The History of Cancer, Treatments and Nutraceuticals: A Natural Form of Cancer Therapy

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Introduction

The term cancer has unfortunately become commonplace in modern vernacular. In fact, approximately one-half of the male population in the United States and one-third of the female population will develop cancer at some stage in their lives [1]. Although cancer has existed for centuries, with some of its earliest descriptions dating back to Ancient Egypt, little remained known about the disease and how to effectively treat it [2]. For centuries, the belief that cancer was an untreatable illness remained unchallenged, thereby slowing the study of cancer as well as the development of its treatments. It was not until the last half a century that a clearer understanding of cancer and its key characteristics were uncovered. Such an understanding has consequently led to the adaptation and continual refinement of varying cancer therapies including surgery, radiation, chemotherapy and targeted therapy. Yet, despite improvements, the therapies face limitations in their ability to arrest cancer, causing cancer to remain a leading cause of death in the world today. The aforementioned cancer treatments can also have severe and even lethal side effects, causing patients and doctors to question whether treatment was worth enduring at all. Consequently, the development of new forms of treatment is necessary in order to combat this deadly disease. As such, a new form of therapy using plant-derived products called nutraceuticals is a great focus of cancer research today. It is the hope that unlike other forms of cancer treatments, the use of nontoxic, natural nutraceuticals will effectively kill cancerous cells without causing harmful effects to healthy cells. Researchers are hopeful that nutraceuticals will in fact revolutionize cancer treatments.

Discussion

Past Theories of Cancer

The history of cancer is a short one. Although cancer has existed for thousands of years, it has only become a leading cause of death in the last century. Prior to the 20th century, the average lifespan was 50 years with most deaths attributed to infectious diseases. Cancer was considered a rare disease that only accounted for a small percentage of mortalities, due to its usual onset later in life [3]. While some of the earliest cancer evidence was found in fossilized bone tumors, human mummies in Ancient Egypt and ancient manuscripts dating back to 1600 B.C.E., little was known about the disease and its causes until the end of the 20th century when new technologies and biological discoveries paved the way for understanding cancer and how to treat it [4].

Despite evidence of cancer found throughout history, cancer remained a mystery shrouded in theories and speculations. Egyptian surgeons, who were aware of the disease, associated cancer with the actions and whims of their gods. Much later, in ancient Greece, Hippocrates (460-370 BCE), the "Father of Medicine," believed that the four *humors* or bodily fluids were in control of a person's medical health. Hippocrates articulated that if a person's *humors*, which were comprised of blood, phlegm, yellow bile and black bile, were balanced, he was healthy, but if his black bile was found in excess, the result was cancer. The humoral theory of cancer remained an unchallenged hypothesis for 1,300 years until the Renaissance [2].

At the close of the Renaissance period, the lymph theory, developed by Hoffman and Stahl, eventually replaced the humoral theory. A greater understanding of the human body was gained due to the conduction of autopsies, which was first introduced by English

physician William Harvey in 1628. The fact that anatomic dissection conducted by Andreas Vesalius and others failed to uncover the presence of black bile in the body ultimately disproved the humoral theory [5]. Harvey's observations of the blood circulatory system, alongside Gasparo Aselli's discovery of lymph in 1622, led to the assumption by George Stahl and Frederick Hoffman that life was dependent on the proper movement of blood and lymph throughout the solid body. According to Stahl and Hoffman, it was the fermenting and degeneration of lymph of differing densities and acidities that caused cancer. John Hunter (1728-1793), a Scottish surgeon, corroborated this theory, hypothesizing that cancerous tumors grew from lymph that was discarded from the blood [2].

With the rising popularity and improvement of the light microscope in the 18th century and the discovery of cells, the blastema theory quickly dispelled the lymph theory. The blastema theory was the first to state that cancer is a disease of cells rather than lymph. Yet, when German pathologist Johannes Müller made this groundbreaking discovery in 1838, his theory remained flawed due to his belief that cancerous cells arose from budding elements called blastemas found among healthy tissue. Müller's theory was eventually modified by another German pathologist, Rudolph Virchow, who stated that all cells must arise from preexisting cells and that even cancer cells must originate from healthy cells [6]. Virchow proposed the chronic irritation theory, which stated that the onset of cancer was attributed to chronic irritation of the body. Virchow also described how cancer spread throughout the body by means of an unknown fluid. He was later corrected in the 1860's by Karl Thiersch, a German surgeon, who proved that cancer travels throughout the body via the spread of cancerous cells and *not* via an unidentified fluid. However, at the end of the 19th century, the trauma theory, which was developed by German professor of pathology Hugo

Ribbert, superseded the chronic irritation theory. Ribbert believed that trauma was the cause of cancer, but was unable to produce evidence to support his theory. Despite the evidence against it, the trauma theory remained popular until the 1920's [7].

The trauma theory was ultimately replaced by the theory of infectious disease, a theory deeply rooted in past history. In the 17th century, two Dutch doctors Zacutus Lusitani (1575-1642) and Nicholas Tulp (1593-1674) claimed that cancer was contagious. This theory continued to be believed throughout the 17th and 18th centuries so much so that the first cancer hospital, which was established in 1779 in Reims, France, was forced to be erected outside of city boundaries, due to fear of contagion. Yet this theory was also eventually dismissed in the 1970's, when it became apparent that the majority of cancer cases in Western cultures contained no signs of viral infections [2].

New groundbreaking discoveries of both cellular and molecular processes as well as the development of new technologies throughout the 20th and 21st centuries have paved the way for the modern cellular theory of cancer. Through molecular and cell biology research as well as clinical trials, scientists have been able to gain a greater understanding of what cancer is and how to effectively treat it. Discoveries made in relation to genetics as well as cell division and its regulation have all played vital roles in understanding the nature and behavior of cancer. Building upon much of the scientific knowledge acquired by the hard work of their predecessors, scientists have learned more about the nature of cancer in the last two decades than what was learned in the preceding centuries. Thus, recent cancer research and clinical trials are responsible for the generation of a rich and complex body of knowledge that attempts to decipher the key characteristics of cancer [9, 10].

