The Molecular Nature of Tumorigenesis

Molecular aspects of cancer continue to be uncovered by researchers around the world. Theses aspects will need to be exploited in the next generation of cancer treatments. Specific tumor suppressor genes such as p53 can serve as novel therapeutic targets as well. Although the scientific understanding of the actual steps in tumorigenesis is rather muddled, the differences between cancer and normal cells can be exploited throughout the tumor development process and used in therapy to destroy as much of the tumor as possible. Finally, differences in cancer mortality by age group might provide some insight into the primary causes of pediatric and adult cancers.

In 2000, Robert Weinberg and Douglass Hanahan published an article titled The Hallmarks of Cancer in the journal Cell. In it, they espoused six aspects that cancer cells exhibit. This revolutionary article was the first to provide and articulate a very comprehensive framework necessary to understand the molecular nature of cancer.

Enabling Replicative Immortality

Cancer cells enable replicative immortality. While normal cells exhibit a finite number of cell divisions, as theorized by Leonard Hayflick in 1961, cancer cells have developed molecular mechanisms to essentially immortalize themselves [1]. During the process of cell division, normal mammalian cells undergo a shortening of the end of chromosomes, or telomeres. About 90% of cancer cells activate the enzyme telomerase to protect the ends of their chromosomes. Telomerase works by extending the repeating ends of chromosomes so the replicative machinery can properly copy the free 3-prime end of the new strand [2]. This process

is often aided by the loss of tumor suppressor genes such as p53 [1]. In recent years, research has uncovered additional functions of telomerase that are independent of telomere length maintenance: enhancement of cell proliferation, resistance to apoptosis, DNA damage repair, RNA-dependent RNA polymerase function, and association with chromatin [3]. The remaining 10% of cancer cells prevent themselves from entering into replicative senescence through alternative methods that are not very well understood such as breakage-fusion-breakage patterns [2]. Regardless of method, cancer cells become immortalized by preventing the shortening of their chromosomes during mitosis. This feat is easily observed in the tumor that was surgically removed from Henrietta Lax. Her cancer cells continue to divide to this day, well beyond the typical lifetime of a mammalian cell. Telomerase inhibitors are an active area of therapy development. However, some mammalian cells such as intestinal crypt cells, embryonic stem cells, immune cells, and other cells that need to readily divide, require telomerase to function properly and anti-telomerase drugs hamper these important cells' ability to provide cellular longevity.

Evading Growth Suppressors

Normal cellular proliferation is a tightly controlled process in which pro- and anti-proliferative signals are coordinated throughout the cell cycle. Checkpoints throughout the cell cycle provide critical regulations that halt cell proliferation in the event of DNA damage or other cellular problems. Antigrowth signals exert a majority of their effect at the G_1 checkpoint [4]. Antigrowth signals at this checkpoint can either force the cell into terminal differentiation or induce the cell into the G_0 state [5]. However, most cancer cells have developed mechanisms to

circumvent normal growth suppressors in an effort to continue proliferating. Retinoblastoma protein (Rb) and p53 are two proteins that have cell cycle control that are often rendered dysfunctional in tumor cells [6]. Rb serves as a gatekeeper at the G_1 checkpoint and p53 regulates apoptosis in cells that have incurred DNA damage. Mutations in these proteins would result in ongoing cell proliferation regardless of DNA damage or cellular stress. This leads to uncontrolled growth and proliferation. Current therapeutic development is involved in cyclin-dependent kinase inhibitors that would help better control the kinase cycle evident throughout mitosis.

Inducing Angiogenesis

Cancer cells, just like normal mammalian cells, are constantly in need of oxygen and nutrients that are delivered through the vasculature. Cancer cells induce angiogenesis, the formation of new blood vessels [1]. Tumor angiogenesis is a multistep process that involves input from several pro-angiogenic factors and is imperative for sustained tumor growth and metastasis [7]. The angiogenic switch occurs when tumor cells overexpress vascular endothelial growth factor [8]. Unabated angiogenesis allows delivery of oxygen and nutrients and production of growth factors that permit tumor expansion and local invasion. Modern research also suggests that tumor vasculature can serve as a portal for metastasis allowing the cancer to spread into systemic circulation [7]. Modern therapeutic development is aimed at developing inhibitors of VEGF signaling.

