The Cell Biological Basis of Cancer

A thesis submitted to the Miami University
Honors Program in partial fulfillment of the
requirements for University Honors with Distinction

By
Ankur K. Patel
Miami University
Oxford, Ohio
May 2012

ABSTRACT

THE CELL BIOLOGICAL BASIS OF CANCER

By ANKUR K. PATEL

This literature review explores the current knowledge of cancer in humans on the cellular level. The review will delve into the critical points on the topic of cancer, with a focus on the biological processes and attributes that characterize cancer cells. These critical points include the generation of cancer, the effect of cancer on a cell's function, proliferation, tumor growth and development, avenues for preventing the spread of cancer cells as well as treating cancerous conditions, and exploring the use of model organisms and systems in biomedical research to study the disease. Specifically, the review first discusses the genetic instability that characterizes cancer. Next, a discussion of oncogenes and tumor suppressor genes reveals well-known mutational pathways by which cancerous conditions arise. Disruption of signaling pathways, as well as the mechanisms of angiogenesis and invasion that characterize malignancies, are also discussed. Finally, the review will look into cancer therapy and the role that biological and computational models play in elucidating cellular abnormalities that can be exploited in pharmacological treatment.

The Cell Biological Basis of Cancer

by Ankur K. Patel

Approved by:

Joyce Fernandes, Advisor
Dr. Joyce Fernandes

, Reader
Dr. David Pennock

Katia del Rio-Tsonis, Reader
Dr. Katia Del Rio-Tsonis

Accepted by:

, Director,
University Honors Program

Acknowledgements

I would like to thank my thesis advisor, Dr. Joyce F. Fernandes, whose patience and guidance made this project possible. I am also grateful for my readers Dr. David Pennock, whose passion for science motivated me every step of the way, and Dr. Katia Del Rio-Tsonis, whose expertise ensured this project would be completed with the utmost quality.

Lastly, I would like to thank my parents for their support in all of my endeavors.

A.K.P.

Table of Contents

List of Figures

CHAPTER ONE

INTRODUCTION

Cancer is a biological disease exemplified by a complex interaction of genetic and environmental factors that coordinate carcinogenesis (Brennan, Offiah, McSherry, & Hopkins, 2009). It is one of the most serious diseases that affect the global population (Zhang, Pan, Cobb, & Anderson, 2007), accounting for 13% of all deaths in 2008 (Cancer, 2012). Cancer is a disease characterized by disruptions in normal cellular functions (Hanahan & Weinberg, 2000; Kreeger & Lauffenburger, 2010). Mutagenic events that affect a cell's genetic material are known to cause deregulation of the pathways that govern the cell's most fundamental processes (Kreeger & Lauffenburger, 2010) (see Figure 1). Specifically, the onset of cancerous conditions is due to an accumulation of multiple genetic mutations which lead to the deregulation of signaling pathways that control cell growth, apoptosis, and DNA repair (Bild, et al., 2006; Kreeger & Lauffenburger, 2009). Once these pathways are transformed to remove the effects of cellular controls, cancer cells are able to proliferate and grow in the absence of normal restrictions.

There are certain, well-defined means by which normal cells transform into cancerous ones. Oncological research in the latter half of the century has indicated that almost all cancerous cells display a relatively few number of acquired molecular, biochemical, and cellular features that result from alteration of key pathways (Kreeger & Lauffenburger, 2010). This may seem like a strong generalization considering that there are over 100 unique

types of cancer, not including further subtypes of malignancies that have been identified (Hanahan & Weinberg, 2000). However, it is not such a stretch when realizing that the field of cellular biology emphasizes the similarity between all types of living cells. Mammalian cells, for example, are all alike in their mechanistic regulation of normal cellular processes, such as division, differentiation, and programmed cell death (apoptosis). Therefore, it is unsurprising that there are certain rules that govern the transformation of normal human cells to cancerous ones (Hanahan & Weinberg, 2000).

The universal nature by which cancer occurs is further evidenced by the ongoing identification of specific mutation sites on the human genome that are found in many forms of cancer. Researchers are also attempting to classify the genes crucial to carcinogenesis into specific classes by studying cancerous phenotypes in experimental models (Hanahan & Weinberg, 2000). Two specific gene classes are predominantly discussed – oncogenes and tumor suppressor genes. Cancer is caused by accumulation of activated oncogenes and inactivated tumor suppressor genes that subsequently confer the abnormal attributes that characterize cancerous cells (Tran, et al., 2008). Proto-oncogenes, which are precursors to oncogenes, are altered by dominant mutations, consequently conferring a gain of function such as proliferation to a normal cell. These specific genes which are known to enhance the proliferative capability of cells are termed oncogenes in their mutated form. Tumor-suppressor genes, on the other hand, are altered and inhibited (Tran, et al., 2008).

As a result of mutations in both classes of genes, fundamental cellular processes such as metabolism, growth, proliferation, and death are altered in cancer cells (Hanahan & Weinberg, 2000; DeBerardinis & Cheng, 2008). These altered pathways give cancerous cells

the ability to grow in number, forming tumors at the local site (Blagosklonny, 2003). Cells are able grow uncontrollably by avoiding the regulatory effects of the multiple mechanisms present in a cell that are controlled by key proto-oncogenes and tumor suppressor genes (Macleod, 2000). Local tumors become carcinomas when they travel and invade foreign tissues in the body (Blagosklonny, 2003).

Recent attempts to organize cancer progression into distinct, well-defined categories have been made. Tran, et al. (2008) state that certain pathological features characterize cancer; specific developmental steps such as independent proliferation, immortalization, inhibited differentiation, induced growth in the vascular network (angiogenesis), invasive capability, resistance to apoptosis, and genomic instability are mentioned. Hanahan and Weinberg (2000) catalog all cancer genotypes as the accumulation of six fundamental alterations in cellular physiology. They propose that perhaps all types of human cancers depend on alterations to cellular physiology that confer autonomy from outside growth signals, insensitivity to antigrowth signals, mechanistic avoidance of apoptosis, boundless proliferative potential, angiogenesis, and invasion to foreign tissues through capillary walls and basement membranes (metastasis) (Jayshree, Sreenivas, Tessy, & Krishna, 2009). Further, Zhang, et al. (2006) outline cancer as a disease state conditioned on five major developmental steps; initiation, promotion, malignant conversion, progression, and metastasis. All of these models display some similarities in the features of cancer development. For example, each model agrees on the ideas of the spread of to foreign tissues. In general, excessive proliferation is perhaps the most often associated phenotype with cancer progression (Kreeger & Lauffenburger, 2010).

Although the aforementioned cancer models have similarities, the path that is taken during oncogenesis is highly varied (Hanahan & Weinberg, 2000). Cancer in general is characterized by a heterogeneous pathology (Brennan, Offiah, McSherry, & Hopkins, 2009; Kreeger & Lauffenburger, 2009). For example, mutagenesis of specific oncogenes and tumor suppressor genes may occur early in some tumor progression pathways and late in others (Hanahan & Weinberg, 2000). Hanahan and Weinberg (2000) state that although all cancers can be characterized by the acquisition of a set of hallmark capabilities, the means by which those traits are acquired vary, both mechanistically and even chronologically (see Figure 2). The order in which cancerous traits are acquired may contrast between the variety of cancer types and subtypes that have been identified, and there is even wide variation in capability acquisition between tumors of the same type. Furthermore, some progressions of metastasis may require a different number of developmental steps, as specific genetic events may simultaneously confer more than one capability to a cancerous cell (Hanahan & Weinberg, 2000).