Defining Cancer

Although much of the complexity of cancer remains unknown, researchers have recently gained greater comprehension of the disease by uncovering several defining features of cancer. Cancer is the uncontrolled growth of abnormal cells that results from the breakdown of cell division regulation. The controlled division of a healthy individual cell within a multicellular organism is imperative for the coordination and functionality of the organism as a whole. The lack of controlled growth and division will lead to the derailment and redirection of the cell's entire metabolism, thereby hindering its ability to meet the needs of the organism. The transformation of a healthy cell into a malignant one is a multi-step process that results from an accumulation of genetic errors. These genetic alterations determine the defining qualities of most cancer cells: uncontrolled growth, cell death evasion, lack of specialization and metastasis. Together, these four trademarks of cancer enable a deadly disease to proliferate and affect the lives of many.

The DNA (Deoxyribonucleic Acid) damage of healthy cells can lead to the uncontrolled growth and division of abnormal cells within the body known as cancer. While there are more than 100 distinct forms of cancer that vary greatly in their behavior and responses to treatment, all forms of cancer develop from genetic changes, termed mutations. Although healthy cells generally have the wherewithal to catch such DNA glitches through many rather redundant cell division regulation processes and either repair the damage or induce cell death, cancer cells lack this ability. In cancer cells, changes in DNA sequencing or structure are not repaired and continue to accumulate inside the cell. Rather than decrease the functional ability of the cell like most mutations, many of these mutations enable cancer

cells to reproduce at a faster rate than healthy cells [3]. Thus, cancer results from an alteration of a normal biological process known as cell division.

In healthy cells, cell division is a crucial cellular process through which a single cell splits into two identical daughter cells. Cell division is beneficial to the human body in that it enables the growth and repair of tissues and organs. The process of division is the culmination of a cyclical sequence of events known as the cell cycle. In the cell cycle, consisting of G1, S, G2, and M phases, cells experience growth, DNA replication, chromosomal segregation and division into two completely new cells. The Gap 1 (G1) phase of the cycle ensures that everything required on both an intracellular and extracellular level is in place for DNA replication of the synthesis (S) phase to occur. The G2 phase is responsible for the accumulation of nutrients and growth needed for the culmination of this cycle, known as the mitosis (M) phase, during which the replicated DNA splits into two distinct nuclei. The newly formed daughter cells can consequently enter the G1 phase again, thereby continuing the cycle [11].

While cell division can be highly beneficial to the human body, increased rates can be harmful and so it must remain a regulated process. In order to maintain a healthy body, cells can only divide a specific number of times before they are programmed to die through a process known as apoptosis. This is because DNA replication and cell division must remain conservative processes leading to the formation of identical progeny cells. If genomic changes were to be transmitted to the next generation of cells, the consequences would be severe, resulting in damaged cells that do not function properly [11, 12]. Thus, regulation of the cell cycle is imperative.

Proto-oncogenes are a class of genes that is largely responsible for the regulation of the cell cycle. In healthy cells, proto-oncogenes code for the production of proteins that help regulate cell growth and division by telling the cell when to divide. There are four different kinds of proto-oncogenes including growth factors, growth factor receptors, signal transducers, and transcription factors. Growth factors are proto-oncogene proteins used as signaling molecules that enable cells to communicate with each other and tell the cell when it is time to start dividing. In order for proliferation to be a specific process, causing only certain cells to divide at the correct time, the cell must have specific growth factor receptors, another product of proto-oncogenes, on its outer surface. When a growth factor protein effectively binds to its receptor, it causes a signal to be transmitted from the cell surface to the nucleus via a series of complex intermediary pathways, which are called signal transduction cascades. These signaling pathways involve many proto-oncogene proteins that are involved in passing the signal from one protein to the next until it reaches the nucleus. In the nucleus, other proto-oncogene proteins known as transcription factors then activate the expression of genes involved in cell division [13]. In this way, the process of cell division is controlled by proto-oncogenes that are responsible for its initiation.

Like proto-oncogenes, tumor suppressor genes play a critical role in cell division regulation. Originally discovered in the 1960s by Henry Harris, tumor suppressor genes monitor the rate of cellular division and slow it down [14]. A subgroup of these genes, known as DNA repair pathway genes, is responsible for recognizing and repairing errors made in the replication of DNA. If the newly formed copy of DNA is not identical to the original strand, it is the job of these genes to fix the mistake [15]. Proto-oncogenes and tumor suppressor genes thus play a key role in regulating the cell cycle.

Researchers have discovered that mutations in proto-oncogenes and tumor suppressor genes cause cells to defy normal regulation and experience the uncontrolled growth characteristic of cancer. When these genes are mutated, it causes the complex, built-in control system of the cell cycle to run amuck, due to the fact that they are no longer capable of slowing or halting the cell cycle. The mutated form of proto-oncogenes, called oncogenes, are dominant in nature, meaning that a single mutant copy of the gene can lead to the constant activation of the cell cycle [9]. In contrast, tumor suppressor genes are recessive in nature and require two mutated copies of the gene in order to produce a cancerous effect [15]. Approximately 100 oncogenes, which were initially discovered by 1989 Nobel Prize laureates John Michael Bishop and Harold Varmus, have been identified, but hundreds still remain unknown today [3]. According to research, specific proto-oncogenes products called Ras proteins are involved in about 25% of human malignancies. Additionally, retinoblastoma (Rb) and p53 are two significant types of tumor suppressor genes that are commonly mutated in cancer cells. While Rb is normally involved in cell cycle initiation and progression as well as the maintenance of genome stability, it is commonly found to be mutated in a form of eye cancer called Retinoblastoma as well as other cancers [11]. Tumor suppressor gene p53 also plays a critical role in mediating cell cycle arrest and DNA repair. The mutated form of p53, which was discovered in the year 1979, is expressed in 40% of all cancers [16,17]. It is thus the deregulation of genes like proto-oncogenes and tumor suppressor genes that contribute to the uncontrolled growth of cancer.