Resisting Cell Death

Cancel cells have developed molecular mechanisms to subvert programmed cell death. Whereas normal cells turn on apoptosis during times of DNA damage or other irreparable stress, cancer cells generally avoid invoking a response when faced with these same stresses [9]. Cancer cells accomplish this by overexpressing anti-apoptotic proteins and silencing pro-apoptotic proteins. These two methods account for extrinsic apoptotic methods such as death domains like tumor necrosis factor alpha and intrinsic methods invoked by tumor suppressor gene p53 [10], [11]. Cancer cells can also alter cellular autophagy and necrosis to aid in resisting cell death [2]. Pro-apoptotic BH3 mimetics are an active area for therapy development.

Sustaining Proliferative Signaling

Tight regulation of growth signaling ensures cellular homeostasis in normal cells. Growth signaling is highly regulated. Proliferative signals are activated whenever necessary and deactivated when they are no longer necessary. These regulations are often compromised in cancer cells. Cancer cells fundamentally can proliferate without a controlled signaling input. They can do this in a variety of ways: increasing growth factor production, stimulating normal cells in the microenvironment to provide cancer cells with growth factor, increasing the number of receptors on the cell surface, altering receptors structurally to facilitate cancer cell signaling, and activating proteins in the downstream signaling pathway [1]. Recent studies also show that cancer cells can disrupt negative feedback mechanisms as well [12]. This is readily seen in the oncoprotein Ras. Mutations in Ras disrupt its intrinsic GTPase activity that prevents it from hydrolyzing GTP [2]. This locks Ras in the “on” position and signals constant proliferation.

Epithelial growth factor receptor inhibitors are currently being researched as possibly therapy to disrupt this sustained proliferative signaling.

Activating Invasion and Metastasis

Ultimately, cancer's effectiveness boils down to it ability to activate invasion and metastasis to distant tissue. Tissue invasion and metastasis are integral to cancer's ability to escape from the primary site and invade distant organs. The process is not well understood molecularly, but it is thought to involve changes to the extracellular matrix [13]. The process includes four general steps: local tissue invasion, intravasation, transition through blood and lymphatic system, and colonization of foreign tissue. Tumor cell migration is promoted through a paracrine loop involving CSF-1, EGF, and their corresponding receptors in the tumor microenvironment [14]. The kinetics of this process can take many years and decades before visible tumors actually arise. Inhibitors of HGF and c-Met are important research directives to the development of new novel therapies.

After about another decade of research and development, Robert Weinberg and Douglass Hanahan co-authored another paper published in Cell in 2011 known as The Hallmarks of Cancer: the next generation. In it, they affirmed their previous six hallmarks and demarcated four new emerging hallmarks of cancer.

Avoiding Immune Destruction

Cancer cells have developed methods to subvert destruction by the immune system [15]. Normally, tumor formation is proactively prevented by an essential cellular process known as

immunosurveillance. Preclinical studies have suggested regular immune system activity eliminates a vast majority of cancer cells by recognizing and destroying them before they are able to form a detectable tumor mass [16]. However, cancer cells can avoid this process through cancer immunoediting. This process includes three key phases: elimination, equilibrium, and escape [17]. The elimination phase is characterized by a properly functioning immune system that recognizes and destroys cancer cells. However, some cancerous cells are able to avoid immune destruction and slip into the equilibrium phase. In equilibrium, the immune system controls cancer growth but does not completely eliminate the transformed cells. Finally, when tumor cells are no longer responsive to immune destruction, they progress into the escape phase. These escaped mutants are no longer effectively recognized or destroyed by the immune system and are thus able to proliferate at uncontrollable rates [18]. Studies have shown that patients with certain cancers actually have a much better prognosis with increased immune response [19]. This idea is readily exploited in immunotherapy and targeted therapy treatment options. Current therapy research aims to investigate immune response by treating patients with biological response modifiers such as cytokines, interleukins, and monoclonal antibodies to help ramp up the patient's immune system.