CHAPTER TWO

CELLULAR STABILITY

It is well known that cancer is caused by changes in the human genome that enhance cellular proliferation through alteration of normal pathways and mechanisms. However, this process does not occur overnight. In fact, most solid human tumors are not detected until 20 years after the initial carcinogenic exposure that lead to the cancerous conditions (Loeb, Loeb, & Anderson, 2003). This is because the genome of normal cells is maintained through the actions of multiple mechanisms and checkpoints. Normally, random mutations of a cell's genetic material are offset by cellular machinery that maintains the normal sequence of nucleotides (Loeb, Loeb, & Anderson, 2003). Therefore, the progression of cancer is reliant on an equilibrium that is shifted towards mutagenic changes.

A non-cancerous cell maintains its genomic material in a variety of ways throughout the cell's life processes. Cellular DNA is subjected to many processes including replication and transcription, and cells have an array of DNA repair mechanisms that help to restore the normal nucleotide sequence in the event of mutations (Loeb, Loeb, & Anderson, 2003). In non-cancerous cells, DNA replication is exceptionally accurate, with an overall error rate of one base misalignment for every billion nucleotides that are polymerized (Loeb, Loeb, & Anderson, 2003). This accuracy can be explained by many factors, most notably by the functioning of DNA polymerase, the enzyme that constructs the identical strand of DNA. First, the presence of nucleotide discrimination at the active site of DNA polymerase

prohibits mismatching of base pairs. The large free energy difference between matching and mismatching base pairs further ensures that the replicated strand is constructed in a perfectly complimentary manner to the original DNA template. DNA polymerase also functions in correcting mismatches after the new strand is generated; in a phenomenon referred to as proof-reading, excision of incorrectly placed nucleotides immediately after polymerization helps to further enhance replication accuracy (Loeb, Loeb, & Anderson, 2003). The importance of precision in the functioning of DNA polymerase has been studied by observing the effect of a dysfunctional polymerase in a cell. Loeb, Loeb, and Anderson (2003) show that swapping wild-type genes that code for DNA polymerase with mutated alleles leads to the expression of an error-prone polymerase that lacks proofreading activity, which is shown to induce epithelial cancers in mice. Mutations in DNA polymerase-expressing genes that decrease base discrimination without affecting catalytic activity are considered to be the most potent alterations to DNA polymerase activity (Loeb, Loeb, & Anderson, 2003).

The functioning of DNA polymerase is merely one example of how a cell maintains the correct nucleotide sequence. When DNA becomes damaged, cells also possess mechanisms to limit the overall effect of the damaged DNA and to prevent daughter cells from inheriting dysfunctional genetic material (Kreeger & Lauffenburger, 2009; Loeb, Loeb, & Anderson, 2003). Cells carry an assortment of repair enzymes and DNA monitors that function to monitor many cellular processes, most notably mitosis, or cellular division (Hanahan & Weinberg, 2000). The activation of these checkpoint pathways can result in arrest of the cell cycle, allowing DNA repair mechanisms time to activate and function.

Certain checkpoint pathways can also trigger apoptosis when DNA damage is deemed too extensive to fix (Loeb, Loeb, & Anderson, 2003). Therefore, through a complex array of many DNA monitoring and repair enzymes, as well as through mechanisms such as replication that occur with high accuracy, normal cells' unceasing maintenance of genomic integrity ensures that the DNA sequence remains immaculate. With all the cellular mechanisms that function in sustaining the correct nucleotide sequence, mutations are rare events when considering the length of a human life (Hanahan & Weinberg, 2000).

CHAPTER THREE

GENETIC INSTABILITY AND CANCER

However, although mutation events seem to be unlikely, multiple mutations are known to occur in tumor cells (Hanahan & Weinberg, 2000). Researchers have found that in some human cancers, tumor cells average approximately 10 mutations that can occur in each of several hundred genes (Stites & Ravichandran, 2009). This is in part due to the fact that damage to cellular DNA can be a continual and process caused by a variety of mutagens (Loeb, Loeb, & Anderson, 2003). Reactive metabolites and certain environmental species are known to induce an alteration in the structure of DNA, and these chemical alterations of the genomic material can lead to mutations and cancer. Carcinogens may directly cause DNA damage, giving rise to mutations or other adverse chromosomal events, by generating harmful reactive oxygen species that are known to alter DNA. Furthermore, carcinogens are also implicated in epigenetic modifications - changes in gene expression that leave the genetic material unchanged (Marsit, et al., 2006). The process of tissue repair, which involves inflammation and reparative cellular proliferation to replace damaged cells, generates reactive oxidative species as well. DNA alteration from oxidative damage can alter 10,000 nucleotide bases per cell per day (Loeb, Loeb, & Anderson, 2003). The variety of methods by which genetic damage can occur increases the possibility of mutagenesis.

As previously mentioned, DNA damage can occur through a variety of mutagenic events. However, cancer progression is usually not possible with just a single mutagenic

event (Loeb, Loeb, & Anderson, 2003; Tran, et al., 2008). Rather, cancer development is an intricate process that hinges on multiple mutageneses (Bild, et al., 2006). This is evidenced by that fact that there is an exponential increase in the incidence of tumors as a function of age (Loeb, Loeb, & Anderson, 2003), which implicates the role of continuing mutagenesis in accumulating damage to DNA and cellular machinery. Further proof of rate-determining mutagenic events in the formation of cancer has been found in experiments that have transformed rodent cells in culture to study cancer. These experiments show that at least two genetic changes are necessary for tumor formation, and studies involving human cells show that additional changes in the genetic code are required for human cancers (Loeb, Loeb, & Anderson, 2003).

The idea that cancerous genotypes are characterized by accumulated mutations in the genetic material has led to several hypotheses. Loeb, Loeb, and Anderson (2003) propose the mutator phenotype hypothesis, claiming that malignant phenotypes arise from mutations in genes that, in their normal state, maintain genetic stability. The affected genes normally function in controlling the accuracy of DNA replication and repair, maintaining the original nucleotide sequence, and regulating damage checkpoints and cellular responses such as apoptosis. It is argued that a normal mutation rate cannot account for the large number of mutations that are often found in tumor cells. The mutator phenotype hypothesis proposes that an initial mutation that consequently increases the mutation rate may account for the large volume of genetic alterations seen in cancerous cells. The hypothesis suggests that mutations in genes coding for proteins involved in maintaining genetic stability would prevent further mutations from being prevented or fixed, initiating a cascade of dangerous

mutagenesis throughout the genome. Cells are turned cancerous after accumulating mutations, and as cancerous cells proliferate, there are rounds of cellular selection that select for cells with mutations that confer advantages in growth. (Loeb, Loeb, & Anderson, 2003).

Other propositions to explain the volume of mutagenic events in cancer have been made. One proposal claims that accumulation of a large number of genetic alterations by cancerous cells may be detrimental, as most mutations are detrimental in themselves (Loeb, Loeb, & Anderson, 2003). Another proposal points to aneuploidy, a condition characterized by cells possessing an incorrect number of chromosomes, as the initiator of malignancy; yet another model emphasizes the singular role of clonal selection and expansion while simultaneously claiming that increases in the mutation rate is still unable to mathematically account for the thousands of mutations in colon cancer cells. While the true underlying mechanism by which mutations accumulate has yet to be determined, it is agreed that cancer's progression depends largely on genetic alterations that confer phenotypic modifications and advantages to cancerous cells in their biochemical processes (Loeb, Loeb, & Anderson, 2003).

CHAPTER FOUR

ONCOGENES

As mentioned earlier, oncogenes have a significant role in the induction and maintenance of cancerous conditions in the body (Tran, et al., 2008). Activation of oncogenes requires dominant mutations of the normal form of the gene, termed a proto-oncogene. They can be activated with alteration of a single allele because the activity of the non-mutated allele may be insufficient to sustain normal cellular function (Lu & Bast, 2009). Mutation of oncogenes, or oncogene activation, can confer many different capabilities to a cancerous cell since the specific role of an oncogene may vary (Jones & Thompson, 2009). Oncogenes are so sufficiently involved in tumor formation and maintenance that some tumors actually require oncogenic activation for prolonged sustenance (Tran, et al., 2008). In a phenomenon known as oncogene addiction, cancerous cells rely on oncogenes and their products to play their specified role in order to function and survive (Jones & Thompson, 2009). For example, oncogenic addiction may force cancer cells to rely on a certain metabolic pathway for growth (Jones & Thompson, 2009).

