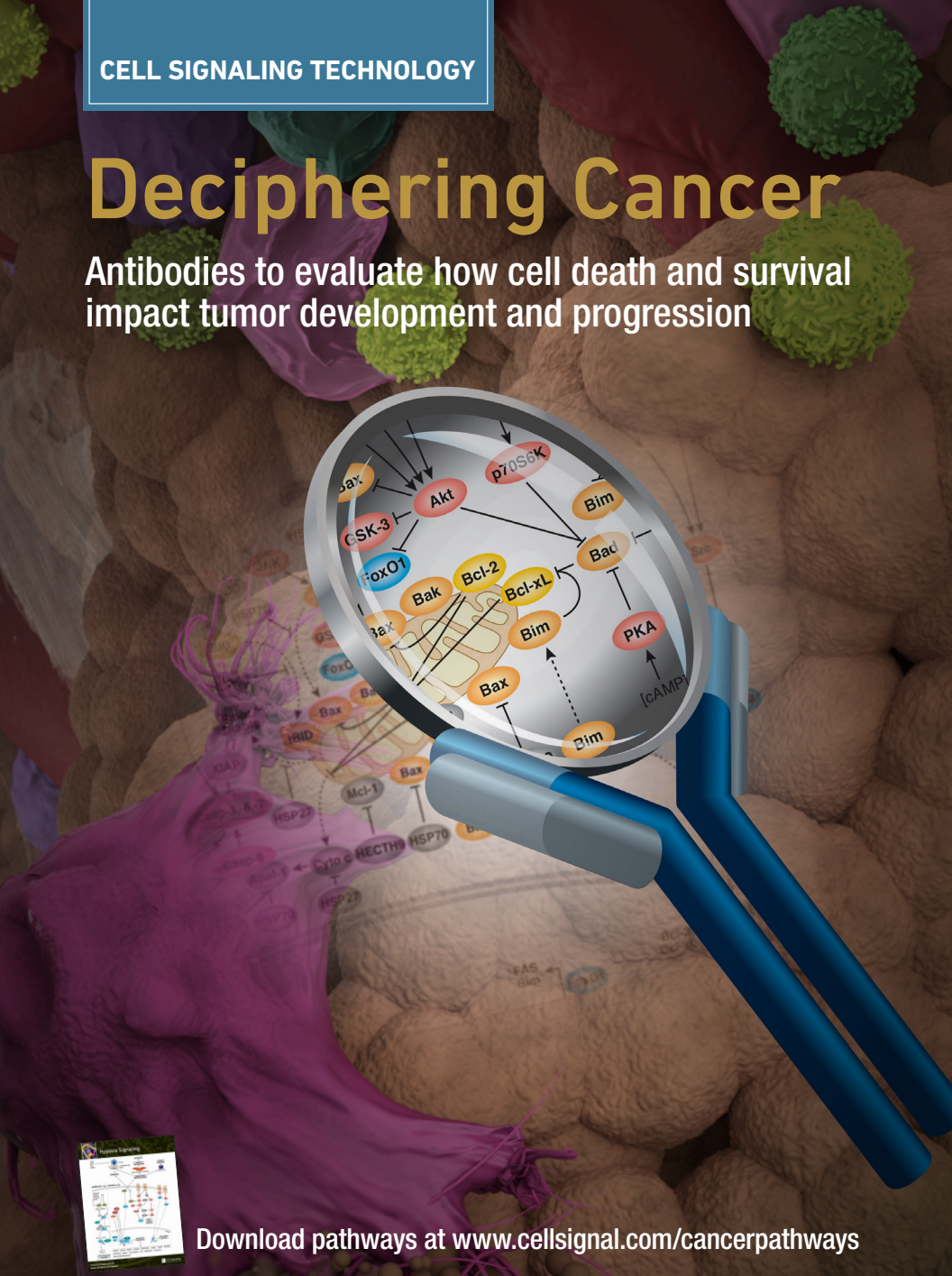

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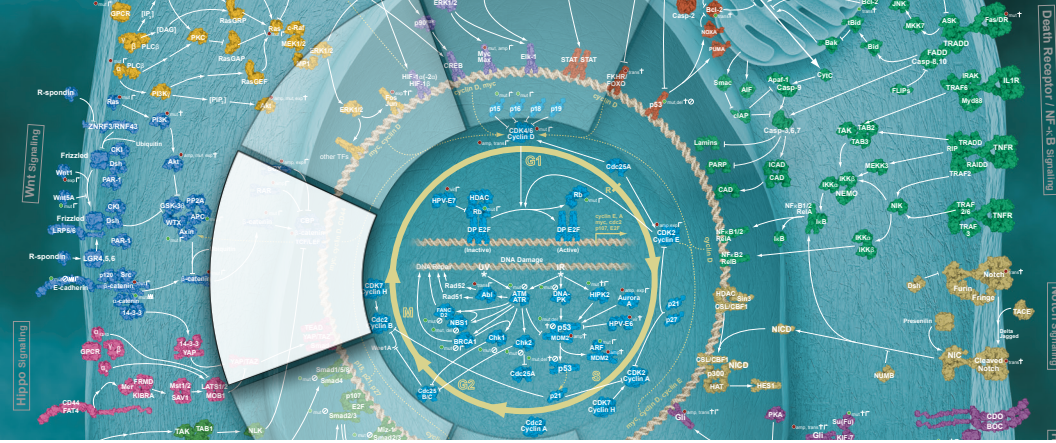


Introduction

When Doctors Robert Weinberg and Douglas Hanahan first published "The Hallmarks of Cancer" in *Cell*, the disease research world changed forever. At the time, most researchers were focused on the nature of aberrant signaling in their specific research area. These visionaries thought, what if the complexity of cancer can be broken down into smaller subsets of general underlying principles? For them, it seemed a useful exercise to step back and look at the commonality between what all researchers had discovered.