Deregulating Cellular Energetics

Surprisingly, cancer cells undergo a dramatic reprogramming of their energy metabolism [15]. In aerobic conditions, normal mammalian cells undergo oxidative phosphorylation in the mitochondria as the final step in transforming glucose into the energy currency ATP. In anaerobic conditions, such as during a sprint, glucose will be converted into pyruvate through

glycolysis and undergo lactic acid formation. This lactic acid buildup is what caused muscle fatigue and tenderness. Cancer cells on the other hand, undergo what is known as the Warburg Effect, aerobic glycolysis [2]. Even in the presence of abundant oxygen, about 85% of cancer cells produce their ATP through glycolysis only [2]. Oxidative phosphorylation provides roughly sixteen times more energy per glucose molecule. This begs the question: is aerobic glycolysis required to maintain the cancer phenotype, or is it merely a side effect of some other phenomena? One argument for the former would stem from biological feedback conditions that would favor proliferation. Glycolytic intermediates are precursors to nucleotide and lipid biosynthesis. An accumulation of the end product lactate would cause a buildup of these precursors and would provide an environment ripe for proliferation [20]. Future research is necessary to delve deeper into the questions surrounding aerobic glycolysis of cancer cells. Aerobic glycolysis inhibitors provide a good therapeutic target for modern clinical research.

Tumor-Promoting Inflammation

As previously mentioned, the tumor microenvironment is constantly being sense by the immune system. Unfortunately, recent research has indicated that the constant influx of immune system cells mimics inflammatory conditions that might aid in tumor growth [15]. Inflammation may help supply the tumor microenvironment with the following: growth factors, survival factors, pro-angiogenic factors, extracellular matrix modifying enzymes, and inductive signals that activate the epithelial-mesenchymal transition [21]. This is often seen later in tumor progression; however, early inflammation can lead to the release of chemicals that lead to genetic mutation and accelerate tumor formation. Tumor-promoting inflammation is currently

on the forefront of cancer research and selective anti-inflammatory drugs are being targeted for therapeutics. It is recommended that people over the age of fifty take one baby aspirin a day to help reduce their risk of certain cancers and tumor-promoting inflammation.

Genome Instability and Mutation

Oncogenic processes are facilitated by multiple alterations throughout the cancer genome. Cancer cells foster tumorigenesis by taking advantage of their hypermutability. They accomplish this by increasing sensitivity to mutagenic agents and by breaking down cellular DNA repair mechanisms. Accumulation of these mutations is accelerated by altering DNA-maintenance machinery responsible for: detecting DNA damage, activating repair machinery, directly repairing DNA damage, and inactivating or intercepting mutagenic molecules [15]. By inactivating these caretaker genes, tumorigenesis is accelerated by genomic instability. One such pathway, the BRCA1 signaling pathway, which is the pathway that is disrupted in a majority of breast cancers, is well characterized in the literature [22]. Poly ADP ribose polymerase inhibitors are important in treating this facet of cancer because several forms of cancer are more dependent on PARP than regular cells.

Ultimately, cancer is a multi-faceted disease whose complexity only hampers therapy development. Often, patients are given a cocktail of drugs to try to alleviate as many of the hallmarks of cancer as possible. However, cancer can progress through many alternative paths each producing the same end result: metastasis. This is why early detection is a key component in the success of cancer treatment.

Fundamental differences exist between pediatric and adult cancers. Figure 1 shows cancer mortality as a function of age.

