

Chapter 1

Cancer and somatic evolution

1.1 What is cancer?

The development and healthy life of a human being requires the cooperation of more than ten million cells for the good of the organism. This cooperation is maintained by signals and cellular checkpoints which determine whether cells divide, die, or differentiate. The phenomenon of cancer can be defined on various levels. On the most basic level, cancer represents the collapse of this cooperation. This results in the selfish, uncontrolled growth of cells within the body which eventually leads to the death of the organism. The first chapter will discuss several aspects of cancer biology. This forms the background for the mathematical models which are presented in this book. Of course, cancer biology is a very complicated topic and involves many components which are not mentioned here. A comprehensive review of cancer biology is given in standard textbooks, such as [Kinzler and Vogelstein (1998)].

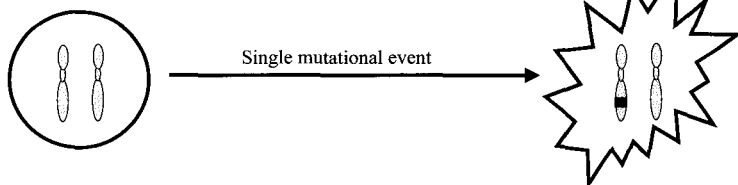
It is commonly thought that cancer is a disease of the DNA. That is, uncontrolled growth of cells is the result of alterations or mutations in the genetic material. More precisely, the emergence of cancer may require the accumulation of multiple mutations which allow cells to break out of the regulatory networks which ensure cooperation. This concept is referred to as *multi-stage carcinogenesis*. Once a cancerous cell has been created it can undergo a process known as *clonal expansion*. That is, it gives rise to descendants by cell division, and the population of cells grows to higher numbers. During this process, cells can acquire a variety of further mutations which leads to more advanced progression. A cancer is typically comprised of a variety of different genotypes and represents a “mosaic” of cell lineages. The growth of a single, or *primary*, cancer does not usually

lead to the death of the organism. Some cancer cells can, however, acquire the ability to enter the blood supply, travel to a different site, and start growing in a different organ. This process is referred to as *metastasis*. It is usually the metastatic growth which kills the organism.

1.2 Basic cancer genetics

Specific genes ensure that the integrity of cells is maintained and that uncontrolled growth is prevented. When these genes are mutated, cells become prone to developing a cancerous phenotype (also referred to as *transformation*). These genes can be broadly divided into three basic categories [Vogelstein *et al.* (2000a)]: oncogenes, tumor suppressor genes, and repair genes.

(a) Oncogene (gain of function)



(b) Tumor suppressor gene (loss of function)

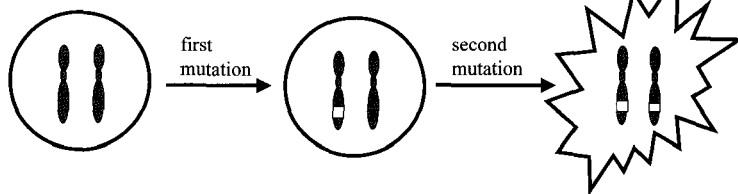


Fig. 1.1 The concept of (a) oncogenes and (b) tumor suppressor genes. Oncogenes result in a gain of function if one of the two copies receives an activating mutation. Tumor suppressor genes can be inactivated (loss of function) if both copies are mutated.

In healthy cells, oncogenes (Figure 1.1) promote the regulated proliferation of cells in the presence of the appropriate growth signals. The best example is the renewal of epithelial tissue such as the skin or the lining of

the gastrointestinal tract. When oncogenes become mutated they induce the cell to divide continuously, irrespective of the presence or absence of growth signals. This can result in unwanted growth and cancer. Examples of oncogenes include Ras in colon cancer or BCL-2 in lymphoid cancers. Only a single mutation is required to activate an oncogene because it causes a “*gain of function*”. Normal cells have two copies of every gene and chromosome; one derived from the mother, the other derived from the father. If any of the copies becomes activated, the cell attains the new behavior.