The initial publication proposed six hallmarks of cancer cells. Robert Weinberg said in an interview with *The Naked Scientists* that they had no way of knowing the paper would be so impactful or cited so many times after its initial publication in 2001. What they expected to fade quietly into pub history became a seminal contribution to the research community. So, in 2011 they expanded their theory to include four other hallmarks to give us the ten hallmarks we recognize today.

Weinberg and Hanahan wanted to contribute something new and useful, a tool for researchers to further discovery. It's with that spirit that we introduce *The Researcher's Guide to the Hallmarks of Cancer*. Here we present an introduction to Weinberg and Hanahan's hallmarks, in which we've also identified key targets for research to get you started.

Which pathways are hijacked or manipulated in each of the hallmarks? Which proteins can be targeted to combat the adverse mechanisms of cancer cells? These are important questions, and this guide helps provide the answers.

Chris Sumner
Communications Specialist
Cell Signaling Technology

GET TECHNICAL

Resisting Cell Death

One thing we know about cancer cells: they can resist death. They evade apoptosis, the mechanism that programs cell death once cells become damaged. Normally, apoptosis helps keep an organism healthy through growth and development, maintaining body tissue by removing infected or damaged cells. But cancer cells do not follow this process, no matter how abnormally they grow.

The cancer cells may alter the mechanisms that detect the damage or irregularities, preventing proper signaling and apoptosis activation. Cancer cells may also introduce defects in the downstream signaling itself, or the proteins involved in apoptosis, which would also prevent proper apoptosis (1,2).

Apoptosis is also significant in the hallmark Evading Growth Suppressors, but that refers to apoptosis triggered by external signals. With this hallmark, we're referring to intrinsic apoptosis, in which the apoptotic program never begins.

To discover how cancer cells evade cell death, we must first investigate the different pathways through which apoptosis may occur.

Apoptosis can be induced through the activation of death receptors.

i. Caspase-8: apoptosis is induced through several receptors that activate caspase-8 and lead to the release of the caspase-8 active fragments, which then cleave and activate downstream caspases.

ii. RIP kinases: (near the receptors in this pathway) are important regulators of cellular stress that trigger pro-survival and inflammatory responses through the activation of NF-kB, as well as pro-apoptotic pathways.

iii. Bcl-2: exerts a survival function in response to a wide range of apoptotic stimuli through inhibition of mitochondrial cytochrome c release.

iv. p53: the "master switch," a tumor suppressor protein that plays a major role in cellular response to DNA damage and other genomic aberrations. Activation of p53 can lead to either cell cycle arrest and DNA repair or to apoptosis. p53 can be phosphorylated and acetylated at multiple sites by a number of proteins, including Chk2 and ATM.

References:

1. Hanahan D, Weinberg RA (March 2011). "Hallmarks of Cancer: the next generation". *Cell*. 144 (5):646-74. doi: 10.1016/j.cell.2011.02.013.
2. Elmore, S (June 2007). "Apoptosis: a review of programmed cell death". *Toxicologic Pathology*. 35 (4): 495–516. doi: 10.1080/01926230701320337. PMC 2117903. PMID 17562483

Deregulating Cellular Energetics

Cancer cells need a lot of energy to grow fast—to do so, they show abnormal metabolic pathways.

Most mammalian cells use glucose as a fuel source. Glucose is metabolized by glycolysis in a multistep set of reactions, resulting in the creation of pyruvate. In typical cells under normal oxygen levels, much of this pyruvate enters the mitochondria, where it is oxidized by the Krebs Cycle to generate ATP to meet the cell's energy demands.

However, in cancer cells or other highly proliferative cell types, much of the pyruvate from glycolysis is directed away from the mitochondria to create lactate through the action of lactate dehydrogenase (LDH/LDHA)—a process typically reserved for the low oxygen state. In contrast to mitochondrial glycolysis, lactate production in the presence of oxygen is termed "aerobic glycolysis" or the Warburg Effect. Several signaling pathways contribute to the Warburg Effect and other metabolic phenotypes of cancer cells. If you look at the pathway, you might notice PI3K/Akt, mTOR, Erk1/2 MAPK, ULK1 (Autophagy), p53, HIF1α (Hypoxia – Angiogenesis), and AMPK all represented.

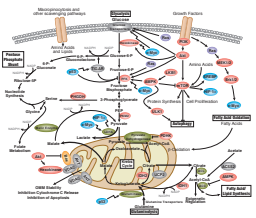
Cancer cells frequently use glutamine as another fuel source, which enters the mitochondria and can be used to replenish Krebs Cycle intermediates or to produce more pyruvate through the action of malic enzyme, or to produce building blocks to help the increased cell growth.

Cancer cells can become addicted to glutamine, with glutamine itself promoting cell proliferation. For this reason, glutamine metabolism is becoming a hot research area for cancer researchers. Because glycolysis is an intricate process, influenced by so many factors, there are many pathways to research, such as the Warburg Effect pathway, Energy Metabolism pathway, and Glutamine Signaling pathway.