Mutations of proto-oncogenes and tumor suppressor genes not only enable the cell to constantly grow and divide, but also facilitate another defining characteristic of cancer known as apoptotic evasion. The choreographed sequence of cellular death can be triggered

by a variety of physiological stresses and abnormalities, including irreparable DNA damage, oxygen deficiency, nutrient deprivation or oncogene signaling malfunction, that can, in turn, cause the deterioration of the cell and all of its internal components. During apoptosis, the cellular membranes are disrupted, the nuclear and cytoplasmic skeletons are deteriorated, the cytosol is expelled, the nucleus is fragmented and the chromosomes are degraded. This controlled cellular suicide is a very systematic and selective process in order to protect the multicellular organism from dangers like viral infection and tumor formation. In the average human adult, approximately 50 to 70 billion cells die each day as a result of apoptosis [18]. Apoptosis usually involves sensor proteins, which monitor the cell's intracellular and extracellular state to determine whether it should live or die, followed by effector caspase proteins that, when signaled, cause the cell to be disassembled. Specific proto-oncogenes and tumor suppressor genes serve as sensor proteins for the apoptotic pathway. Proto-oncogenes, like members of the *bcI*-2 and *myc* protein families, produce both pro- or anti- apoptotic proteins. Similarly, tumor suppressor genes, like p53, initiate apoptosis when severe chromosomal abnormalities are detected. Mutations to these proto-oncogenes and tumor suppressor genes enable cancer cells to outwit and circumvent the apoptotic pathway. The functional inactivation of the p53 sensor protein at the start of the cell cycle is one of the most common ways cancer cells avoid cellular death. Since other apoptotic signals that detect additional abnormalities must pass their signals onto p53 in order to induce cell death, they too become impaired from effectively inducing apoptosis when p53 is mutated. Likewise, cancerous cells' ability to increase expression of anti-apoptotic proto-oncogenes, like *bcI*-2, enable cancer cells to evade apoptosis even when an unhealthy environment exists [9, 10].

By use of alterations in these specific genes, cancer cells gain the capability to evade cell death and achieve limitless replicative potential.

Other mutations in regulatory genes facilitate the ability of cancer cells to remain unspecialized in function. Normal cells that are fully specialized in function can no longer continue to divide. However, in order for cancer cells to maintain their replicable ability, cancer cells are arrested at an early stage of maturation and remain eternally young. As a result, abnormal cancer cells are unable to carry out specific functions in the body necessary for producing a healthy and stable internal environment. It is the growing presence of these dysfunctional abnormal cells in the form of tumors that impacts other healthy tissues and organs, interfering with their ability to fulfill their respective jobs [10].

Still other regulatory gene mutations enable cancer cells to acquire the ability to metastasize and spread throughout the body. Cancer can secrete digestive enzymes called proteases that enable the disease to invade nearby tissues. In order to travel to distant locations in the body, cancer cells must have access to the blood circulatory system. Therefore, the process of angiogenesis, by which new blood vessels are formed, is crucial to cancer cells. By secreting growth factors, cancer cells themselves are able to promote the formation of easily penetrated capillary blood vessels. In this way, they are able to gain access to the circulatory system and travel throughout the body. Cancer cells also need to constantly increase vascularization in the areas surrounding tumor formation in order to provide oxygen and nutrients that will support their continuous growth. Control of the angiogenesis process is consequently crucial for the further development and progression of cancerous tumors [3]. Thus, cancer cells have gained the capabilities to spread throughout the body and sustain their incessant growth.

It is the accumulation of mutations in the multiple cell regulatory systems that enables the cell to gain cell cycle autonomy and continue to divide. The fact that cancer often develops later on in life and the rate of cancer incidence increases dramatically with age attests to the notion that tumorigenesis is a multi-step process that involves the accrual of DNA alterations at multiple sites. This buildup of mutations in a single cell, known as tumor initiation, thereby causes abnormal proliferation and formation of mutant clones. Due to the mutant cell's ability to increase its capacity for proliferation, survival, and metastasis, it has a selective advantage over other healthy cells. The body consequently selects cancer cells over healthy cells in a process known as clonal selection. This process continues throughout tumor development, producing cells that are continuously mutating in order to better themselves in their ability to rapidly grow and increase malignancy. It is because of this selection process that, with time, cancer cells become increasingly aggressive. Thus, it is the accumulation of genetic mutations that consequently leads to the development of cancer [9, 10].

Causes of Cancer

While genetic mutations are held responsible for the unfettered and rampant state of cancer, the question arises as to what initially induces these genetic changes to take place. Some of these genetic alterations are inherited, but most occur sporadically due to the effects of environmental factors over extended periods of time. With inherited gene defects held responsible for only 5-10% of all cancers, environmental agents are accountable for the other 90-95% of cancer incidences [19]. Prior to the attainment of a real understanding of cancer itself, many associations between cancer-inducing substances, termed carcinogens, and the onset of cancer were documented throughout history. Physician Percival Pott, for example, associated the high incidence of testicular cancer in chimneysweepers with their dangerous

occupation in 1775, without any knowledge of mutations [2]. Although it eventually became apparent that specific substances can cause cancer, it was not understood how that happens until the 1970's when researcher Bruce Ames of the University of California recognized the correlation between the cancer-causing ability of certain chemical substances and their mutagenicity [8]. It was only then that it became clear that cancer-inducing substances cause mutations in healthy cells that consequently lead to the development of cancer.