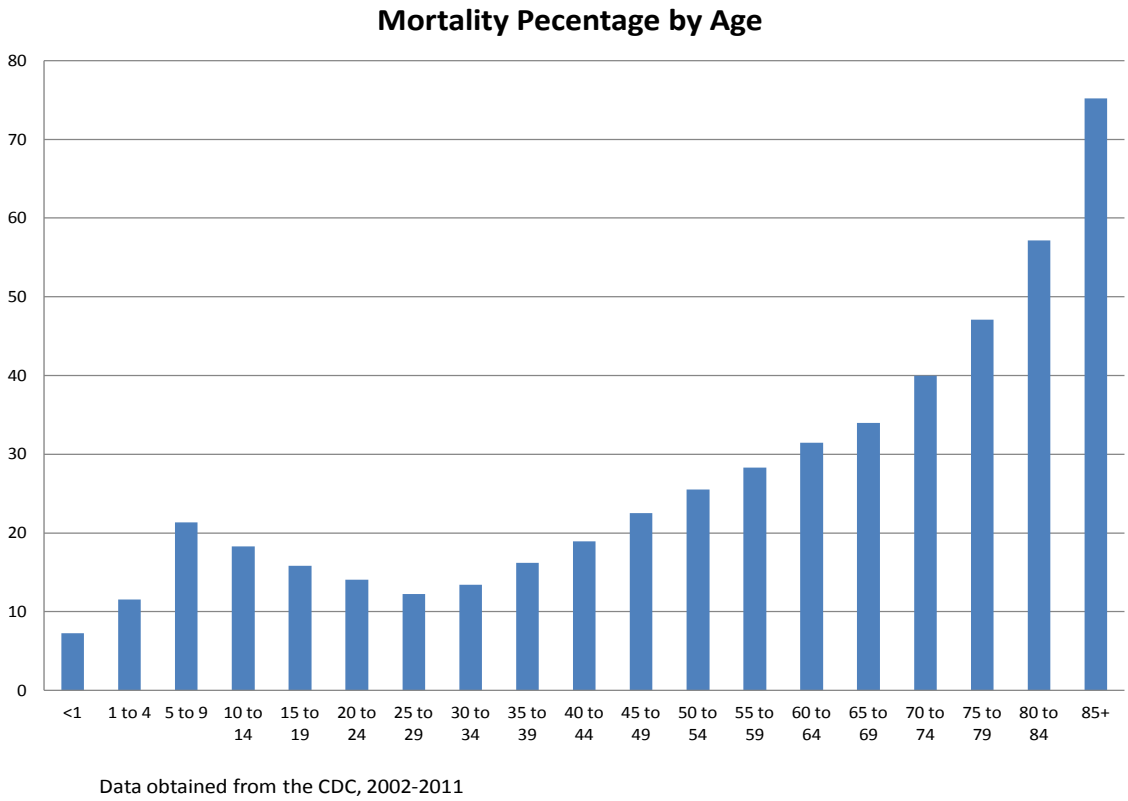

Figure 1: Cancer Mortality by Age [23]

This data was obtained from the CDC. The mortality percentage was calculated by taking the number of people in each age range that died from cancer and dividing it by the total cancer incidence of the same age group. The data shows a bimodal growth that defies expectation. When breaking down the pediatric age groups and observing the specific cancers affecting each group, some trends can be noted. Early cancer mortality appears to be a direct result of the growth rate. For example, many osteosarcomas, lymphomas, leukemias, and brain

cancers occur in the age range five to nine. These correlate to the organs and systems that are developing most rapidly in this age range. Also, in the age ranges from ten to nineteen, many puberty-associated cancers (breast, cervical, testicular) cause a majority of the cancer mortalities. After the age of 25, the cancer mortalities follow a predictable pattern that correlates to the natural accumulation of mutations. Putting all of this together, cancer-associated mortalities result from: in utero mutagens before the age of four, growth rate associations between the ages of five and twenty five, and a natural accumulation of mutations over the age of twenty five. Further statistical analysis may provide insight into factors that cause cancer or may help prevent it.

Genetic predisposition and environmental exposure also play roles in cancer-associated mortalities. However, they appear rather consistently over all age ranges. For example, retinoblastoma deficiencies show up often in children while breast cancer predispositions often take effect after the age of forty [2]. Also, the Chinese have an extremely low rate of stomach cancer. However, second generation Chinese immigrants in California showed increased rates of stomach cancer comparable to the rest of the California population [2]. These are just a few examples of how genetic predisposition and environmental exposure affect an individual over the course of a lifetime.