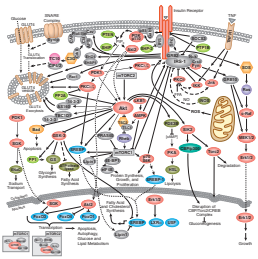
References:

1. Hanahan D, Weinberg RA (January 2000). "The Hallmarks of Cancer". *Cell.* 100 (1): 57–70. doi:10.1016/S0092-8674(00)81683-9
2. Hanahan D, Weinberg RA (March 2011). "Hallmarks of Cancer: the next generation". *Cell.* 144 (5):646-74. doi: 10.1016/j.cell.2011.02.013

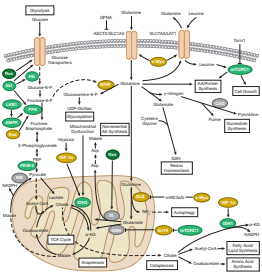
Warburg Effect



Energy Metabolism



Glutamine Signaling



Inducing Angiogenesis

Cancer cells stimulate the growth of blood vessels to supply nutrients to tumors. Angiogenesis is the formation of new blood vessels from pre-existing blood vessels. This plays an important role in tumor growth.

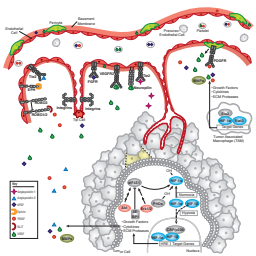
Benign tumors can exist in a dormant state, which can be driven by inadequate access to sufficient blood supply. However, the "angiogenic switch" occurs when angiogenesis is activated in a dormant tumor, and growth factors are secreted to induce sprouting and chemotaxis of endothelial cells toward the tumor mass.

Within the hypoxic environment of the tumor mass, hypoxia inducible factor-1 (HIF-1) is stabilized and activates the expression of multiple genes contributing to the angiogenic process, including vascular endothelial growth factor (VEGF) and fibroblast growth factor (bFGF), or platelet-derived growth factor (PDGF).

References:

1. Hanahan D, Weinberg RA (January 2000). "The Hallmarks of Cancer". *Cell.* 100 (1): 57–70. doi:10.1016/S0092-8674(00)81683-9
2. Hanahan D, Weinberg RA (March 2011). "Hallmarks of Cancer: the next generation". *Cell.* 144 (5):646-74. doi: 10.1016/j.cell.2011.02.013

Tumor Angiogenesis



> "Benign tumors can exist in a dormant state, which can be driven by inadequate access to sufficient blood supply."

Sustaining Proliferative Signaling

Cancer cells stimulate their own growth, which means they become self-sufficient in growth signals and no longer depend on external signals, such as epidermal growth factor (EGF/EGFR). Proliferation depends highly on these three important pathways: Akt, MAPK/Erk, and mTOR.

Akt, also known as Protein Kinase B (PKB), represents a family of serine/threonine protein kinases, Akt1, Akt2, and Akt3. Akt1 (v-Akt) was originally discovered as a proto-oncogene and plays a critical role in regulating diverse cellular functions, which include metabolism, growth, proliferation, survival, transcription, and protein synthesis. Akt is activated by various extracellular and intracellular signals, most involving the lipid kinase phosphoinositide 3-kinase (PI3K). It is also a major regulator of cell survival by inhibiting the apoptotic effects of signaling pathways and proteins (like FoxO1).

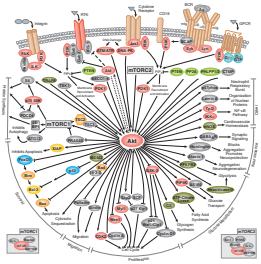
Mitogen activated protein kinases (MAPKs) are the central component in numerous signaling cascades that transmit growth, proliferation, and survival signals from the cell surface to the nucleus. Stimulation of receptor-tyrosine kinases (RTKs) by growth factors, engagement of integrins, or changes in cellular homeostasis (eg, stress) promote the activation of signaling pathways involving Erk (extracellular regulated kinase), p38 MAPK, or Jnk (c-Jun N-terminal kinase). Each of these kinases, in turn, promotes activation of transcription factors such as c-Jun, Ets, Alk, and ATF resulting in cellular growth, survival, repair, and proliferation.

mTOR (mammalian target of rapamycin) is a protein kinase that functions as an ATP and amino acid sensor to balance nutrient and energy availability with cellular growth. mTOR can also be activated or inhibited by other signaling pathways involving Akt, Erk, and AMPK. mTOR in turn regulates a series of metabolic enzymes and other protein kinases that modulate lipid metabolism and biogenesis cellular growth and proliferation and autophagy.

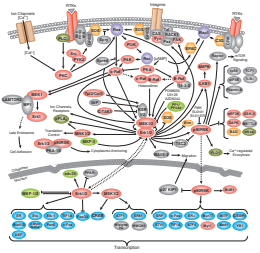
References:

1. Hanahan D, Weinberg RA (January 2000). "The Hallmarks of Cancer". *Cell.* 100 (1): 57–70. doi:10.1016/S0092-8674(00)81683-9
2. Hanahan D, Weinberg RA (March 2011). "Hallmarks of Cancer: the next generation". Cell. 144 (5):646-74. doi: 10.1016/j.cell.2011.02.013.

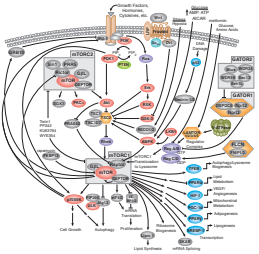
PI3K/Akt

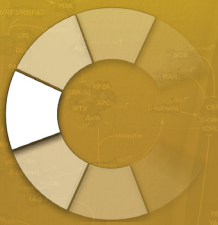


MAPK/Erk



mTOR





Enabling Replicative Immortality

Cancer cells can revert to a pre-differentiated, stem-cell-like phenotype, allowing uninhibited cellular division and other metabolic adaptations that enable survival in adverse conditions.