Ever since the function of c-Src was elucidated (the first proto-oncogene to be described), the discovery of many more oncogenes has revealed the variety of roles they play in cancer development and the wide variety of cancer types that rely on oncogenes (Ma & Adjei, 2009). For example, various oncogenes participate in inhibiting apoptotic pathways (Macleod, 2000; Jayshree, Sreenivas, Tessy, & Krishna, 2009), while other oncogenes

promote tumorigenesis by negatively inhibiting genes that control cellular differentiation (Zhang, Pan, Cobb, & Anderson, 2007). Oncogenes are able to alter specific mechanisms by imitating signals for normal growth, and the discovery of specific oncogenes has led to an appreciation of how cancer cells seize signaling pathways used by growth factors to stimulate proliferation (Hanahan & Weinberg, 2000). Additionally, oncogenic activation is essential for cellular immortalization, and conferring the overall malignant phenotype in HPV-caused cancers for example (Jayshree, Sreenivas, Tessy, & Krishna, 2009). Oncogenes have also been implicated in altering cellular metabolism to support proliferation (Jones & Thompson, 2009), and studies over the last decade have revealed the intricate connections between oncogenic activation and altered glucose and glutamine metabolism in tumors (DeBerardinis & Cheng, 2010). For example, researchers have observed that tumor cells in cancer-induced mouse models display high rates of glucose consumption and lactate secretion as a result of oncogenic activation. Scientists have termed this phenomenon as the Warburg Effect (DeBerardinis & Cheng, 2010).

A particular proto-oncogene called c-Myc is known to code for proteins that regulate multiple metabolic pathways crucial to growth in non-cancerous cells and cancerous cells, for example, in lung tumors (Jones & Thompson, 2009; Zhang, Pan, Cobb, & Anderson, 2007). In non-cancerous cells, a transcription factor called Myc regulates many processes that regulate cellular growth and proliferation (Tran, et al., 2008), as well as entry into the cell cycle (Zhang, Pan, Cobb, & Anderson, 2007). Myc is normally stimulated by growth factor binding. In a mutated form, oncogenic c-Myc is overexpressed (Tran, et al., 2008), resulting in increased rates of glycolysis and expression of enzymes that function in nucleotide and

amino acid metabolism (Zhang, Pan, Cobb, & Anderson, 2007), thereby altering specialized biosynthetic activities in a way that favors cell division and cancer growth (Zhang, Pan, Cobb, & Anderson, 2006; Tran, et al., 2008). One function of mutated Myc is providing cancerous cells with a rich supply of NADPH, so that cells can maintain high levels of anabolic synthesis, which leads to cancerous conditions as cells grow uncontrollably (Jones & Thompson, 2009).

Tran, et al. (2008) re-examined two classic examples of oncogenes, the aforementioned Myc and K-Ras. Ras mutations have been found in over 20% of human cancers (Ma & Adjei, 2009). The Ras family of genes encodes for proteins that function in transmitting signals from receptors to downstream regulators of survival and growth (Blagosklonny, 2003; Tran, et al., 2008). Ras genes are extremely crucial in cancer development (Ma & Adjei, 2009), as mutated forms of Ras genes result in a higher prevalence of Ras signaling proteins (Bild, et al., 2006; Stites & Ravichandran, 2009; Tran, et al., 2008). Ras proteins have been found to be downstream of many receptors associated with cancer and upstream of various signaling pathways also associated with cancer (Kreeger & Lauffenburger, 2009; Stites & Ravichandran, 2009).

K-Ras is one of three genes that codes for Ras protein signals, and is the most commonly mutated Ras gene in cancers (Ma & Adjei, 2009; Stites & Ravichandran, 2009). K-Ras is generally thought to function in stabilizing Myc, and the two cellular molecules in their mutated forms are thought to cooperate to stimulate tumorigenesis (Tran, et al., 2008). To study the level of cooperation of and oncogenic addiction to mutated Myc/K-Ras in mice lung tissue and lymphocytes, Tran, et al. (2008) used dual (Myc and K-Ras) oncogenic

activation and individual (Myc or K-Ras) activation to induce cancer in mice, as well as dual and individual *inactivation* to induce tumor regression. Lymphomas were induced by individual Myc mutagenesis, by individual K-Ras mutagenesis, and by dual Myc/K-Ras mutagenesis; tumor regression upon inactivation of either or both oncogenes (Tran, et al., 2008) was also observed. However, different results were reported for lung tumor regression. Inactivation of Myc failed to completely regress lung tumors induced by mutated Myc, while lung tumors induced by K-Ras mutagenesis and K-Ras/Myc dual-mutagenesis regressed completely upon inactivation of either or both oncogenes. Only partial regression was observed with Myc inactivation, suggesting that lung tumors do not depend on Myc for tumor maintenance (i.e., lung tumors are not oncogenetically addicted to Myc). Subsequent findings revealed that K-Ras mutagenesis and inactivation is rate-limiting and dominant in the induction and regression of lung tumors, respectively (Bild, et al., 2006; Tran, et al., 2008). The results of the study also suggest that K-Ras and Myc have different "cooperation levels in different tissues (Tran, et al., 2008); researchers observed that Myc and K-Ras fail to cooperate induce tumorigenesis at a more accelerated pace than either oncogene individually in lung tissue, but significant cooperation is present during tumorigenesis in lymphocytes. The results of this study (see Figure 3) show the variation of oncogene function in different tissues and setting (Tran, et al., 2008), while simultaneously showing how cancer cells seize normal signal transduction pathways to stimulate proliferation through the function of oncogenes (Hait, 2009).

CHAPTER FIVE

TUMOR SUPPRESSOR GENES

Much like oncogenes, mutations in tumor suppressor genes also lead to the deregulation of cellular signaling pathways, which eventually leads to changes in gene expression (Bild, et al., 2006). In non-cancerous cells, tumor suppressor genes function in inhibiting the attributes that characterize cancer growth. These tumor-inhibiting functions of specific genes are well-documented, and labels such as 'gatekeepers, caretakers, and landscapers' have been used to describe the role of tumor suppressor genes (Macleod, 2000). Some tumor suppressor genes code for proteins and enzymes that directly monitor the integrity of the genetic material by repairing DNA damage; others function in regulating the extracellular microenvironment around cells (Macleod, 2000). Generally, tumor suppressor genes have roles in prohibiting cellular growth independent of growth signals, insensitivity to anti-growth signals, avoidance of apoptosis, acquisition of limitless replication, and metastasis and angiogenesis (Lu & Bast, 2009). It is easy to see then how tumor suppressor genes are important in prohibiting the phenotypes of cancer; cells that display abnormal growth rates and loss of apoptotic regulation have been observed to transform into cancerous cells (Zhang, Pan, Cobb, & Anderson, 2007). Much like in the case of proto-oncogenes, tumor suppressor genes play various roles in maintaining normalcy in cells. Mutations of these genes lead to the classical conditions that characterize cancer.