Tumor suppressor genes (Figure 1.1), on the other hand, are responsible for stopping growth in normal cells. Cell growth has to be stopped if a cell becomes damaged or mutated, or if cell death is required for normal tissue homeostasis. This is done either by preventing the cell from completing the cell cycle (*cell cycle arrest* or *senescence*), or by inducing a cellular program which results in cell death (*apoptosis*). In this way, altered cells cannot succeed to grow to higher levels and cannot induce pathology. When tumor suppressor genes become inactivated, the growth of altered cells is not prevented anymore, and this promotes the development of cancer. Because this type of gene needs to be inactivated rather than activated (i.e. a *loss of function event*), both the paternal and the maternal copies of the gene have to be mutated. Therefore, two mutational events are required for the inactivation of tumor suppressor genes. Because many cancers are initiated via the inactivation of a tumor suppressor gene, it is thought that cancer initiation often requires two hits. This idea was first formulated by Alfred Knudson and is called the “two hit hypothesis”. Examples of tumor suppressor genes are the gene which encodes the retinoblastoma protein and which is inactivated in retinoblastomas, APC which is inactivated in colon cancer, and p53 which is inactivated in more than 50% of all human cancers.

Finally, repair genes are responsible for maintaining the integrity of genomes. When DNA becomes damaged, for example through the exposure to UV radiation or carcinogens contained in food, those genes make sure that the damage is removed and the cell remains healthy. If repair genes become mutated, cells can acquire new genetic alterations at a faster rate, and this promotes the process of carcinogenesis. For example, mutations in oncogenes or tumor suppressor genes are generated faster. Cells which have mutated repair genes are sometimes referred to as “mutator phenotypes” or “genetically unstable cells”. Examples of repair genes are mismatch repair genes and nucleotide excision repair genes. Their inactivation promotes a variety of cancers. Loss of repair function usually requires

two hits, although a single mutation might result in reduced function in the context of certain repair genes.

1.3 Multi-stage carcinogenesis and colon cancer

Cancer initiation and progression requires the sequential accumulation of mutations, most importantly in tumor suppressor genes and in oncogenes. The case study where this is understood in most detail so far is colorectal cancer. The colon consists of a collection of so-called *crypts*.

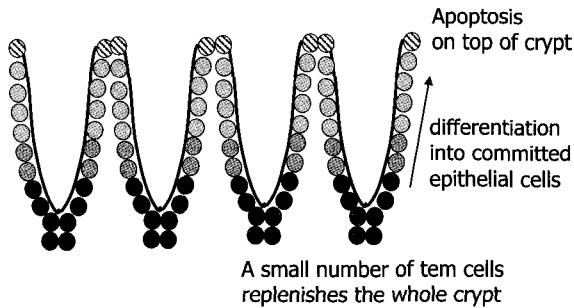


Fig. 1.2 Schematic diagram of crypts in the colon.

Crypts are involutions of the colonic epithelium (Figure 1.2). Stem cells are thought to be located at the base of the crypts. These are undifferentiated cells which can keep dividing and which give rise to differentiated epithelial cells. It is thought that stem cells divide asymmetrically. That is, stem cell division creates one new stem cell and one cell which embarks on a journey of differentiation. The differentiating cells travel up the crypt, perform their function, and die by apoptosis after about a week. Because the epithelial cells are relatively short lived, stem cell division has to give rise to new differentiated cells continuously in order to replenish the tissue. For this process to function in a healthy way, it is crucial that the differentiated cells die by apoptosis. If this cell death fails, we observe an accumulation of transformed cells around the crypts, and this gives rise to a mass of cells called a *dysplastic crypt* (Figure 1.3).

This is the first stage of colon cancer. In molecular terms, the death of differentiated cells is induced by the APC gene. APC is a tumor suppressor gene. Data suggest that the majority of colon cancers are initiated through

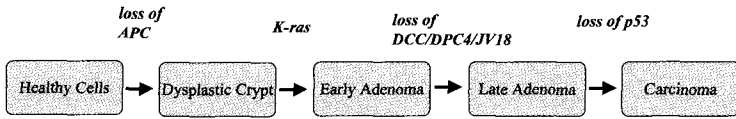


Fig. 1.3 Diagram describing the multi-stage progression of colon cancer. Drawn according to [Kinzler and Vogelstein (1998)].

the inactivation of the APC gene (Figure 1.3). A dysplastic crypt is also sometimes referred to as a polyp. As a subsequent step, many colon cancers activate the oncogene *K-ras* which allows the overgrowth of surrounding cells and an increase in the size of the tumor. This stage is called the early adenoma stage (Figure 1.3). In more than 70% of the cases, this is followed by the loss of chromosome 18q which contains several tumor suppressor genes including *DDC*, *DPC4*, and *JV18-1/MADR2*. This results in the generation of late adenomas (Figure 1.3). In the further transition from late adenoma to the carcinoma stage, *p53* is typically lost in more than 80% of the cases (Figure 1.3). Further mutations are assumed to occur which subsequently allow the colon cancer cells to enter the blood system and metastasize. Note that this sequence of event is not a hard fact, but rather a caricature. The exact details may vary from case to case, and new details emerge as more genetic research is performed.