Tumor suppressor gene p53 is an extremely important protein that is often mutated late in the tumor progression pathway. Its mutation plays a major role in tumor progression because it provides a cellular environment conducive to many of the hallmarks of cancer, especially avoiding programmed cell death. When functioning normally, p53 acts to prevent

tumorigenesis through a variety of signaling pathways. P53 responds to cellular stresses to induce cell cycle arrest, DNA repair, blockage of angiogenesis, or apoptosis. When mutated, p53 loses these pertinent abilities [2]. This begs the question: Why has evolution entrusted so many vital alarm systems to a single protein?

P53 is the single most mutated protein in all tumors, showing up in at least 33% of all cases. This number is even larger when referring to cancer as a whole and not just an individual tumor. The 33% is artificially low because many tumors are removed early in tumor progression before p53 is mutated. It is theorized that the p53 gene is mutated in nearly 100% of all cancer mortalities [2].

P53 functions normally as a homotetramer comprised of four identical subunits. When one of the subunits is mutated, the functional protein has slightly altered abilities. If all four subunits were mutated, this would result in a completely null, non-functional protein. Cancer cells actually benefit more from slightly altered p53 function rather than complete loss of function [2].

P53 basal levels are kept surprisingly low in cells. They are controlled by another protein known as mdm2. Normally, p53 promotes the transcription of mdm2, its own destroyer. Mdm2 functions to ubiquitylate p53 and mark it for destruction by the proteosome. For this reason, p53 has a half-life of about twenty minutes [2].

In times of DNA damage, p53 becomes phosphorylated. This serves two functions: p53 no longer transcribes mdm2, effectively reducing its destroyer, and it is no longer recognized by mdm2, effectively stopping its degradation. This allows for a fast response to DNA damage or

other stresses by dramatically increasing p53 concentration throughout the cell. When the stress or damage is resolved, p53 is dephosphorylated, mdm2 is transcribed, and p53 returns to basal levels [2].

If the DNA damage or stress is too large to overcome, p53 can initiate apoptosis, programmed cell death. P53 sends pro-apoptotic signals to the mitochondria, causing the release of cytochrome c. The released cytochrome c complexes with seven Apaf-1 proteins to form the apoptosome, or wheel of death. Apoptosome formation triggers a cascade of caspase activations that result in the formation of executioner caspases. The executioner caspases fully rupture the mitochondria leading to the disruption of the electro transport, loss of ATP production, release of reactive oxygen species, and the loss of mitochondrial structural integrity, thereby amplifying the apoptotic process. At some point during evolution, the mitochondrion was co-opted to harbor the messenger of death! Cancer cells are often very hard to destroy via apoptosis because many of them have developed methods to subvert the apoptotic machinery [2].

The treatment of cancer has improved drastically over the past few decades. High resolution MRIs are becoming so revealing that the incidence rate of cancer is actually rising because previously undetectable tumors are being diagnosed. The effects of the improvements on cancer mortality are currently unknown, but improvements in early detection should lead to reductions in the mortality rate. Current treatments often involve a combination of two or more of the following: bone marrow transplant, chemotherapy, radiation, surgery, and targeted therapy/immunotherapy [24].

Bone marrow transplants are often used when cancer has damaged the patient's bone marrow in the surrounding areas. The process is accomplished by radiating the damaged area and replenishing the diseased cells with healthy ones. Undamaged bone marrow is often grafted from another site in the patient and used to replace compromised marrow. However, if this option is unavailable, bone marrow can be used from an outside donor. This is the less preferred option because many complications can arise from the allogeneic donor [24].