One manner in which most tumor suppressors are different than proto-oncogenes is the method by which each is altered to promote cancerous phenotypes. While proto-oncogenes are altered to become active and confer a new function, tumor suppressor genes are altered to become inactive and confer a loss of function. Further, unlike proto-oncogenes, both alleles of tumor suppressor genes must be altered to cause the loss of function. Mutation of the first allele simply serves as a predisposition to tumor formation, while mutagenesis of the second tumor suppressor allele results in tumor initiation (Macleod, 2000). However, while a single non-mutated tumor suppressor gene allele is able to inhibit the malignant phenotype (Lu & Bast, 2009), recent studies have shown that simple epigenetic modification of a single allele of a tumor suppressor gene may be sufficient in inducing tumorigenesis and deregulating cellular proliferation if the mutated tumor suppressor gene allele gets deleted (Lu & Bast, Jr., 2009; Ma & Adjei, 2009). This form of genetic modification, often called epigenetic silencing, is like turning a gene on or off in regards to whether or not that gene will be expressed in a cell (Ma & Adjei, 2009).

A well-known 'caretaker' tumor suppressor gene that is altered in cancer formations is the p53 tumor suppressor gene (Hanahan & Weinberg, 2000; Zhang, Pan, Cobb, & Anderson, 2006), which encodes for the nuclear p53 protein (Lu & Bast, 2009). Researchers have estimated that at least half of all human tumors contain cells that have defective checkpoint pathways due to mutated p53 (Kreeger & Lauffenburger, 2009; Loeb, Loeb, & Anderson, 2003). In fact, genetic alteration the p53 gene is the most common genetic modification in ovarian cancer (Lu & Bast, 2009). Referred to as a 'guardian of the genome' (Macleod, 2000), p53 is a transcription factor (Jones & Thompson, 2009) whose main role is

to prevent genetic instability by regulating transcription of several genes that function in the cell cycle (Macleod, 2000). A variety of stimuli, such as DNA damage, hypoxia, and oxidative stress can lead to p53 activation via post-translational modifications to p53 (Hanahan & Weinberg, 2000; Jones & Thompson, 2009); the specific gene transcription that is carried out by activated p53 depends on the context and degree of the stimulus (Jones & Thompson, 2009). Cells respond to p53-induced alterations in genetic expression by arresting progress in the cell cycle, senescence, differentiation, or activating the apoptotic cascade (Lu & Bast, 2009) if the damage is deemed excessive (Hanahan & Weinberg, 2000). Normal levels of p53 in a cell fluctuate in response to stress, and computational models have shown the feedback circuits present in networks that monitor DNA damage help to regulate p53 levels (Kreeger & Lauffenburger, 2010)

The tumor suppressor gene that codes for p53 is often altered by a point mutation in one allele, and chromosomal deletion of the other allele (Lu & Bast, 2009). In most cases, this leads to genetic alterations in the DNA-binding domain of p53 (Lu & Bast, 2009). It has also been shown that via a dominant-negative effect, mutant p53 can form a complex with wild-type versions of the protein, thereby inhibiting normally-functioning p53 protein by causing an inactivating conformational change of its DNA-binding domain (Lu & Bast, Jr., 2009; Macleod, 2000). Cancer progression via dysfunctional p53 is further enhanced, notably in ovarian cancer, by overexpression of mutated p53 (Lu & Bast, 2009).

CHAPTER SIX

TUMOR GROWTH AND ANGIOGENESIS

The aforementioned mutations in oncogenes and tumor suppressor genes confer crucial alterations in cellular growth. As cells accumulate a succession of genetic mutations, they are selected for due to advantageous growth attributes (Blagosklonny, 2003; Hanahan & Weinberg, 2000). There is direct selection for cells that display growth independent of growth signals and insensitivity to apoptotic signals (Blagosklonny, 2003). It is important to note that cells in a tumor still undergo apoptosis, albeit at a reduced rate relative to the rate of proliferation. The number of cells in a tumor significantly under represents the number of cell generations that were required to reach that number (Hanahan & Weinberg, 2000). However, tumors are able to grow because cancer cells are able to shift the equilibrium between apoptosis and proliferation to the side that favors excessive growth (Kreeger & Lauffenburger, 2010).

Growing tumors must be able to meet the increased energetic demands of cancer cells as they undergo excessive proliferation. The development of hypermetabolic abilities in cancer cells, via the mutagenic pathways discussed earlier, allows cancer cells to produce lipids, proteins, and nucleic acids at a higher rate (DeBerardinis & Cheng, 2010). Specifically, high rates of glycolysis allow the levels of anabolic growth required by cancer cells (Jones & Thompson, 2009). Cancer cells may also activate autophagic pathways and use degradation products to fuel growth (Jones & Thompson, 2009). This reprogramming of cellular

metabolic pathways confers a further selective growth advantage, and tumor formation is initiated as cancer cells are successively selected for.

The nutritional requirements of cells, especially cancerous ones, necessitates that there be nutrient delivery systems present throughout the body – specifically networks of blood and lymphatic vessels (Nishida, Yano, Nishida, Kamura, & Kojiro, 2006). The pre-cancerous vasculature present in tissues requires most cells to reside within 100 micrometers of a capillary blood vessel (Hanahan & Weinberg, 2000). However, as a tumor enlarges at its ends through excessive mitosis and the central core of parental cell becomes increasingly further away from vascular systems, the central core becomes necrotic due to lack of sufficient nutrients. The tumor must then develop ways to recruit nutrients to continue fueling growth (Kreeger & Lauffenburger, 2010) and remove waste products (Nishida, Yano, Nishida, Kamura, & Kojiro, 2006), as the pre-cancerous network of blood and lymph vessels is insufficient for tumor growth due to tumor expansion often outpacing the delivery capabilities of the accessible vasculature (Jones & Thompson, 2009). In fact, tumors are unable to continue growing beyond a volume of two cubic millimeters unless they develop methods to obtain additional nutrients (Ma & Adjei, 2009). In response to tumor growth, cancerous cells often stimulate angiogenesis and lymphagenesis to relieve nutrient deficiency (see Figure 4) (Jones & Thompson, 2009). Angiogenesis is the formation of new blood vessels, while lymphagenesis is the formation of lymphatic vessels; both processes are examples of neovascularization (Nishida, Yano, Nishida, Kamura, & Kojiro, 2006).

In normal cells that are growing to form tissues, neovascularization is a highly regulated process (Hanahan & Weinberg, 2000). The ability of cancerous cells to override

regulations to induce and maintain angiogenesis is thought to result from activation of an angiogenic 'switch' that may be turned on by increased expression of angiogenic factors and decreased expression of angiogenic inhibitors (Hanahan & Weinberg, 2000). Turning on this 'switch' involves altered gene expression of inhibitors and activators such as vascular endothelial growth factor (VEGF). In general, cancer cells stimulate altered gene expression to shift the equilibrium between angiogenic activating and inhibiting factors to induce the angiogenic phenotype in response to detection of decreased profiles of oxygen and other nutrients (Kreeger & Lauffenburger, 2009; Nishida, Yano, Nishida, Kamura, & Kojiro, 2006).

Nishida, et al. (2006) outline the basic steps that lead to angiogenesis in cancer. First, local tissue basement membrane is degraded due to tumor growth, resulting in hypoxia, or reduced oxygen availability. This may trigger some cancer cells to begin to overexpress angiogenic factors. Next, angiogenic factors stimulate endothelial cells to produce matrix metalloproteinases (MMPs) that degrade the extracellular matrix (ECM), allowing the endothelial cells to begin to migrate. Third, endothelial cells proliferate and stabilize to form new blood vessels as they embed in surrounding tissues. Finally, sustained angiogenesis is influenced by the continued expression and activity of angiogenic factors.

Proliferative cells utilize more than a dozen growth factors to initiate angiogenesis (Hanahan & Weinberg, 2000; Nishida, Yano, Nishida, Kamura, & Kojiro, 2006). VEGF (vascular endothelial growth factor) is a potent angiogenic factor that is overexpressed in a variety of tumors (Hanahan & Weinberg, 2000). One study has observed the VEGF family

of factors and their specific receptors expressed in about half of the investigated tumors (Nishida, Yano, Nishida, Kamura, & Kojiro, 2006).