This is a clear example of cells acquiring sequential mutations in a multi-step process while they proceed down the path of malignancy. This gives rise to an important question. The multi-step process requires many mutations. The inactivation of each tumor suppressor gene requires two mutations, and the activation of each oncogene requires one mutation. The physiological mutation rate has been estimated to be 10^{-7} per gene per cell division. Is this rate high enough to allow cells to proceed through multi-stage carcinogenesis during the life time of a human? Some investigators argue that the process of clonal expansion involves a sufficient number of cell divisions in order to account for the accumulation of all the mutations. A competing argument says that the accumulation of the oncogenic mutations requires a loss of repair function and the generation of mutator phenotypes (i.e. genetically unstable cells). Genetic instability is a defining characteristic of many cancers. It is reviewed in the following section.

Small sequence instabilities involve subtle genetic changes which can dramatically speed up the process of cancer progression. Defects in mismatch repair mechanisms give rise to microsatellite instability or MSI. This involves copying errors in repeat sequences (Figure 1.4). MSI is most common in colon cancer. It is observed in about 13% of sporadic cases and is the mechanism of cancer initiation in the hereditary non-polyposis colorectal cancer (HNPCC). Another type of small scale instability comes about through defects in nucleotide excision repair genes. These are responsible for the repair of DNA damage caused by exogenous mutagens, most importantly ultraviolet light. It is thus most important in the development of skin cancers. A defect in such repair mechanisms has been found in a disease called *xeroderma pigmentosum*, which is characterized by the development of many skin tumors in sun exposed areas.

Instabilities which involve gross chromosomal alterations are called chromosomal instability or CIN (Figure 1.4). Cells which are characterized by CIN show a variety of chromosomal abnormalities. There can be alterations in chromosome numbers which involve losses and gains of whole chromosomes. This results in aneuploidy. Alternatively, parts of chromosomes may be lost, or we can observe chromosome translocations, gene amplifications, and mitotic recombinations. Many cancers show evidence of chromosomal instability. For example, 87% of sporadic colon cancers show CIN. The reason why CIN is observed in so many cancers is unclear. CIN can be advantageous because it helps to inactivate tumor suppressor genes where both functional copies have to be lost. Assume that one copy of a tumor suppressor gene becomes inactivated by a point mutation which occurs with a rate of 10^{-7} per cell division. The second copy can then be lost much faster by a CIN event (Figure 1.4). For example, CIN could speed up the generation of an APC deficient cell in the colon. On the other hand, CIN is very destructive to the genome. Therefore, even though a cell with an inactivated tumor suppressor gene can be created with a faster rate, clonal expansion of this cell can be compromised because of elevated cell death as a consequence of chromosome loss. The costs and benefits of CIN, as well as the role of CIN in cancer progression, will be discussed extensively in this book.

While it seems intuitive that genetic instability can be advantageous because it leads to the faster accumulation of oncogenic mutations, this is not the whole story. Genetic instability can be advantageous because of an entirely different reason. If cells become damaged frequently, they will enter cell cycle arrest relatively often in order to repair the damage. Therefore, in

the presence of elevated damage, repair can compromise the growth of cells. On the other hand, cells which are unstable avoid cell cycle arrest in the face of damage and keep replicating while accumulating genetic alterations. This can lead to an overall higher growth rate of unstable compared to stable cells. The role of DNA damage for the selection of genetic instability will be discussed later in the book.

1.5 Barriers to cancer progression: importance of the microenvironment

So far we have discussed the processes of multi-stage carcinogenesis in some detail. We have thereby concentrated on an approach which is centered around the genetic events which allow cells to escape from growth control and to become cancerous. However, experiments have revealed that the interactions between tumor cells with their tissue micro-environment may be equally important in the process of carcinogenesis [Hsu *et al.* (2002); Tlsty (2001); Tlsty and Hein (2001)]. The stroma surrounding the tumors shows in many cases changes in the patterns of gene expression, in the cellular composition, and in the extracellular matrix. This allows cancers to grow and progress. The development of cancer can thus be seen as a conspiracy between tumor cells and their altered environment which allows uncontrolled growth. Under non-pathogenic conditions, the tissue environment can prevent tumor cells from growing to significant levels.