While there are multiple signaling pathways involved in these changes, two key components enable replicative immortality, Hippo and WNT. There are multiple pathways involved in this characteristic of cancer cells. Below, we'll focus on two of these key pathways: Hippo signaling and Wnt signaling.

Hippo signaling is an evolutionarily conserved pathway that controls organ size by regulating cell proliferation, apoptosis, and stem cell self-renewal. In addition, dysregulation of the Hippo pathway contributes to cancer development. Important targets include:

- YAP and TAZ are key mediators to Hippo signaling. YAP acts as a transcriptional co-activator, can be phosphorylated at multiple sites and translocates from the nucleus to the cytoplasm
- When the Hippo pathway is turned off, YAP is phosphorylated, translocates to the nucleus and is associated with various transcription factors, including the TEAD YAP/TEAD complexes regulate the expression of genes involved in cell proliferation and apoptosis.

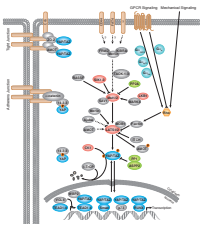
The Wnt/β-Catenin pathway is another evolutionarily conserved mechanism that contributed to cancer's ability to replicate indefinitely. This pathway regulates stem cell pluripotency and cell fate decisions during development. Wnt signaling has also been shown to promote nuclear accumulation of transcriptional regulators implicated in cancer, such as TAZ. Key regulators include:

- β-Catenin is a key downstream effector in the Wnt signaling pathway. It is implicated in two major biological processes in vertebrates: early embryonic development and tumorigenesis. It can translocate to the nucleus.
- LEF1 and TCF bind to Wnt response elements to provide docking sites for β-catenin, which translocates to the nucleus to promote the transcription of target genes upon activation of Wnt signaling.

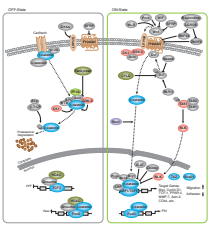
References:

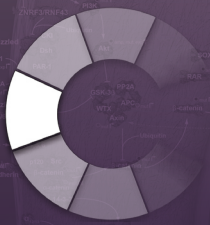
1. Hanahan D, Weinberg RA (January 2000). "The Hallmarks of Cancer". Cell. 100 (1): 57–70. doi:10.1016/S0092-8674(00)81683-9
2. 2 Hanahan D, Weinberg RA (March 2011). "Hallmarks of Cancer: the next generation". Cell. 144 (5):646-74. doi: 10.1016/j.cell.2011.02.013.

Hippo Signaling



Wnt Signaling





Evading Growth Suppressors

Cancer cells resist inhibitory signals that might otherwise stop their growth. The major pathways involved are Autophagy and Death Receptor Signaling (Apoptosis), both of which can ultimately lead to cell death, and reduction in tumor growth.

Autophagy is a dynamic cellular recycling system that results in the degradation of cytoplasmic contents, abnormal protein aggregates, and excess or damaged organelles so that the building blocks (e.g., amino acids) can be used to create new cellular components. Therefore, autophagy is a survival-promoting process. Cancers can upregulate autophagy to survive microenvironmental stress and to increase growth and aggressiveness. The autophagy process involves the formation of an autophagosome, which then fuses with lysosomes to form an autophagolysosome. The process is regulated by mTOR/AMPK/PI3K/MAPK pathways.

Proteins of Interest:

- Atg16L1 plays a key role in helping to form a huge complex that then is needed to form the autophagosomes, the structures that deliver cytoplasmic components to the lysosomes
- Sequestosome 1 (SQSTM1, p62) is a ubiquitin binding protein that interacts with ubiquitin and provides a scaffold for several signaling proteins. It triggers degradation of proteins through the proteasome or lysosome
- Autophagy marker Light Chain 3 (LC3) becomes associated with autophagic vesicles. The presence of LC3 in autophagosomes and the conversion of LC3 to LC3-II have been used as indicators of autophagy
- Atg1/ULK1 can act as a convergence point for multiple signals that control autophagy

Apoptosis (programmed cell death) can be induced through the activation of death receptors.

Proteins of Interest:

- Caspase-8: Apoptosis is induced through several receptors which activate caspase-8 and lead to the release of the caspase-8 active fragments, which then cleave and activate downstream caspases.
- RIP kinases are important regulators of cellular stress that trigger pro-survival and inflammatory responses through the activation of NF-κB, as well as pro-apoptotic pathways
- Bcl-2 exerts a survival function in response to a wide range of apoptotic stimuli through inhibition of mitochondrial cytochrome c release

References:

1. Hanahan D, Weinberg RA (January 2000). "The Hallmarks of Cancer". *Cell.* 100 (1): 57–70. doi:10.1016/S0092-8674(00)81683-9

2. 2 Hanahan D, Weinberg RA (March 2011). "Hallmarks of Cancer: the next generation". *Cell.* 144 (5):646-74. doi: 10.1016/j.cell.2011.02.013.

Avoiding Immune Destruction

Some cancer cells adapt mechanisms to evade detection and destruction by the host's immune system. One way cells do this is by hijacking normal mechanisms of immune checkpoint control and modulation of the innate immune response via STING.

Immune checkpoints refer to the built-in control mechanisms of the immune system that maintain self-tolerance and help to avoid collateral damage during a physiological immune response. It is now evident that tumors engineer microenvironments to evade immune surveillance and attack, particularly by modulating certain immune-checkpoint pathways.