CHAPTER SEVEN

METASTASIS

During the development of most cancers, tumor cells are able to leave the primary growth site and invade adjacent tissues (Hanahan & Weinberg, 2000). In a process known as metastasis, cancer cells circulate through the vascular system and implant and proliferate at foreign sites (Nishida, Yano, Nishida, Kamura, & Kojiro, 2006). The process of metastasis is complex and involves several steps (Hanahan & Weinberg, 2000). In order to be capable of invasion, cancer cells must be able to first grow and spread locally (via tumor growth and angiogenesis, as previously discussed), survive in the blood and lymphatic vessels, and survive in foreign tissue (Kreeger & Lauffenburger, 2009; Paňková, Rösel, Novotný, & Brábek, 2010). Cancer cells with these properties are able to implant a foreign tissue where nutrients and space do not immediately limit further proliferation (Hanahan & Weinberg, 2000).

The initiation of metastasis, which involves cancer cells leaving the primary tumor site to invade local sites, begins with movement through the local extracellular matrix (ECM) (Paňková, Rösel, Novotný, & Brábek, 2010). Navigation through the ECM is facilitated in most cases by downregulation of protease inhibitors (Hanahan & Weinberg, 2000) and an increase in the expression of MMPs that degrade the extracellular matrix (Paňková, Rösel, Novotný, & Brábek, 2010) and the basement membrane (Kreeger & Lauffenburger, 2010). Paňková, et al (2010) examine the different forms of migration used by cancer cells to

overcome the ECM. Some cancerous cells are able to spread collectively as epithelial tissues by retaining cell-to-cell junctions. Other cells may migrate individually, and the transition between the epithelial migration to individual cancer cell migration is called epithelial-mesenchymal transition (EMT) (Paňková, Rösel, Novotný, & Brábek, 2010). Cancer cells are able to transition from epithelial sheets in order to invade individually, as evidenced by studies that have found tight junction proteins and other cell-cell adhesion molecules to be dysfunctional in various human cancers (Brennan, Offiah, McSherry, & Hopkins, 2009; Hanahan & Weinberg, 2000). The most widely observed dysfunctional cell adhesion molecule in cancer is E-cadherin, which fails to function in most epithelial cancers (Hanahan & Weinberg, 2000).

Paňková, et al (2010) specifically examined two distinct modes of individual invasion and migration. Mesenchymal invasiveness by individual tumor cells is characterized by the formation of leading pseudopods that facilitate movement at 0.1-0.5 micrometers per minute. Cancer cells utilizing this form of invasion can be distinguished by a long, spindle-like shape (see Figure 5). Cancerous cells displaying a second type of invasion, called amoeboid-like movement, undergo cycles of cell-body expansion and contraction to move through gaps in the extracellular matrix at speeds ranging from 2 micrometers per minute to 25 micrometers per minute. Tumor cells exhibiting amoeboid-like movement present as rounded cells in a 3D substrate. The mesenchymal and amoeboid modes of movement are not mutually exclusive; in response to changes in the microenvironment, cancerous cells are able to rapidly switch from one mode of invasiveness to the other by regulating specific pathways. The key difference between the two modes of invasion is that cancerous cells

displaying amoeboid movement do not necessarily participate in ECM degradation. Rather, the mechanical forces that cause the contraction-relaxation cycles typical of amoeboid movement are strong enough to structurally change the ECM, allowing the cells to migrate easily. This finding was interesting in that it disagreed with the widely-held notion that tumor cell invasion involves ECM proteolysis. However, further studies need to be done to test the viability of amoeboid-like, proteinase-independent invasion in vivo (Paňková, Rösel, Novotný, & Brábek, 2010).

The second step of metastasis involves a further spread of cancer cells that travel to secondary sites such as distant organs via the lymphatic system or the bloodstream (Kreeger & Lauffenburger, 2009; Nishida, Yano, Nishida, Kamura, & Kojiro, 2006; Paňková, Rösel, Novotný, & Brábek, 2010). By spreading to foreign tissues, cancer cells are able to implant and proliferate in a new environment that is not limited in nutrient and oxygen availability (Hanahan & Weinberg, 2000). This spread of cancer cells to foreign sites is the most life-threatening aspect of cancer (Paňková, Rösel, Novotný, & Brábek, 2010) and has been implicated in the majority of cancer fatalities (Kreeger & Lauffenburger, 2010) and possibly up to 90% of all cancer deaths (Hanahan & Weinberg, 2000). Research has shown that additional mutations may be required to convert local growth and invasion to extravasion to foreign tissues (Kreeger & Lauffenburger, 2010). Metastasis is hardly a random process, and scientists also suspect that some genetic mutations may also give cancer cells preferences for target organs (Kreeger & Lauffenburger, 2010).

CHAPTER EIGHT

MODEL SYSTEMS

In all of the studies discussed, certain model systems were employed to study the genotypes and phenotypes that characterize cancer. In recent oncological research, transgenic mouse models have been widely used as a valuable means to indentify events leading to cancer development in humans (Tran, et al., 2008). There are multiple advantages to studying cancer pathogenesis in mouse models. Mouse models allow temporal control of oncogene expression and have thus been exploited in elucidating tumorigenic pathways (Tran, et al., 2008). Furthermore, murine models allow for quick, reproducible tumor induction.

Carcinogenesis in mice is similar to that in humans in that spontaneously occurring cancers in rodents is also relatively common (Rosenberg, Giardina, & Tanaka, 2009). Some of these cancers, and the mechanism by which they arise, show high degrees of similarity in mice and human systems (Rosenberg, Giardina, & Tanaka, 2009). For example, the transition between non-invasive adenomas to invasive carcinomas in colorectoral cancer occurs via a related sequence of events in humans and mice. Furthermore, chemical agents that induce tumors in rodent systems target genes and genetic pathways that have been found to be targeted in human cancers (Rosenberg, Giardina, & Tanaka, 2009). With the availability of detailed genetic information on individual mouse lines, scientists are able to exploit mouse models to develop experiments that have high degrees of external validity to

human systems (Rosenberg, Giardina, & Tanaka, 2009). Certain genetically-defined mice lines also experience different sensitivities to carcinogens, so cancer studies utilizing mouse models are able to recreate the variation in cancer development that is known to present during human carcinogenesis (Rosenberg, Giardina, & Tanaka, 2009). As a result of the all these similarities in cancer development in mice and humans, mouse models have proven useful in testing therapeutic agents (Rosenberg, Giardina, & Tanaka, 2009).

Other models to study cancer that have been utilized offer distinct advantages over mouse models. In the last decade, computational (or mathematical) models have become a potent tool for elucidating complex biochemical networks that are invariably affected by cancerous conditions. Stites & Ravichandran (2009) examine the advantages of computational and mathematical models in elucidating the aforementioned stages of cancer development. Unlike murine models, computational models can catalog intricate network properties to better understand the effect of a single mutagenic event on a cell's function. Many mathematical models are able to incorporate large volumes of quantitative information that can describe the complexity of a cell's biological pathways. These models can be manipulated in more ways than can traditional models and can simultaneously administer multiple biochemical reactions. For example, computers have been able to calculate the time a population of cells requires in accumulating the two mutagenic events that inactivate most tumor suppressor genes. Furthermore, scientists have recently modeled mechanisms describing the progressive acquisition of mutations, the role of the microenvironment of the tumor, and how a tumor is affected by targeted therapy. The latter ability of mathematical models serves as a large advantage over traditional models; scientists are able to study the

effect of a potential drug to evaluate whether its activity in treating cancer is significant enough to warrant large-scale production. Computational analysis may also identify the most effective strategy to target specific pathways. Overall, mathematical analysis allows for in-depth study of the intricate biochemical pathways that are often deregulated in cancer cells, and these models may be used to develop more effective treatments (Stites & Ravichandran, 2009).