Interestingly, autopsies have revealed that people who die without ever developing cancers show microscopic colonies of cancer cells which are referred to as *in situ* tumors [Folkman and Kalluri (2004)]. Data suggest that >30% of women in the age range between 40 and 50 who do not develop cancer in their life-time are characterized by small colonies of breast cancer cells. Only 1% of women in this age range, however, develop clinically visible breast cancer. Similar patterns have been observed in the context of thyroid or prostate cancers. The reason for the inability of cancer cells to grow to higher numbers and give rise to pathology is important to understand. The defensive role of the tissue microenvironment in which the cancer tries to grow could be a key factor. For example, cancer cells require the formation of new blood supply in order to obtain oxygen and nutrients, and to grow beyond a relatively small size [Folkman (2002)]. The formation of new blood supply is termed angiogenesis (Figure 1.5).

Our understanding about the role of angiogenesis in the development of

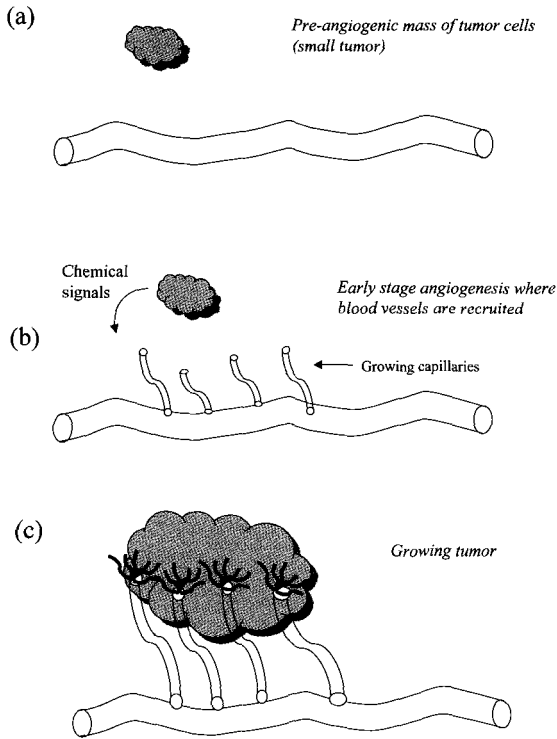


Fig. 1.5 Diagram explaining the concept of angiogenesis. (a) When a cancerous cell is created it can expand up to a small size without the need for blood supply. At this stage, the growth of an *avascular tumor* stops. (b) When angiogenic cell lines emerge, they send out chemical signals called promoters. This induces blood vessels to grow towards the tumor. (c) This process leads to the complete vascularization of the tumor, allowing it to grow to larger sizes.

cancers has been advanced significantly by a variety of studies from Judah Folkman's laboratory [Folkman (1971); Folkman (2002)]. Whether new blood supply can be formed or not appears to be determined by the balance between angiogenesis inhibitors and angiogenesis promoters. Healthy tissue produces angiogenesis inhibitors. Examples of inhibitors are thrombospondin, tumstatin, canstatin, endostatin, angiostatin, and interferons. At the time of cancer initiation, the balance between inhibitors and promoters is heavily in favor of inhibition. Data suggest that even cancer cells themselves initially produce angiogenesis inhibitors which strengthens the defense of the organism against the spread of aberrant genes. In order to

grow beyond a small size, angiogenic tumors have to emerge. These are tumor cells which can shift the balance away from inhibition and in favor of promotion. This can be brought about by the inactivation of angiogenesis inhibitors, or by mutations which result in the production of angiogenesis promoters. Examples of promoters are growth factors such as FGF, VEGF, IL-8, or PDGF. If the balance between inhibitors and promoters has been shifted sufficiently in favor of promotion, the cancer cells can grow to higher numbers and progress towards malignancy (Figure 1.5). The mechanisms by which blood supply is recruited to the tumor, and the ways in which inhibitors and promoters affect cancer cells are still under investigation. New blood supply can be built from existing local endothelial cells. On the other hand, angiogenesis promoters may induce a population of circulating endothelial progenitor cells to be recruited to the local site where the blood supply needs to be built. Blood supply can affect cancer cells in two basic ways. First it can influence the rate of cell death. That is, in the absence of blood supply cells die more often by apoptosis as a result of hypoxia, and this is relaxed when sufficient blood supply is available. On the other hand, lack of blood supply can prevent cancer cells from dividing. In this case they remain *dormant*, That is, they do not divide and do not die. These dynamics will be discussed extensively.