Chemotherapy is a general term for any medication used to destroy or stop the growth of cancer. Cytotoxic chemotherapy agents are given to help decrease the size of tumors to make them safer and easier to remove. Chemotherapy can also be used to enhance the cancer-killing ability of other treatment options. Chemotherapy can be delivered to the bloodstream to reach systemic cancers or it can be given directly to the site of the cancer. Chemotherapy medicine works to prevent cells from growing by: preventing the copying of cellular components needed for cells to divide, replacing or eliminating essential enzymes or nutrients the cancer cells need to survive, or triggering cancer cells to self-destruct [24].

Radiation treatment utilizes high energy x-rays to destroy rapidly growing cells. Radiation therapy works by destroying or damaging rapidly growing cells. It hinges upon the fact that normal cells are better able to repair themselves than are cancer cells. Doctors hope to shrink the size of a tumor by delivering radiation to the tumor's exact location. Unlike chemotherapy, radiation does not cause cell damage throughout the body [24].

Surgery involves cutting into the patient's body to access parts beneath the skin. It is used to treat cancer in a variety of ways including diagnosis, tumor removal, or to support a

child undergoing cancer treatment. There are two main types of surgery: open and minimally invasive. Open surgery is often used to remove cancerous tumors through incisions directly above the tumor. Minimally invasive surgery involves small incisions that utilize specifically designed instruments. Surgery is further divided according to the purpose it serves in treatment. Primary surgery removes all or of the tumor, exploratory surgery involves a second look into the body to see how treatments have worked, and supportive care surgery is designed to help patients cope with their treatment [24].

Immunotherapy and targeted therapy are two of the most active areas of modern cancer therapy research. Immunotherapy is designed to help ramp up the body's natural immune system and help it better fight cancer. Biological response modifiers such as interleukins, monoclonal antibodies, and cytokines are given to change the way the body responds to cancer. Active therapies involve stimulating the natural immune system to work harder and more efficiently while passive therapies involve giving manmade proteins to supplement a patient's immune system. Targeted therapies are designed to stop the growth and spread of cancer by attacking the molecular aspects of cancer. Targeted therapies can be used alone or in combination with other therapies such as chemotherapy and radiation [24].

In an era where treatment development appears to have stalled, taking a prophylactic rather than a reactive approach to cancer will pay large dividends. Using tobacco of any form puts one on a collision course with cancer. Smoking has been linked to lung, bladder, cervical, and kidney cancers while chewing tobacco has been implicated to cause cancers if the oral cavity and pancreas. Avoiding tobacco, including secondhand smoke, is one of the most

important health decisions one can make. Current research shows that eating a healthy diet can have dramatic health effects, including reduced risks of cancer. It is important to eat a diet high in fruits and vegetables that provide important nutrients and antioxidants. Fat and red meats should be limited because they have a high tendency to lead to obesity which increases the risk of cancer. Alcohol consumption should also be moderated as alcohol has been implicated in breast, colon, lung, kidney, and liver cancers. Maintaining a healthy weight and being physically can reduce one's risk for breast, lung, prostate, colon, and kidney cancer. The physical, social, mental, and emotional benefits of physical activity are endless. People who are more physically active tend to have lower rates of obesity which are major causes of cardiac and cancer deaths, the two biggest killers worldwide. Protection from the sun is another key way to prevent cancer, specifically melanomas that are showing up with regularity worldwide. It is important to avoid the midday sun and tanning booth while wearing plenty of sunscreen and protecting the face and head areas with a covering hat. Immunizations that protect against hepatitis and human papillomavirus reduce risks for liver and cervical cancers respectively. Another effective cancer prevention tactic is to avoid risky behaviors that can lead to infection and, in turn, increase the risk of cancer. These risky behaviors include unprotected sex, multiple sexual partners, and sharing needles. Finally, regular medical care and self-exams can increase the chances of early detection. Treatment is most successful in instances of early detection [25].

In conclusion, a reduction in cancer incidence rather than improvements in therapy offer the greatest prospects for substantial decreases in cancer associated mortality. For example, it is estimated that in the year 2000 alone, 850,000 people died prematurely from lung cancer that was purely attributable to smoking [26]. These nearly one million people could

have lived longer, healthier lives had they made the decision to never start smoking. Thus, prevention is the cure!

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