CHAPTER NINE

TREATMENT

Understanding of the abnormal intracellular processes that characterize cancer cells, through the use of model systems previously discussed, has brought to light a variety of possible mechanisms to treat cancer (Brennan, Offiah, McSherry, & Hopkins, 2009; Ma & Adjei, 2009). Studies have shown that gene expression profiles of tumors allow scientists to predict subsequent pathway deregulation (Bild, et al., 2006; Hanahan & Weinberg, 2000). Elucidation of these deregulated signaling pathways in cancer cells has led to the idea of targeting key proteins that function in the crucial pathways that give cancer cells their hallmark capabilities (Hanahan & Weinberg, 2000; Stites & Ravichandran, 2009).

Hait (2009) examines details surrounding targeted and non-targeted therapies. In general, targeted therapy refers to attacking a specific molecule with a particular drug to inhibit that molecule's function. These agents selectively inhibit a target molecule that functions abnormally in cancer cells. Often, targeted therapies affect events early in deregulated signaling pathways that confer abnormal growth. In contrast to targeted therapies, non-targeted therapies involve drugs discovered by phenotypical screening without prior knowledge of the target molecule. These therapies often affect proteins or nucleic acids downstream of most signaling pathways.

Each mode of therapy offers advantages and disadvantages. Agents used in targeted therapies usually have low toxicities, but have proven to possess limitations in treating solid

tumors. In some cases, drugs that target proteins involved in the beginning parts of pathways are subject to downstream mechanisms of resistance that may render targeted therapies less effective (see Figure 6). Time costs are also associated with targeted therapies as molecular targets need to be identified prior to drug synthesis. However, previous methods of target identification, which took 40 years in some cases, have been improved upon. Nontargeted therapies, on the other hand, include some of the most effective drugs; however, their high toxicity is a large disadvantage. Both forms of therapy are prevalent in oncological treatment strategies (Hait, 2009).

Traditional targeted and non-targeted methods of dealing with cancer involve interfering with mitosis, DNA synthesis, and cellular repair systems (Loeb, Loeb, & Anderson, 2003; Ma & Adjei, 2009). For example, specific drugs can be synthesized to target error-prone DNA polymerases without inhibiting normal DNA replication (Loeb, Loeb, & Anderson, 2003). Other drugs function in stimulating the remains of the apoptotic pathway in cancer cells, while yet others attempt to inhibit angiogenesis (Hanahan & Weinberg, 2000; Nishida, Yano, Nishida, Kamura, & Kojiro, 2006). Brennan, et al (2009) suggest tight junction proteins as optimal targets to prevent metastasis. Other researchers praise the potential to exploit the metabolic changes that characterize cancer cells to deter cancer growth (DeBerardinis & Cheng, 2009; Jones & Thompson, 2009).

Cancer treatments that do not rely on the use of drugs are also prevalent. Surgery is used to removed localized solid tumors, while toxic chemotherapy is used for tumors that cannot be removed surgically (Nishida, Yano, Nishida, Kamura, & Kojiro, 2006). However, these methods of direct cancer suppression are often used in combination with drug therapy,

and it has been shown the combination therapy increases survival (Nishida, Yano, Nishida, Kamura, & Kojiro, 2006). Combining chemotherapy and drug treatment is a popular alternative because it is less toxic than a complete chemotherapy regimen (Loeb, Loeb, & Anderson, 2003).

CHAPTER TEN

CONCLUSION

The content of this literature review summarizes many of the basic concepts dealing with the cellular biology of cancer. Cancer is generated via mutagenic events often affecting key oncogenes and tumor suppressors. These genetic alterations, coupled with changes in gene expression, allow cancer cells to participate in unregulated proliferation. Cancer cells begin to accumulate mutations and metabolic adaptations which allow them to be selected for over normal cells. As cancer cells grow into large tumors, they are able to induce extension of the vasculature which provides them the nutrients required to sustain excessive proliferation. Cancerous conditions are furthered by metastasis to foreign tissues. Model systems have been developed to study all of the abnormalities that characterize cancer. Finally, different modes of cancer treatment are being developed, each with distinct advantages and disadvantages.

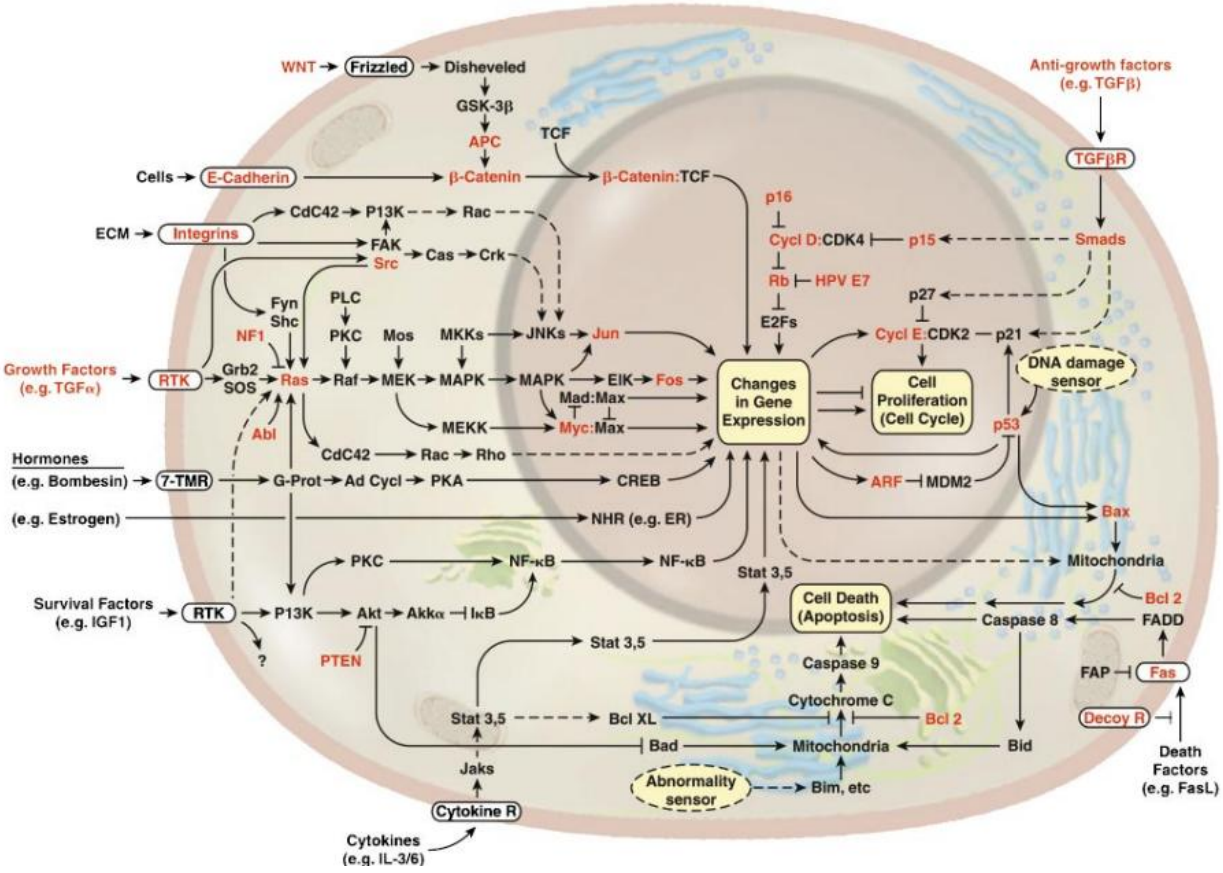

Figure 1. The onset of cancer involves alterations to intricate and complex pathways in the cell that control and monitor gene expression, cell proliferation, DNA integrity, and cell death (Hanahan & Weinberg, 2000). Highlighted in red are genes known to have altered functions in cancerous cells.