1.6 Evolutionary theory and Darwinian selection

Theodosius Dobzhansky who, according to Stephen J. Gould, was the greatest evolutionary geneticist of our times, wrote that “nothing in biology makes sense except in the light of evolution”. This also applies to our understanding of cancer. The process of carcinogenesis includes all the essential ingredients of evolutionary theory: reproduction, mutation, and selection (Figure 1.6).

As outlined in detail above, the entire process of cancer initiation and progression is concerned with the accumulation of mutations which allow the cells to break out of normal regulatory mechanisms. Such cells will grow better than healthy cells and are advantageous. In evolutionary terms, they are said to have a higher *fitness*. The more oncogenic mutations the cells acquire, the better they are adapted to growing in their environments, and the higher their fitness. Cancer cells which grow best can be selected for and can exclude less fit genotypes. Cancer cells can even adapt their “evolvability”: genetically unstable cells may be able to evolve faster and adapt

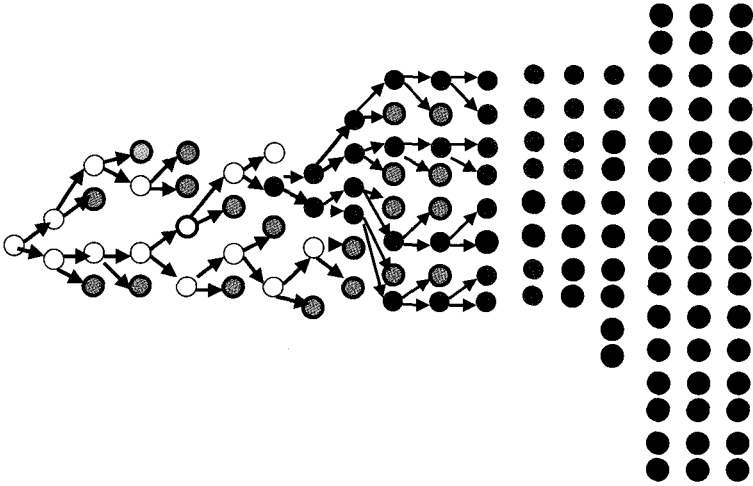


Fig. 1.6 Diagram explaining the concept of somatic evolution and cancer progression. Cancer originates with the generation of a mutant cell. This cell divides and the population grows. This is called clonal expansion. Further mutations can subsequently arise which have a higher fitness. They grow and expand further. Consecutive mutations and rounds of clonal expansion allow the cancer to grow to ever increasing sizes.

better than stable cells. This can be very important in the face of many selective barriers and changing environments. Barriers can include inhibitory effects which are exerted by the tissue microenvironment, or an adaptive immune system which can specifically recognize a variety of tumor proteins and mount new responses as the tumor evolves. The environment in our bodies can change over time and render different genotypes advantageous at different stages. An example is aging which involves the continuous rise in the rate of DNA damage as a result reactive oxygen species which are produced as a byproduct of metabolism.

The somatic evolution of cells will be a central component of the mathematical models which are discussed in this book. A large part of the chapters will investigate the selective forces which can account for the emergence of genetic instability in cancer. Is instability selected for because it allows faster adaptive evolution of cells as a result of the enhanced ability to acquire oncogenic mutations? Can instability reduce the fitness of cells because it destroys the integrity of the genome? Can a rise in the level of DNA damage select for unstable cells because the costs of arrest and senescence are avoided? What are the pathways to cancer? Further, the book

will examine the evolution of angiogenic cell lines, the relationship between immunity, somatic evolution and cancer progression, and will conclude with some implications for treatment strategies.

The central philosophy of the book is twofold: to introduce mathematicians to modeling cancer biology, and to introduce cancer biologists to computational and mathematical approaches. The book is written in this spirit, presenting both the analytical approaches and the biological implications. It is important to note that we do not aim to cover the entire subject of computational cancer biology. That would be impossible because the subject is characterized by an enormous complexity and can be addressed on a variety of levels. Instead, we concentrate on one particular aspect of cancer biology; that is, we concentrate on studying the process of carcinogenesis and consider it in the light of somatic evolution. We aim to introduce readers to the basic mathematical methodology as well as to some interesting biological insights which have come out of this work.