Many environmental agents including radiation, chemicals and viruses damage DNA and induce mutations. Radiation, especially in the form of solar ultraviolet rays, x-rays and gamma rays, is one of the major causes of skin cancer. Radiation also induces other forms of cancer like leukemia, lymphoma, thyroid cancers, sarcomas, lung cancers and breast carcinomas. Approximately 10% of all cancers are caused by both ionizing and nonionizing radiation [19]. Studies indicate that many skin cancers, due to solar UV radiation exposure, exhibit mutations specifically in their p53 tumor suppressor genes [20, 21]. Similarly, chemical carcinogens like tobacco, coal tars and their derivatives, hydrocarbons, aniline, asbestos and others are attributed to causing cancer. Such chemicals can come in the form of air, water and food pollution. In 1964, the US Surgeon General issued a report identifying smoking as the primary cause of lung cancer. Today, it is accountable for 87% of lung cancer deaths and 25-30% of all cancer deaths [19]. National regulations have been setup by the government in order to limit exposure of these substances and ensure the public's safety. Additionally, cancer development can occur due to the introduction of viruses' DNA sequences into the DNA of a healthy cell. Peyton Rous's discovery at the Rockefeller Institute in 1911 linking viruses to cancer in chickens, which earned him the 1966 Nobel Prize, paved the way for the discovery of several viruses capable of inducing cancer in

humans. Long-term infection of hepatitis B or C viruses have been linked to liver cancer, while a form of the herpes virus known as the Epstein- Barr virus can lead to non-Hodgkin lymphoma and nasopharyngeal cancer. Forms of the human papilloma virus (HPV) are known to cause many different forms of cancer including cervical, vaginal and anal cancers, as well as specific head and neck cancers. Lastly, the rising incidence of the human immunodeficiency virus (HIV) in the 1980s led to the understanding that HIV patients have increased risk of developing cancers, including non-Hodgkin lymphoma and Kaposi sarcoma, due to their suppressed immune systems. As of 2012, the World Health Organization's International Agency for Research on Cancer (IARC) has reported more than 100 physical, chemical and biological cancer-inducing substances, many of which society is unfortunately exposed to on a daily basis [2].

Lifestyle factors such as diet and obesity are also linked to the onset of cancer. Clinical epidemiologic studies have shown that diet and obesity are attributed to 14-20% and 35% of all cancers respectively [22]. Although how diet contributes to cancer is not fully understood, specific foods, food additives and cooking methods have been discerned as cancer risk factors. For example, red meat is found to induce cancers of the gastrointestinal tract and other forms due to carcinogenic compounds found in the meat itself as well as the strong cancerous effects of the smoking and curing processes used in its preparation. Similarly, foods high in fat content, trans fat and refined sugar and flour, are found to induce cancer. While exposure to food additives like nitrite preservatives and azo dyes are also associated with carcinogenesis, chemicals from plastic food containers can integrate themselves into food, thereby increasing the risk of both breast and prostate cancers. In addition to diet, obesity, which has become prevalent in modern Westernized societies, is

associated with increased incidences of colon, breast, endometrium, kidney, esophagus, gastric cardia, pancreas, prostate, gallbladder and liver cancer. Although the connection between cancer and obesity is not fully comprehended, researchers have found many relationships between obesity and cancer including their involvement in multiple signaling pathways and the increased presence of neurochemicals, hormones, and growth factors [19]. It has thus become apparent that what a person chooses to eat on a daily basis can impact his susceptibility to cancer.

Due to their prevalence, cancer-inducing substances exist all around and may even be deemed unavoidable. As such, according to the American Cancer Society, one-half of all men and one-third of all women in the United States will develop cancer at some point during their lives [2]. In order to fight this deadly disease, claiming 1 in every 4 deaths in the United States, the refinement of existing treatments alongside the development of new treatments are imperative [1].

Leading Cancer Treatments

The complexity of cancer eludes our brightest scientists in the development of a curable and nontoxic cancer treatment. Many like Hippocrates, known as the "Father of Medicine" and Galen, a 2nd century Roman doctor, believed that cancer was an untreatable disease with no long-term remedy. This philosophy remained prominent for centuries and therefore only limited developments in cancer research were made throughout the ages. Today, many have dispelled this philosophy and are determined to find the cure for cancer. Thus, with the help of recent discoveries and technologies, cancer treatments have developed tremendously. Some of the leading cancer treatments currently implemented include surgery,

radiation, chemotherapy and targeted therapy.

Surgery is one of the oldest forms of cancer treatment that continues to be used today. While cancerous tumors were treated in ancient times by surgical excisions, surgical procedures are currently used to detect and remove localized tumors in the least invasive ways than ever before. With the help of technology, as well as the use of better surgical instruments, surgery towards the end of the 20th century became much more precise and removed far less healthy tissue during the procedure. While tumors of the arm or leg prior to this time required amputation, surgeons became skilled at removing only bone and soft tissue along with the tumor rather than the entire arm or leg. Likewise, due to development of sonography, patients no longer need to undergo exploratory surgery in order to ascertain the exact location of the cancerous tissue, thereby decreasing the risks of undergoing another surgery entirely. With the help of fiber-optic technology, doctors today can view and work inside the body with only small incision sites as a means of entry. This cutting edge technology enables doctors to perform surgical procedures of the abdomen and chest noninvasively via laparoscopic and thorascopic surgery respectively. Similarly, no incisions are required for the use of the endoscope in its removal of tumors from the bladder, esophagus and colon, as it utilizes the natural entryways of the human body. As technology continues to advance, even less invasive surgical techniques are being developed to destroy cancerous cells in the body without the need to excise them. Scalpels and forceps are quickly becoming tools of the past, as lasers are now being used to precisely cut through tissue or vaporize specific cancer cells. Likewise, liquid nitrogen spray can be used in cryosurgery to freeze and destroy abnormal cells, while radiofrequency ablation transmits radio waves to tumors labeled with small antennas in order to induce cellular death [2, 23]. Thus, modern

technologies attempt to help surgery save as much healthy tissue as possible, while still effectively removing the cancerous cells.