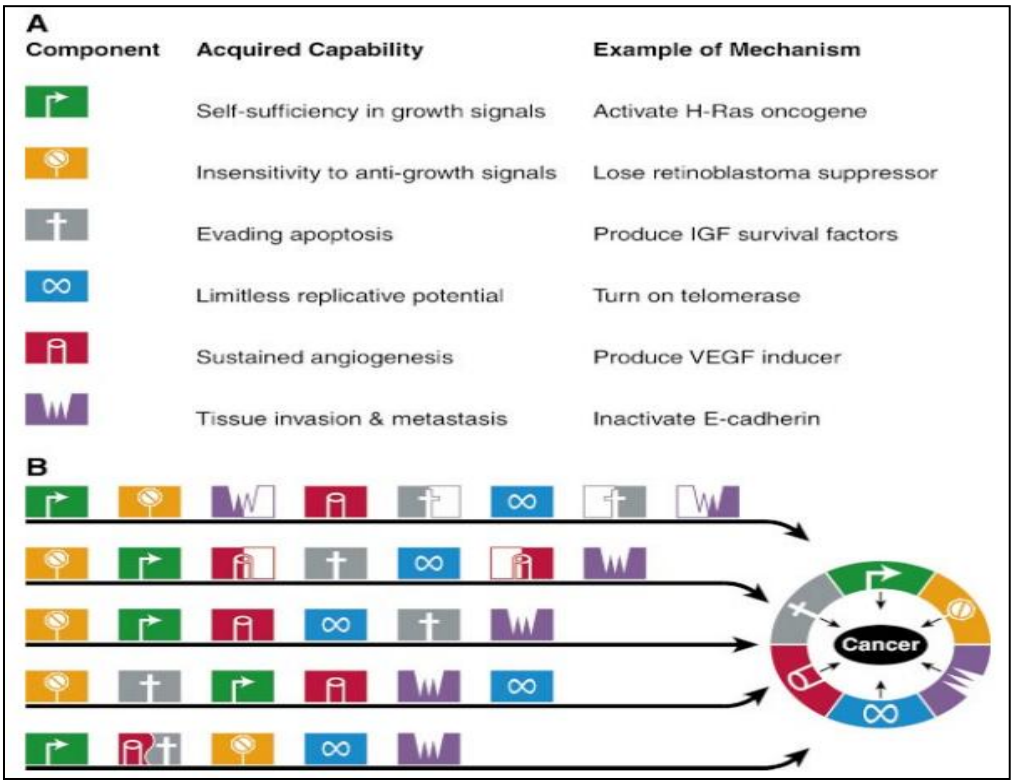

Figure 2. Hanahan and Weinberg (2000) state that cancer progression varies mechanistically in regards to the hallmark traits acquired and chronologically in regards to the order those traits are acquired

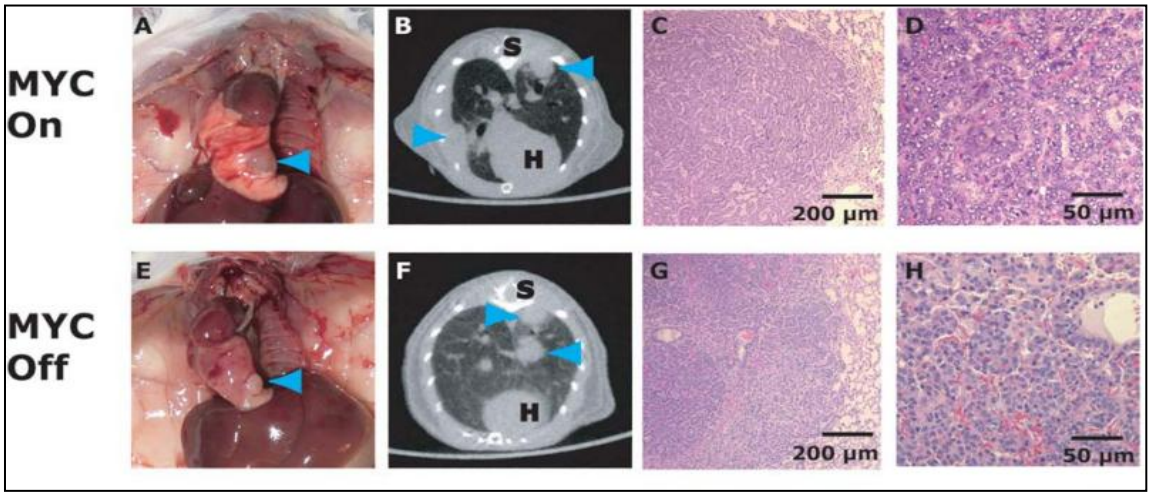

Figure 3. (A) Tran, et al (2008) show that myc expression in mice lung tissue resulted in lung adenocarcinomas, shown radiographically (B) and microscopically (C & D). Inactivation of Myc failed to completely reduce tumors (E-H) .

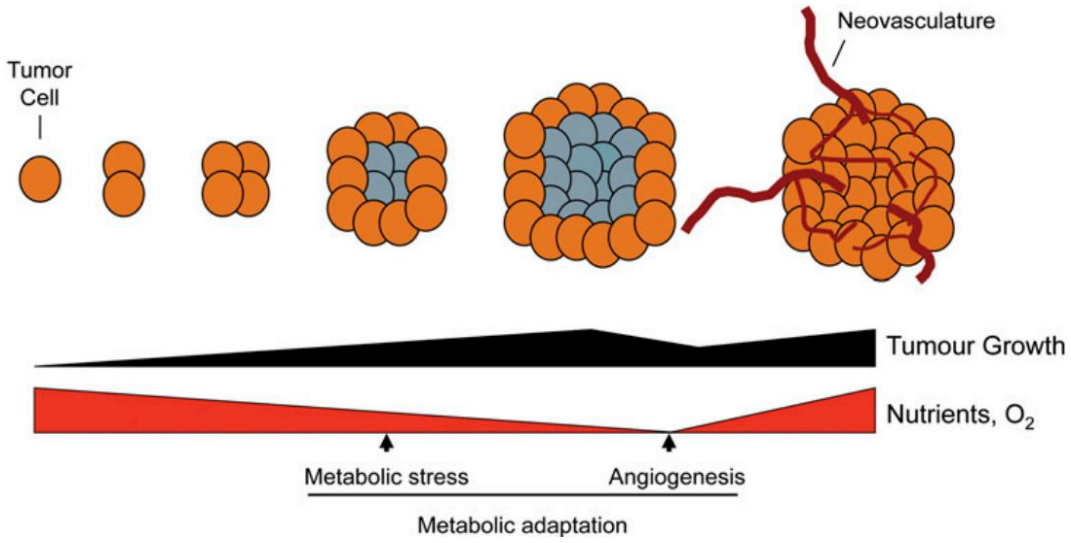

Figure 4. As a tumor increases in cell number and size, cancer cells stimulate angiogenesis to overcome metabolic stress in the form of local nutrient and oxygen deficiency (Jones & Thompson, 2009).