Tumor-specific T cells must discriminate between destruction of the tumor cell and survival of the target cell. Important for discrimination are proteins on both the T-cell and the target cell:

- CD8 is a T cell and the coreceptor for the T cell receptor (TCR). These two distinct structures recognize the Antigen–Major Histocompatibility Complex (MHC). In tumors, T cells are also known as tumor infiltrating lymphocytes (TIL), present in high abundance at the tumor site to influence overall survival. During cancer therapy, TILs are sometimes removed from a patient's tumor and are then treated with substances that activate the lymphocytes to help them better kill the patient's cancer cells.
- PD-L1 and PD-L2 (programmed cell death proteins) are transmembrane proteins that suppress the adaptive arm of the immune system during particular events such as pregnancy, tissue allografts, autoimmune disease and other disease states like hepatitis. PD-1 on the T-cell is activated by the cell surface ligands on the tumor cell. Upregulation of PD-L1 may allow cancers to evade the host immune system.
- TIM-3 is an inhibitory molecule that is induced following T cell activation. As a negative regulatory immune checkpoint, it is detected in different types of immune cells, including T cells, regulatory T cells (Tregs), dendritic cells (DCs), B cells, macrophages, nature killer (NK) cells, and mast cells. TIM-3 inhibits antitumor immunity by mediating T-cell exhaustion.

STING (stimulator of interferon genes) is a key mediator of innate immunity, and the STING pathway has been shown to be involved in the induction of an anti-tumor immune response. STING is responsible for regulation of type-I interferon production and cellular defense against intracellular pathogens (like bacteria or viruses). Key regulators of the STING pathway are:

- Interferon regulatory factors (IRFs) comprise a family of transcription factors that function within the Jak/Stat pathway to regulate interferon (IFN) and IFN-inducible gene expression in response to viral infection
- TING is a signaling molecule associated with the endoplasmic reticulum (ER) and is essential for controlling the transcription of numerous host defense genes (including type I interferons (IFNs) and pro-inflammatory cytokines) following the recognition of aberrant DNA species or cyclic dinucleotides in the cytosol of the cell. Sting can translocate out of the ER upon activation.

Learn more about the pathways and proteins involved in Avoiding Immune Destruction:

- Immune Checkpoints
- STING

References:

1. Hanahan D, Weinberg RA (January 2000). "The Hallmarks of Cancer". *Cell.* 100 (1): 57–70. doi:10.1016/S0092-8674(00)81683-9
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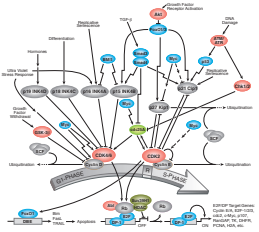
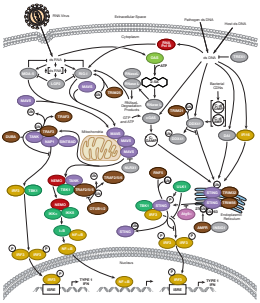
4. Barber, Glen N., "STING: infection, inflammation and cancer". *Nat Rev Immunol.* 2015 Dec; 15(12): 760–770.

Immune Checkpoints



STING



> "It is now evident that tumors engineer microenvironments to evade immune surveillance and attack...."

Genome Instability and Mutation

Not all cancer cells are equal, they evolve in response to selective pressure driven by accumulation of mutations. Cancer cells have to out-compete nearby cells for nutrients and other resources, avoid immune cell attack, and suppress apoptotic self-destruction.

Due to the aberrant proliferation associated with cancer cells, there is an increased tendency of genomic changes and mutations that contribute to the damage of multiple genes regulating cell division and tumor suppression. This is known as genomic instability. Genomic instability has the tendency to compound in cancer cells, since survival-enhancing mutations increase the probability that those mutations will propagate in future cells.

A mutation is an alteration of an organism's DNA sequence. The nucleotides that compose our DNA can be added, replaced, or deleted, and single- or double-stranded breaks can occur within the DNA strand. Complete sections of DNA can also swap positions, be inadvertently replicated, or deleted. Most of these mutations are not cancer-related. They can either be spontaneous or the result of environmental insults like chemicals and radiation. Despite the high probability that such mutations can occur, our DNA is maintained relatively error-free. Our genome surveillance and maintenance systems, mitotic checkpoints and DNA repair mechanisms are always working to mitigate common daily factors that attempt to mutate our genetic code. A defect in any of these systems can increase the DNA's susceptibility to mutations, resulting in genomic instability and an increased risk of malignancy.

One such mechanism is the G2/M DNA damage checkpoint, which serves to prevent the cell from entering mitosis (M-phase) with genomic DNA damage, facilitating genome surveillance and DNA repair. There are several key proteins involved:

- DNA-dependent protein kinase (DNA-PK), a serine/threonine kinase complex composed of a heterodimer of Ku proteins (Ku70/Ku80) and the catalytic subunit DNA-PKcs, is deployed to the site of double-stranded DNA breaks almost instantly to initiate repair via non-homologous end joining.
- BRCA1 and BRCA2, two tumor suppressors that are found in breast and other tissue, contribute to DNA repair, chromosomal stability and transcriptional regulation in response to DNA damage. Studies have shown that, in response to DNA damage, BRCA1 is hyperphosphorylated and translocated to specific sites within the replication fork. BRCA1 has also been shown to regulate the expression levels of several genes activated in response to DNA damage. In addition, BRCA1 is required for the S-phase and G2/M-phase mitotic checkpoints. BRCA2 plays a slightly different role than BRCA1 and is predominantly active in maintaining chromosomal stability and mitotic recombination. Both BRCA1 and BRCA2 have been shown to repair double-stranded DNA breaks via homologous recombination.
- Chk1 and Chk2 are key signaling transducers that are part of a complex network of gene integrity checkpoints, damage detectors and tumor suppressors.
- p53 is also known as the "guardian of the genome" for its role in conserving genomic stability. p53 plays a central role in in a pathway that recognizes and mitigates oncogenic stress by halting proliferation and inducing apoptosis/senescence in an attempt to allay accumulating DNA damage that could lead to malignancy. (Fun fact, elephants have over 20 copies of the p53 gene to protect them from mutations.)