Despite attempts of new technologies to reduce the effects of surgery on the rest of the body, significant side effects and risks still exist. During cancer surgery, as with all surgeries, possible complications may arise, including allergic reactions to anesthesia or incessant bleeding. Surgery can also lead to life-threatening problems with other organs, such as the lungs, heart, or kidneys. Subsequent to the procedure, side effects include pain and possible infection at the surgical site. Other more significant problems include lung infections, internal bleeding, and blood clots that form after the surgery [23]. Although surgery remains the best method of treatment available for many types of cancers despite the risks involved, it remains limited in effectively treating only non-metastasized cancer tumors.

Viewed as an improvement to surgery at the time of its development, radiation is another technique utilized to treat localized cancer tumors. Radiation, which was first developed at the end of the 19th century, uses high-energy ionizing radiation in order to control or kill cancerous cells within a specific location in the body. By damaging the genetic material of the cancerous cells, radiation can hinder cancer cells from continuing to divide and effectively kill them. The use of radiation, in the form of X-rays, gamma rays or charged particles, can either damage the cell's genetic material directly or induce the formation of charged atoms or molecules within the cell known as free radicals that will damage the DNA. Due to their diminished ability to repair DNA damage, cancerous cells are more susceptible to radiation than other healthy tissues, which either repair the damage or are forced to undergo apoptosis. Further research is currently being conducted to develop chemical modifiers in order to make the cancer cells more susceptible to the radiation [24].

Yet, because the same radiation used to annihilate cancer cells from the body can actually also cause cancer to develop, radiation, like surgical procedures, needs to contend with the hurdle of not damaging the nearby healthy tissues. Thus, researchers developed new technologies like conformal radiation therapy (CRT) and intensity-modulated radiation therapy (IMRT) in order to precisely locate the tumor position in 3 dimensions and match its exact shape with radiation beams, so as to limit the exposure of other healthy tissues. Other technologies, like the conformal proton beam radiation therapy, use proton beams rather than x-rays because protons, which are small particles of atoms, cause minimal damage to the tissues they pass through but effectively kill the cells at their destination. Still other forms of radiation, like intraoperative radiation therapy (IORT), circumvent damage to healthy tissues by literally moving them out of the way during surgery and administering radiation. Internal radiation, in which a radioactive source is implanted inside or near the tumor itself, and systemic radiation, in which unsealed radioactive sources are administered orally or intravenously in order to target specific cancerous tumors, are other forms of newly developed techniques. Researchers are thus constantly trying to develop new ways to limit the exposure of healthy tissue to radiation [24].

Although radiation is far less invasive than surgery, it too causes many harmful side effects. For example, people who undergo radiation commonly experience physical, mental and emotional fatigue. They also may experience skin problems including redness, irritation, dryness, swelling and blistering, as well as hair loss in the treated area. Radiation of the head and neck or parts of the digestive system may lead to complications with eating and digesting food properly. More severe side effects include the formation of secondary cancers as well as damage to normal tissues or organs, consequently resulting in decreased functional capacity,

scarring, and fluid buildup [24]. Such serious side effects lead one to ask whether radiation treatment is worth the potential side effects.

While surgery and radiation therapies tend to target and treat a specific area in the body, chemotherapy works throughout the entire body as a form of systemic treatment. Chemotherapy, which was first discovered during World War II (1939-1945) in the form of nitrogen mustard, uses strong cytostatic drugs in order to treat cancer. It is the job of cytostatic drugs to inhibit cells that are dividing uncontrollably by effectively impairing the mitosis (M) stage of the cell cycle. Such drugs prevent mitosis via various mechanisms including damaging DNA and impeding the functionality of some of the cellular machinery involved in cell division. Thus, tumors with high growth rates are much more susceptible to chemotherapy treatment as opposed to low growth rate cancers [25].

Today, there are more than 100 chemotherapy drugs used for cancer treatment. Some of the main categories of chemotherapy include alkylating agents, anti-metabolites, anthracyclines and topoisomerase inhibitors. Alkylating agents attach alkyl substituents ($C_nH_{2n+1)}$ to molecules like proteins, RNA and DNA, thereby immobilizing their functional ability, while antimetabolites block RNA and DNA synthesis by substituting the normal building blocks of RNA and DNA. Additionally, anthracyclines interfere with enzymes crucial for the process of DNA replication and topoisomerase inhibitors impede enzymes called topoisomerase that help in the unwinding of the DNA during its replication or gene expression [26]. With the help of Sidney Farber, regarded as the father of chemotherapy, chemotherapy drugs have effectively treated and cured many different kinds of cancers including Hodgkin disease, childhood acute lymphoblastic leukemia (ALL) and testicular

cancer. Even if chemotherapy cannot cure other forms of cancer, it is still successful in instigating long-term remissions [2].