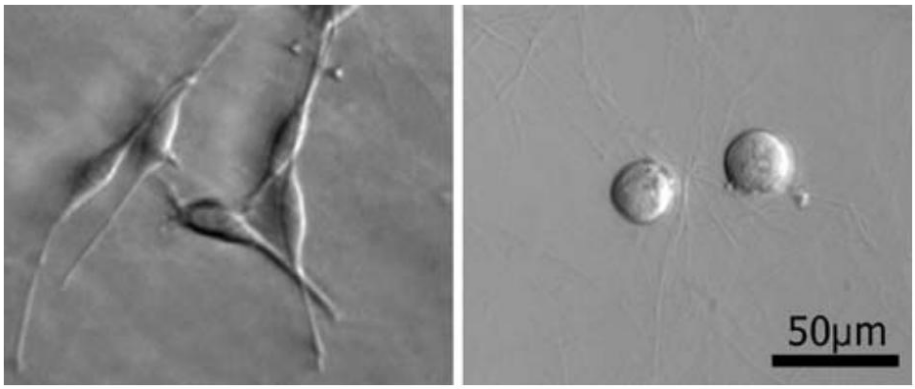

Figure 5. The left pane shows the spindle-like morphology of mesenchymal-invasive cancerous cells. The right pane shows the round morphology of amoeboid-invasive cancerous cells (Paňková, Rösel, Novotný, & Brábek, 2010).

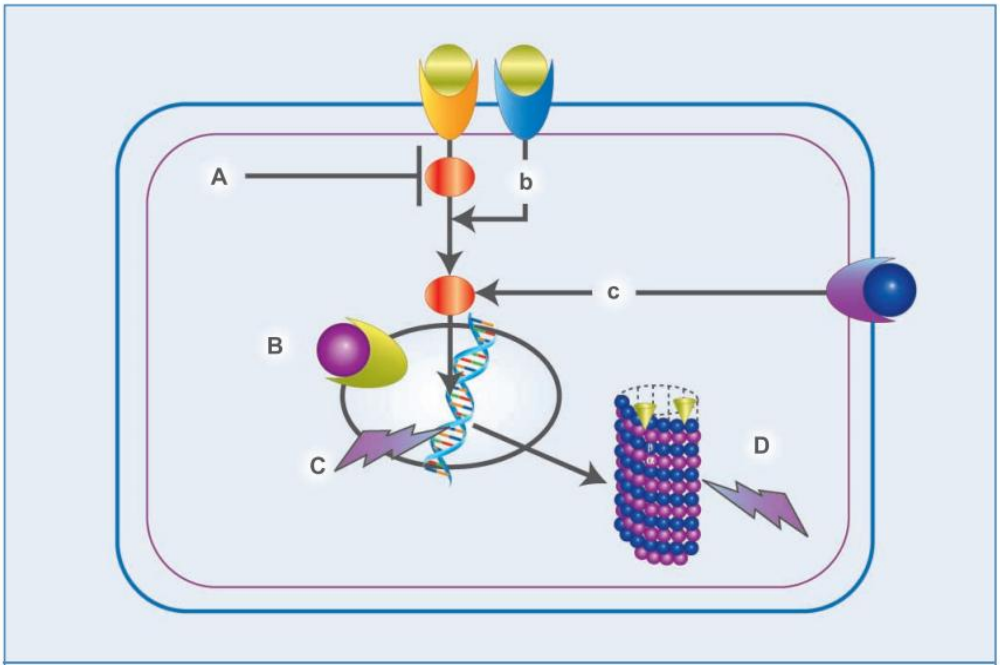

Figure 6. Drugs A, B, C, and D target different points of an example pathway that stimulates DNA synthesis of proteins and assembly of the mitotic symbol for the increased cell division seen in cancer. Drug A targets a tyrosine kinase pathway but is susceptible to interference from activated pathways b or c. Drug B targets a nuclear receptor that regulates gene expression. Drug C targets DNA directly, while Drug D targets newly-synthesize microtubules of the mitotic apparatus. The targets of Drugs B, C, and D are further downstream than that targeted by Drug A and are less susceptible to downstream pathways that may block the drugs' effects (Hait, 2009).

References

Bild, A. H., Yao, G., Chang, J. T., Wang, Q., Potti, A., Chasse, D., . . . Nevins, J. R. (2006). Oncogenic pathway signatures in human cancers as a guide to targeted therapies. *Nature, 43*, 353-357.

Blagosklonny, M. V. (2003). Cell Immortality and Hallmarks of Cancer. *Cell Cycle, 2*(4), 296-299.

Brennan, K., Offiah, G., McSherry, E. A., & Hopkins, A. M. (2009). Tight Junctions: A Barrier to the Initiation and Progression of Breast Cancer? *Journal of Biomedicine and Biotechnology, 2010*, 1-16.

Cancer. (2012, February). Retrieved from World Health Organization: http://www.who.int/mediacentre/factsheets/fs297/en/

DeBerardinis, R. J., & Cheng, T. (2010). Q's next: The diverse functions of glutamine in metabolism, cell biology and cancer. *Oncogene, 29*(3), 313-324.

Forster, C. (2008). Tight Junctions and the modulation of barrier function in disease. *Histochemistry and Cell Biology, 130*, 55-70.

Hait, W. N. (2009). Targeted Cancer Therapeutics. *Cancer Research, 69*(4), 1263-1267.

Hanahan, D., & Weinberg, R. A. (2000). The Hallmarks of Cancer. *Cell, 100*, 57-50.

Jayshree, R. S., Sreenivas, A., Tessy, M., & Krishna, S. (2009). Cell intrinsic and extrinsic factors in cervical carcinogenesis. *Indian Journal of Medical Research, 130*, 286-295.

Jones, R. G., & Thompson, C. B. (2009). Tumor suppressors and cell metabolism: a recipe for cancer growth. *Genes & Development, 23*, 537-548.

Kreeger, P. K., & Lauffenburger, D. A. (2010). Cancer systems biology: a network modeling perspective. *Carcinogenesis, 31*(1), 2-8.

Loeb, L. A., Loeb, K. R., & Anderson, J. P. (2003). Multiple mutations and cancer. *Proceedings of the National Academy of Science, 100*(3), 776-781.

Lu, Z., & Bast, J. R. (2009). Chapter 5: Tumor Suppressor Genes. *Cancer Treatment and Research, 149*, 109-129.

Ma, W. W., & Adjei, A. A. (2009). Novel Agents on the Horizon for Cancer Therapy. *CA: A Cancer Journal for Clinicians, 2009*, 111-137.

Macleod, K. (2000). Tumor suppressor genes. *Curent Opinion in Genetics & Development, 10*, 81-93.

Marsit, C. J., Karagas, M. R., Danaee, H., Liu, M., Andrew, A., Schned, A., . . . Kelsey, K. T. (2006). Carcinogen exposure and gene promoter hypermethylation in bladder cancer. *Carcinogenesis, 27*(1), 112-116.

Nishida, N., Yano, H., Nishida, T., Kamura, T., & Kojiro, M. (2006). Angiogenesis in cancer. *Vascular Health and Risk Management, 2*(3), 213-219.

Paňková, K., Rösel, D., Novotný, M., & Brábek, J. (2010). The molecular mechanisms of transitoin between mesenchymal and amoeboid invasiveness in tumor cells. *Cell and Molecular Life Sciences, 67*, 63-71.

Rosenberg, D. W., Giardina, C., & Tanaka, T. (2009). Mouse models for the study of colon carcinogenesis. *Carcinogenesis, 30*(2), 183-196.

Stites, E. C., & Ravichandran, K. S. (2009). A Systems Perspective of Ras Signaling in Cancer. *Clinical Cancer Research, 15*(5), 1510-1513.

Tran, P. T., Fan, A. C., Bendapudi, P. K., Koh, S., Komatsubara, K., Chen, J., . . . Felsher, D. W. (2008). Combined Inactivation of MYC and K-Ras Oncogenes Reverses Tumorigenesis in Lung Adenocarcinomas and Lymphomas. *PLOS ONE, 3*(5), 1-12.

Zhang, B., Pan, X., Cobb, G. P., & Anderson, T. A. (2007). microRNAs as oncogenes and tumor suppressors. *Developmental Biology, 302*, 1-12.