Aside from the genomic instability that arises from compounding DNA mutations, aberrant epigenetic modifications can also dramatically change functional protein levels and affect genomic integrity. Two epigenetic mechanisms that play an important role in genomic instability are DNA methylation and histone modifications. Hyper- and/or hypomethylation of regulatory regions within genes can mimic DNA mutations and promote tumor progression. In addition, the remodeling of chromatin structure via epigenetic modifications to histones can permit chromosomal rearrangements that lead to chromosomal instability. Together, these epigenetic changes can also affect cell cycle progression and checkpoint regulation, further contributing to genomic instability and cancer progression.

References:

1. Hanahan D, Weinberg RA (January 2000). "The Hallmarks of Cancer". *Cell.* 100 (1): 57–70. doi:10.1016/S0092-8674(00)81683-9
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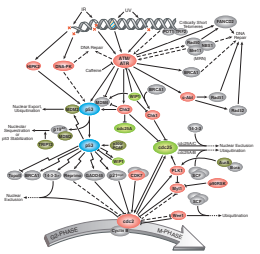
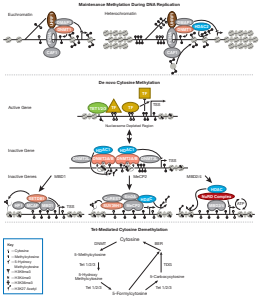
4. Burma, S. et al. (2001) *J Biol Chem* 276, 42462-7.
5. Rogakou, E.P. et al. (1999) *J Cell Biol* 146, 905-16.

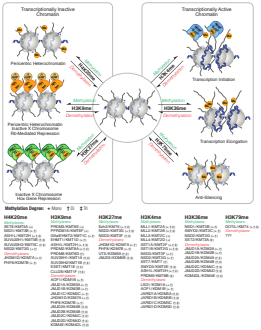
G2M/DNA Damage Contol



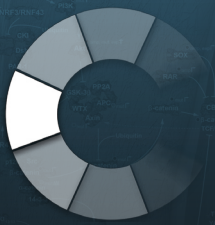
DNA Methylation



Histone Marks



"Due to the aberrant proliferation associated with cancer cells, there is an increased tendency of genomic changes and mutations that contribute to the damage of multiple genes regulating cell division and tumor suppression."



Tumor-promoting Inflammation

Cancer cells hijack inflammatory mechanisms to promote their own growth and survival. During a normal inflammatory response by the innate and adaptive immune system, immune cells carry out their designated task of engulfing and/or destroying foreign invaders.

Within the complex tumor microenvironment, the same infection-fighting immune cells are corrupted by cancer cells. As a result, instead of destroying the transformed cells, the anti-tumor immune cells are subverted into tumor-promoting immune cells that secrete pro-survival, pro-migration, and anti-detection factors that allow tumor growth and metastasis. Important molecules and signaling pathways in mediating the immune response to the tumor microenvironment include NF-κB, inflammasome signaling, tumor-infiltrating immune cell markers, and immune checkpoint signaling.

NF-κB

In immune cells, NF-κB signaling regulates the transcription of genes, influencing innate and adaptive immunity, inflammation, stress responses, B-cell development, and cytokine/chemokine release. In unstimulated cells, NF-κB is in a complex with IκB inhibitory proteins in the cytoplasm. Upon activation, IκB proteins are phosphorylated, then targeted for rapid degradation through the ubiquitin-proteasome system. Removal of IκB proteins releases the sequestered NF-κB, allowing its entry into the nucleus where it can regulate gene expression.

NF-κB signaling in cancer and immune cells within the tumor microenvironment has been particularly implicated in the epithelial-to-mesenchymal transition (EMT) of cells on the tumor border, allowing the detachment and migration of the tumor mass. EMT is a classic hallmark of malignant cancers. Thus, the cross talk between NF-κB signaling in immune-infiltrating cells and cancer cells establishes an environment that promotes tumor growth, invasion, and malignancy in cyclical feedforward manner.

Inflammasome Signaling

The innate immune system is the first line of defense in protection from pathogenic microbes and host-derived signals of cellular distress. One way in which these “danger” signals trigger inflammation is through activation of inflammasomes, which are multiprotein complexes that assemble in the cytosol. The inflammasome promotes the cleavage of caspase-1 and subsequent cleavage of proinflammatory cytokines IL-1β and IL-18. The best characterized inflammasome complex is the NLRP3 complex, which contains NLRP3, ASC (an adaptor protein) and a number of other proteins.

Tumor-infiltrating Markers

The immune system can identify and eliminate cancer cells through both innate and adaptive mechanisms; however, such antitumor responses can be inhibited by the microenvironment through a process known as immunosuppression. Cancer immunotherapy aims to manipulate both immunosuppressive and immunostimulatory mechanisms to increase the anticancer immune response. Therefore, it is important to understand tumor-infiltrating immune cells and their role in tumor growth and suppression.

Tumor-infiltrating immune cells are derived from either a myeloid or lymphoid cell lineage. The abundance and subtype of immune cells within a tumor microenvironment correlate with prognosis. In addition, careful analysis of these two origins can lead to the development of suitable immunotherapeutic strategies for patients.