While chemotherapy drugs cause no pain when administered, they do cause several short and long-term side effects. Although it is the goal of chemotherapy drugs to kill cancer cells, they target all rapidly dividing cells and thus, can cause damage to healthy cells as well. Cells that rapidly divide that are most commonly affected by chemotherapy drugs include skin, blood and hair follicle cells as well as cells that line the digestive and reproductive tracts. Damage to these rapidly dividing cells consequently results in the common side effects of chemotherapy drugs. Some of the short-term side effects include nausea and vomiting. Others include slow or rapid hair loss of the head, face, arms, armpits and groin as well as skin problems including redness, itching, dryness and acne. Some chemotherapeutic drugs cause sores in the mouth and throat. The formation of red blood cells, which are cells that carry oxygen from the lungs to the body, are affected, thereby causing shortness of breath, weakness and fatigue. Similarly, chemotherapy can cause a patients' white blood cell count to plummet, making them more prone to infection. The decrease in platelets, which form blood clots that stop the bleeding of cuts and bruises, can cause incessant bleeding from even a small cut. While the reduction of blood cell formation is generally short-term and will be regenerated with time, some chemotherapeutic drugs unfortunately cause long-term damage to the bone marrow. Problems with cognitive functioning as well as emotional changes are also associated with this form of therapy. Additionally, long-lasting fertility problems, peripheral nerve damage, permanent heart damage as well as increased risk of a secondary cancer may arise after treatment with chemotherapy. While new delivery techniques are being studied and new approaches to

target cancer cells specifically are being attempted, the side effects of chemotherapy are still very weighty [27].

In order to decrease the negative impact upon healthy tissues, an entirely new form of therapy known as targeted therapy was developed in the wake of recent cancer discoveries. Although technically considered a chemotherapy, targeted therapy drugs differs from standard chemotherapy in that they are able to do less damage to healthy cells by targeting the inner workings and acquired adaptations of cancer. Rather than damage all rapidly dividing cells, targeted therapy specifically influences cellular processes in cancer cells that control cell growth, division, metastasis and apoptotic evasion. By targeting specific parts of the cell and interfering with particular molecules that are crucial for tumor growth and progression, targeted therapy drugs block the growth and spread of cancer. Some targeted therapies that have been developed include growth signal inhibitors, angiogenesis inhibitors, and apoptosis-inducing drugs. While growth signal inhibitors prevent the auto-stimulation of cancer cells to constantly divide, angiogenesis inhibitors block the formation of new blood vessels, thereby inhibiting the ability of cancer cells to obtain oxygen and nutrients needed to survive. Lastly, apoptosis-inducing drugs aim specifically at cellular molecules that control cell death and survival. New research is underway in this field to develop drugs that target repair molecules in cancer cells that enable them to fix damage caused by cancer treatment. The recent discoveries and insights of the last half a century regarding the genetic alterations of cancer cells have enabled researchers to be successful in developing drugs that target these gene changes [28].

While targeted therapy is expected to be less harmful to normal cells than the aforementioned forms of treatment, there are still side effects and risks that come along with

this form of therapy. Since the targets that the drugs are designed to inhibit in cancer cells are also present in healthy cells, this form of treatment does cause side effects too. Some of the most common side effects include skin problems like severe rashes, dry skin, and itching. This is because skin cells have a lot of growth factor receptors in order to continuously divide and maintain the skin's surface layer, and therefore skin cells are susceptible to this form of drug as well. For this same reason, targeted therapy also results in changes in hair growth involving hair texture coarseness, loss of scalp hair and increased growth of facial hair for both men and women. Hand-foot syndrome (HFS), which is marked by redness, swelling and pain on the palms of the hands and feet, can be caused by angiogenesis inhibitors' damage to small blood vessels. High blood pressure and problems with bleeding, clotting and wound healing may occur due to the effects of angiogenesis inhibitors, leading to severe conditions like bleeding from the stomach and intestine, blood clots, heart attacks and strokes. Each person who undergoes this form of treatment does not necessarily experience every side effect, and most side effects do slowly subside with recovery time. However, targeted therapy is still a new form of treatment and many of its long-term effects are yet to be discovered. Even though this form of cancer treatment tends to cause less severe side effects than standard chemotherapeutic drugs, the side effects are still significant [28].

Nutraceuticals and Antioxidants

Although the cancer death rate has steadily declined over the past two decades largely due to the major developments in cancer treatments, cancer still remains to be the second leading cause of death in the United States after cardiovascular disease [1]. According to the American Cancer Society, cancer is expected to take the lives of approximately 585,720 Americans in the year 2014, leading to 1,600 deaths per day [29]. With such staggering

statistics, the questions that consequently arise are why current cancer treatments are not effectively curing cancer and more importantly, what should be the future direction of cancer treatment research. Unfortunately, the limitations involved with all forms of cancer treatment as well as their side effects largely impact their efficacy. Due to the fact that 90% of all cancer deaths are linked to tumor metastasis, both surgery and radiation remain limited in action due to their inability to treat cancer beyond its localized region [30]. The lifetime dose limits of radiation that cells can tolerate likewise restricts the intensity and sheer number of times radiation can be used as a treatment. Additionally, the many side effects of chemotherapy, radiation and even targeted therapy occur due to their unspecific nature and inability to differentiate between cancerous and healthy cells. This lack of ability can result in harmful effects on healthy tissue that may even lead to the formation of secondary cancers. It is rather ironic that cancer, which is caused by DNA damage, is treated by creating *more* DNA damage in both cancer and healthy cells. While these forms of treatment may be too general in their methods, targeted therapy in its attempt to produce cancer drugs for a single gene, gene product or signaling pathway, may be just too specific. Although in theory, this form of a cancer treatment should be effective, in reality, such treatments are creating very little therapeutic impact due to cancer's complexity. According to extensive research conducted in the last half a century, cancer is caused by the deregulation of as many as 500 different gene products, thus making the prospects of treating cancer via this technique rather grim [31]. Therefore, in their attempt to kill cancer, these unnatural, synthetic forms of treatment may be causing more harm than good regarding the overall health of a person. In an attempt to find similar therapeutic results without the limitations and potentially harmful

side effects, researchers are now working hard to develop natural, nontoxic cancer drugs in the form of nutraceuticals.

The term nutraceutical, which was coined by Dr. Stephen DeFelice, chairman of the Foundation for Innovation in Medicine in 1989, describes the union between nutrition and pharmaceuticals. Nutraceuticals are plant-derived dietary compounds found in the natural environment that reportedly provide health or medicinal benefits. Although the use of natural plant products to treat disease might be viewed as innovative and cutting edge within the realm of cancer treatment, it is by no means new. Plants have been used for centuries to treat and cure diseases. Ancient civilizations were the first to realize the power and healing capabilities of plants, fruits, and herbs. It was common for them to use food as medicine to treat and prevent diseases. Evidence documents the exploration of the medicinal benefits of plant-derived products for thousands of years. The positive therapeutic effects of nutrition were alluded to approximately three thousand years ago when Hippocrates said, "let food be thy medicine and medicine be thy food" [31]. The relationship between health and diet thus continues to be explored today.

While plant-derived products were often used as alternative medicines, today they are the focus of many cancer studies. Biomedical research conducted over the past twenty years indicates that diet plays a critical role in the prevention and progression of cancer [31]. According to the World Cancer Research Foundation, a diet comprised of fruits, vegetables, spices, cereals, pulses and nuts serves as a protective measure against cancer [22].

Mounting evidence displays that many plant-derived nutraceuticals play crucial roles in cancer prevention due to their unique antioxidant nature. An antioxidant is a molecule that prevents cellular damage caused by reactive oxygen species (ROS). ROS are a form of free

radicals that contain oxygen atoms with one or more unpaired electrons in their outermost shell. In an attempt to fill their outermost shell of electrons and gain stability, these ROS become highly reactive molecules. As a result, ROS interact with integral cellular components including DNA, proteins, fats, and other macromolecules, consequently causing a tremendous amount of damage. Examples of ROS include oxygen ions and hydrogen peroxide (H_2O_2). ROS are the natural by-product of metabolic processes that include oxygen. Under normal circumstances, low concentrations of ROS can be extremely beneficial in maintaining a healthy balanced cellular environment. ROS protect the cell against infection, induce apoptosis and mediate cell signaling [32].

However, environmental agents including many carcinogens like ionizing radiation, tobacco, alcohol and pollution can induce increased levels of ROS that can be severely detrimental to the cell. Increased levels of ROS can lead to a state of oxidative stress, in which there is an imbalance between ROS production and the body's natural antioxidant defenses that are responsible for the detoxification of these damaging molecules and repair of the resulting damage [33]. The increased levels of cellular ROS can cause deleterious modifications to DNA, proteins and lipids that can directly or indirectly cause mutations to occur, leading to cellular disarray. An accumulation of these mutations can lead to numerous human diseases including cardiovascular disease, diabetes and cancer [32]. While there are varying cellular and molecular events that are involved in the transformation of healthy cells to cancerous cells, as previously discussed, researchers believe oxidative stress plays a key role in the overall modulation of the carcinogenesis process. ROS damage such as DNA double-stranded breaks as well as modifications to the DNA itself like deletions, insertions, and translocations, can essentially result in the inactivation of tumor suppressor genes and/or

the activation of oncogenes, thereby causing cancer. Thus, research proves that antioxidants, including those derived from natural products, can effectively neutralize the reactive oxygen species by accepting their outermost electrons and consequently quenching ROS activity. By reestablishing stability within the cell and preventing DNA damage, antioxidants can inhibit ROS-mediated carcinogenesis [34].

Antioxidants are found in an array of different foods including wild strawberries, blackberries, citrus fruits, walnuts, fish and green tea, among others. Such foods have been found to exert anti-cancerous effects against colon, lung, renal, breast and colorectal cancers. Thus, it is believed that nutraceuticals can serve as a chemopreventative measure against cancer subsequent to carcinogen exposure [34].

Grape Seed Extract Experiment

The grape is a form of antioxidant that is the focus of many recent cancer studies. The grape, known scientifically as the *Vitis vinifera*, is native to Southern Europe and Western Asia [35]. Although grapes have grown wild since prehistoric times, archeological records suggest that the cultivation of this valuable horticultural fruit began in Asia as early as 5,000 BCE. References to the grape in both biblical texts and Egyptian hieroglyphics attest to the reverence of this phenomenal fruit. Ancient Greeks and Romans also respected grapes for their winemaking ability. Although grapes were naturally indigenous to certain continents, travel and navigation eventually led to the transportation of the grape across the world, making it readily accessible to all [36]. While the grape vine is rich in flavonoids, polyphenols, anthocyanin, proanthocyanidins, procyanidins and trans-resveratol, grape seed extract (GSE) is a complex mixture of polyphenolic components termed proanthocyanidins composed primarily of gallic acid, catechin, and epicatechin. GSE is prepared from grape

seeds and is a by-product of the grape juice and wine industries. Known for its varying health benefits, GSE is sold in the United States as an over-the-counter dietary supplement in both tablet and capsule forms. Recent scientific studies prove that the GSE is involved in cardioprotective, antidiabetic, anti-microbial and anti-viral activities. Most importantly, research conducted using both *in vitro* and *in vivo* models displays the chemopreventative effects of GSE against various cancers due its polyphenolic phytochemical composition with antioxidant effects [37]. Studies show that the antioxidant nature of GSE protects cells again ROS-mediated damage in human colon adenocarcinoma cells, thereby leading researchers to conclude that GSE suppresses the formation of human colorectal cancer via its ability to inhibit the generation of oxidative stress [34]. Thus, the antioxidant nature of GSE enables it to effectively exert preventative effects against various forms of cancer.