Once a tumor is established, it can circumvent immune detection through various mechanisms including antigen loss, down-regulation of major histocompatibility molecules, alteration of the endogenous antigen presentation pathways, and immune suppression via cytokine secretion. Along with their ability to evade detection by the immune system, tumors can also hijack immune cells to promote self-growth and metastasis. Immune suppression and subversion by a tumor generally occur in a step-wise manner.

Immune Checkpoints

Immune checkpoints refer to the built-in control mechanisms of the immune system that maintain self-tolerance and help to avoid collateral damage during a physiological immune response. Research has shown that tumors engineer microenvironments to evade immune surveillance and attack, particularly by modulating certain immune checkpoint pathways3.

Because T cells are the primary effector immune cells, they express multiple autoinhibitory cell surface receptors, such as lymphocyte-activation gene 3 (LAG-3), programmed cell death protein 1 (PD-1) and cytotoxic T-lymphocyte-associated protein 4 (CTLA-4), that modulate their response. Within the tumor microenvironment, tumor cells can upregulate the ligands to these receptors to enhance tumor tolerance and evade eradication by the immune system.

In recent years, pharmacological modulators of these ligand-receptor interactions, known as immune checkpoint therapies, have been intensely researched and deployed as novel immunotherapy agents to treat cancers. Of particular interest are monoclonal antibodies against PD-1 and CTLA-4. Given the early success of these immune checkpoint therapies in activating anti-tumor immune responses, creating immunotherapies targeting other co-inhibitory and co-stimulatory receptors and their ligands in order appears to be a compelling therapeutic strategy.

Learn more about the pathways and proteins involved in Tumor-promoting Inflammation:

- NF-κB Signaling
- Inflammasome Signaling
- Tumor-infiltrating Markers
- Immune Checkpoints in the TME

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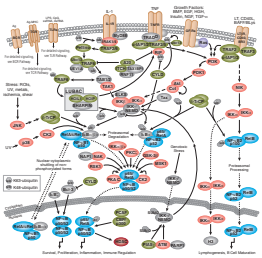
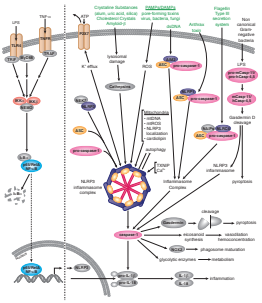
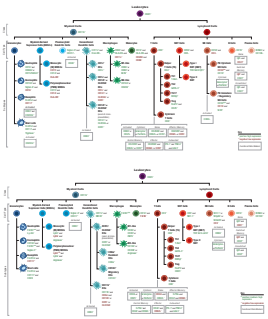
Nf-κB Signaling



Inflammasome Signaling



Tummor-infiltrating Markers



Activating Invasion and Metastasis

Tissue invasion is the mechanism by which tumor cells expand into nearby environments. Metastasis refers to the process of tumor cells breaking away from the primary tumor, migrating to a new location and establishing a new, or secondary tumor, in the new environment. Both of these complex processes leverage existing cellular mechanisms, such as the Adherens Junction Signaling pathway, to enable invasion and migration of the tumor cells.

Adherens junctions are dynamic structures that form, strengthen and spread, degrade, and then re-form as their associated proteins create connections with counterparts from adjacent cells. The Adherens junction serves multiple functions, such as initiation and stabilization of cell-cell adhesion, regulation of the actin cytoskeleton, intracellular signaling, and transcriptional regulation. They can appear as bands encircling the cell (zonula adherens) or as spots of attachment to the extracellular matrix (adhesion plaques).

Under normal conditions, this helps create order among the cells. Cell-cell junctions link cells to adjacent cells in tissue. They also regulate tissue homeostasis in critical cell processes that include tissue barrier function, cell proliferation, and migration. Defects in cell-cell junctions give rise to a wide range of tissue abnormalities that disrupt homeostasis and are common in genetic abnormalities and cancers.

The connection between cell junctions and the cytoskeleton is still under investigation. It may be more dynamic than originally considered and may rely on multiple, weak associations between the cadherin-catenin complex and the actin cytoskeleton or rely on other membrane-associated proteins.

Learn more about Adherens Junction Signaling and the proteins involved.

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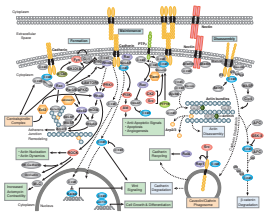
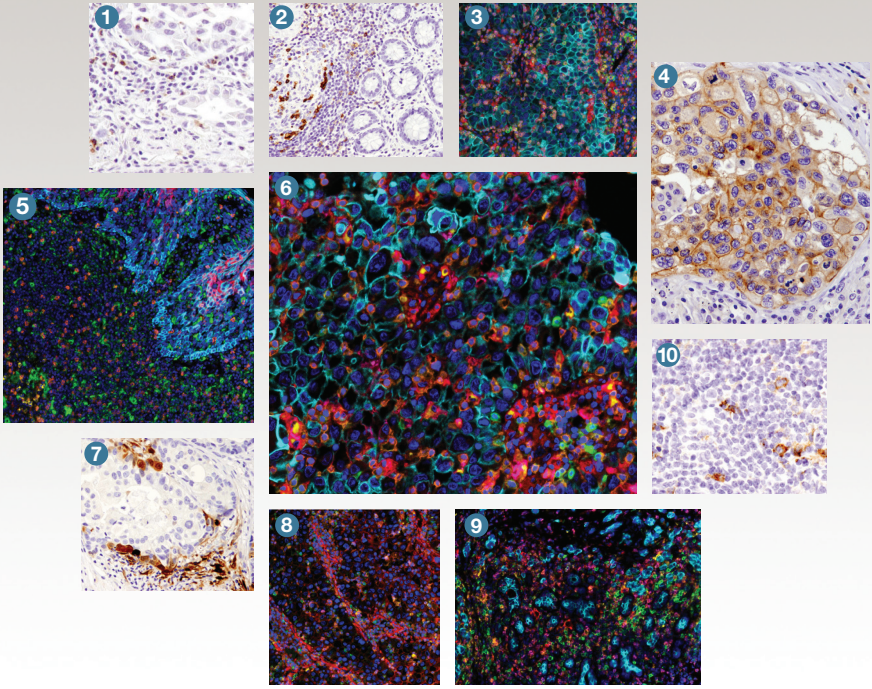
Adherens Junction Signaling