The numerous epidemiological studies regarding the chemopreventative nature of GSE has spurred interest in the use of this natural substance not only to prevent cancer, but also to treat it. Several recent studies demonstrate the toxic nature of GSE against varying forms of cancer cells in both *in vitro* and *in vivo* models. I personally had the opportunity to participate in a study involving *in vitro* research examining the use of GSE as a cancer-treating product. Under the guidance of Dr. Jeffrey Weisburg, I worked with a group of students who tested the effects of GSE on oral cancer cells called oral squamous cell carcinoma (OSCC) in comparison to healthy cells.

OSCC, one of the most common forms of head and neck cancers, is considered to be a leading malignancy in the United States, claiming more than 20,000 lives annually [37]. Unfortunately, this form of cancer has proven difficult to treat due to its relative resistance to chemotherapeutic drugs, ability to metastasize and sensitive location. As a result, despite

recent advances in many forms of treatment, the prognosis of OSCC has predominantly remained the same and has not improved [38]. Thus, the development of new strategies to treat this form of cancer is of urgent need.

Prior to experimentation, we knew that GSE selectively killed tumor cells as compared to normal cells and that the induction of apoptosis was not due to the normal generation of ROS, specifically H_2O_2, but rather was initiated by GSE itself. OSCC cells were obtained and incubated alongside their healthy gingival fibroblast counterparts. Both cell types were exposed to specific amounts of grape seed extract as well as a fluorescent dye for subsequent analysis. In order to detect the effects of GSE on the cell types, a Guava flow cytometer was utilized. A flow cytometer is a form of biotechnology that allows for the analysis of cells at different stages of the cell cycle based on their nuclear DNA content. As cells continue through the cell cycle, their DNA content varies in size due to processes like DNA replication and ultimate segregation. This large laser-based instrument is capable of detecting the DNA content of every individual cell by measuring the intensity of fluorescent dye bound to its DNA. Thus, in order to determine the proportion of apoptotic cells within the heterogeneous population as well as the stage of the cell cycle in which apoptosis occurred, the cells were subject to this form of analysis [3]. Initially, we saw that GSE caused cell cycle arrest of the OSCC cells at the G2/M phase of the cell cycle, while the healthy cells remained viable. We likewise noted that while GSE did generate ROS, it did not generate huge amounts as we had seen with other nutraceuticals like the pomegranate. Thus, GSE appeared to kill the cancer cells more by the agent itself and not as much by the generation of ROS. However, we were unable to replicate these findings and unfortunately could not further confirm these results.

While the results of our laboratory experiment were inconclusive, additional experiments conducted at other research facilities tested the therapeutic use of GSE on OSCC cells. Their research indicates that GSE does in fact stimulate apoptotic cellular death of OSCC cells during the G2/M phase of the cell cycle. Not only did their results reflect our hypothesis, but studies also attribute such apoptotic death to the accumulation of intracellular ROS and consequent irreparable DNA damage caused by GSE itself. By inducing further oxidative stress on the cell in the form of DNA double stranded breaks, GSE effectively triggers cellular DNA damage sensors leading to cell cycle arrest and/or apoptotic death. Simultaneously, GSE also decreases levels of crucial proteins of DNA repair pathways resulting in the inability of the cancerous OSCC cells to repair the genetic damage. By hindering the cancer cells' ability to repair DNA damage, GSE makes the OSCC cells much more susceptible to the toxic effects of GSE. Other studies show GSE's ability to inhibit the cellular invasion of OSCC by targeting growth factors and transcription factors like NF-κ B. In analyzing the cytotoxic nature of GSE, recent studies display the selective nature of GSE due to its ability to exert apoptotic potential on OSCC cells without inducing damage upon the healthy fibroblast cells [35]. Yet, how does GSE cause damage in cancer cells, while simultaneously preventing DNA damage of healthy cells? It appears almost as though GSE serves completely opposite roles when acting in cancer cells versus healthy cells. While GSE serves as an *anti*oxidant by quenching ROS activity in healthy cells, it ironically serves as a *pro*oxidant in cancerous cells by increasing the formation of ROS. Although the reason for this unique nature of GSE and other forms of antioxidants remains unknown, some researchers speculate that it is due to the higher levels of ROS found in cancer cells as opposed to normal cells. In this way, GSE serves as a selective and differential cancer

treatment that can effectively kill cancer cells, without causing harmful effects on healthy cells. Likewise, *in vivo* studies show that GSE is well tolerated as a dietary supplement with no consequent side effects. It is thus considered safe for human consumption. Although the obtained results shed positive light on the use of GSE as an effective adjuvant cancer treatment, much remains to be discovered about the possible long-term benefits of GSE [39]. Therefore, nutraceuticals like GSE are not only utilized to prevent cancer formation, but may also be used to kill and treat cancer via a selective and nontoxic form of treatment.

Conclusion

The scientific world has come a long way in its understanding of cancer and how to treat it. A disease once theorized to be caused by the excess of black bile, degeneration of lymph, budding blastemas, chronic irritation, trauma and infectious disease is now known as a disease of cellular deregulation and uncontrolled growth. Current comprehension as to the internal mechanisms of cancer gives us the hope that great progress is underway towards the improvement of existing treatment techniques and development of new forms of therapies that target the specific characteristics of cancer. Yet, in the wake of new discoveries and innovations, researchers are starting to realize that treatment of this deadly disease may actually lie within the ancient wisdom of the past. After all, it was Hippocrates who once said, "[i]f we could give every individual the right amount of nourishment … we would have found the safest way to health" [31]. Perhaps today's society can tap into the healing capabilities of fruits, vegetables and herbs just as the ancient civilizations once did. While further research is needed to determine the effectiveness of GSE and other plant-derived dietary compounds in treating cancer, the use of nontoxic nutraceuticals holds much hope and promise in the eyes of researchers. Thus, it is possible that nutraceuticals like GSE may serve as one more option in our arsenal against cancer after all.

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