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1. GITR (D9I9D) Rabbit mAb (IHC Preferred) #68014 (lung). **2.** PD-1 (D4W2J) XP® Rabbit mAb #86163 (colon). **3.** TIM-3 (yellow), CD8α (magenta), PD-1 (green), CD68 (Red), LAG3 (orange), Pan-Keratin (cyan) on FFPE NSCLC. **4.** PD-L1 (E1L3N®) XP® Rabbit mAb #13684 (lung). **5.** PD-L2 (orange), PD-L1 (E1L3N®) (red), CD68 (green), PD-1 (yellow), CD8α (magenta) & Pan-Keratin (cyan) on FFPE tonsil. **6.** PD-1 (green), CD68 (yellow), PD-L1 (E1L3N®) (magenta), PD-L2 (red), CD8α (orange), Pan-Keratin (cyan), DAPI (blue) on FFPE NSCLC. **7.** IDO (D5J4E™) Rabbit mAb #86630 (breast). **8.** PD-1 (green), TIM-3 (yellow), B7-H4 (negative), LAG3 (magenta), B7-H3 (red) & VISTA (orange) on FFPE NSCLC. **9.** PD-L1 (E1L3N®) (red), PD-1 (green), TIM-3 (yellow), CD8α (magenta), LAG3 (orange), Pan-Keratin (cyan) on FFPE breast adenocarcinoma. **10.** TIM-3 (D5D5R™) XP® Rabbit mAb #45208 (tonsil).

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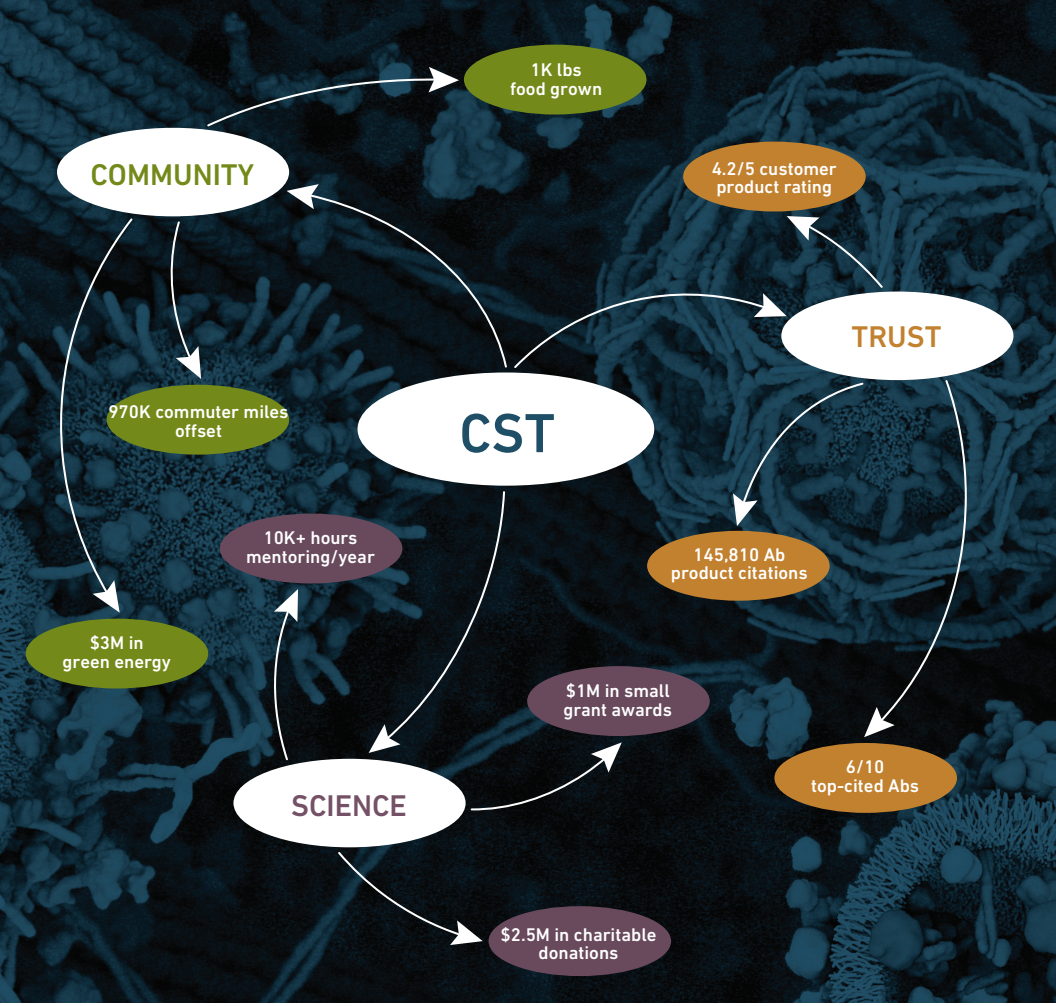

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